Physical characterization and spray performance of non-sterile liquid preparations

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DE ACORDO COM A LEGISLAÇÃO EM VIGOR, NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTA TESE/TRABALHO.

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Assinatura:

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ABSTRACT

The drug product (DP) under development is composed by a non-sterile liquid preparation and a container closure system. This DP is intended to release a drug substance (DS) in an internal mucosa and to promote the permeation of DS into the bloodstream for systemic delivery. For that, the development of methods for the physical characterization of the formulation and the spray performance evaluation are required. The tests performed were selected according to the Critical Quality Attributes (CQA) identified for the technology/ product and were based on pharmacopoeias chapters, as well as, on pharmaceutical guidances.

The appearance of the formulation was visually inspected and the results revealed clear to slightly opaque formulations. The density (specific gravity) and pH measurement are critical attributes to predict the stability of DP. Viscosity was important to estimate the capability to obtain *sprayable* formulations and to predict the stability of the DP over time. Adhesion test is crucial to distinguish adhesive over non-adhesive formulations. To study the release profile of the DS, the *in vitro* drug release test was evaluated and the results depict that the DS was partially or totally released within the first two minutes and allowed comparative studies.

The spray performance was investigated using controlled settings with the aid of a specialized equipment – Texture Analyser. The spray actuation test defined the actuation (ActF) and maximum (MaxF) intensity forces required to actuate different spray devices. Pump delivery test was performed to define priming details of the spray device. The results recommended to discard the first 5 to 6 actuations before the administration of the first dose of DP to the patient. After the initial priming, if the product is stored for more than 1 month without use, it is recommended to perform 1 repriming actuation before the administration to the patient (up to 6 months). The tail-off occurs when the dose emitted from the spray device starts decreasing. During the pre-stability evaluation, the weight loss of the spray device containing formulation has also been evaluated. The results showed that, besides the presence of volatile excipients, the weight loss was within an acceptable limit referred by the authorities.

Overall, the study of the physical features and the investigation of spray performance support the development of non-sterile liquid preparations and are essential to ensure the quality of pharmaceutical product and patient safety. The results obtained help the formulation development team to define the specification according to the CQA for the intended use.

RESUMO

O produto em desenvolvimento é composto por uma preparação líquida não estéril e pelo recipiente de isolamento. Este produto deve libertar a substância ativa numa mucosa interna e promover a sua permeação para a corrente sanguínea favorecendo a ação sistémica. Para tal, foi necessário desenvolver métodos para a caraterização física da formulação e para a avaliação do desempenho do dispositivo spray. Estes testes foram selecionados de acordo com os "Critical Quality Attributes" (CQA) identificados para o produto/tecnologia e basearam-se nas farmacopeias e "guidances" farmacêuticas.

A aparência da formulação foi avaliada visualmente e os resultados revelaram que as formulações ideais são límpidas ou ligeiramente opacas. A densidade e o pH da formulação são atributos críticos que preveem a estabilidade do produto. A medição da viscosidade das formulações permitiu avaliar a capacidade destas serem dispensadas via spray e estimar a estabilidade do produto. O método de adesão desenvolvido possibilitou a distinção entre formulações adesivas e não adesivas. Por fim, o teste de libertação *in vitro* da substância ativa permitiu a realização de testes comparativos entre produtos, tendo-se ainda verificado que a libertação da substância ativa ocorre parcial ou totalmente em 2 minutos.

O desempenho do spray foi efetuado utilizando configurações bem definidas com o auxílio de um equipamento especializado - Texture Analyser. O teste de actuação do dispositivo spray define as intensidades de força de actuação (ActF) e máximas (MaxF) necessárias para os diferentes dispositivos spray analisados. O peso libertado por actuação foi avaliado de forma a definir o perfil de pesos libertados para cada dispositivo spray. Os resultados obtidos recomendam descartar as primeiras 5/6 actuações antes da primeira administração. E, nos casos em que o produto é armazenado sem qualquer utilização de 1 a, pelo menos, 6 meses também é recomendado descartar a primeira actuação antes da administração ao doente. A exaustão do dispositivo spray caraterizou-se pela diminuição da dose emitida. Por fim, a monitorização da perda de peso do produto através de estudos de pré-estabilidade mostrou que, apesar da presença de solventes voláteis, a perda de peso encontra-se nos limites aceitáveis pelas autoridades.

Em suma, o estudo das caraterísticas físicas e a avaliação do desempenho do dispositivo spray apoiam o desenvolvimento de preparações líquidas não estéreis e são essenciais para assegurar a qualidade do produto e a segurança do doente. Além disso, os resultados permitem definir a especificação do produto de acordo com os CQA para o uso pretendido.

CONTENTS

Acknowledgements .... **iii**

Abstract .... **v**

Resumo .... **vii**

List of figures .... **xiii**

List of tables .... **xix**

Acronyms .... **xxi**

Chapter 1 Introduction **1**

1.1. Contextualization **2**

1.2. Bluepharma Indústria Farmacêutica SA **3**

1.3. Objectives **5**

1.4. Pharmaceutical dosage forms **7**

1.4.1. Background 7

1.4.2. Formulations for mucosal administration 9

1.5. Product quality and product performance **11**

1.5.1. Characterization of the formulation 15

1.5.1.1. Definition/Description/Appearance 15

1.5.1.2. Specific Gravity/Density 17

1.5.1.3. pH 17

1.5.1.4. Viscosity 17

1.5.1.5. Adhesion 19

1.5.1.6. *In vitro* release test (IVRT) 21

1.5.2. Spray performance 24

1.5.2.1. Spray Actuation test 24

1.5.2.2. Pump Delivery/Shot weight 24

1.5.2.3. Mean delivered dose 25

1.5.2.4. Tail-off characteristics 25

1.5.2.5. Minimum fill/Net content 25

1.5.2.6. Weight loss (stability) 25

1.5.2.7. Priming and Repriming (stability) 26

1.5.2.8. Number of doses/actuations 26

Chapter 2 Practical activity **29**

2.1. Materials **30**

2.2. Equipments **30**

2.3. Procedures **31**

2.3.1. Characterization of the formulation 31

2.3.1.1. Definition/Description/Appearance 31

2.3.1.2. Specific Gravity/Density 31

2.3.1.3. pH 33

2.3.1.4. Viscosity 33

2.3.1.5. Adhesion 34

2.3.1.6. *In vitro* release test 37

2.3.2. Spray Performance 37

2.3.2.1. Spray actuation test 37

2.3.2.2. Pump delivery/Shot weight 38

2.3.2.3. Mean delivered dose 38

2.3.2.4. Tail-off characteristics 38

2.3.2.5. Minimum fill/Net content 39

2.3.2.6. Weight loss (stability) 39

2.3.2.7. Priming and Repriming (stability) 39

2.3.2.8. Number of doses/actuations 39

Chapter 3 Results and discussion **41**

3.1. Day 1 Evaluation **42**

3.1.1. Characterization of the formulation 42

3.1.1.1. Definition/Description/Appearance 42

3.1.1.2. Specific Gravity/Density 44

3.1.1.3. pH 45

3.1.1.4. Viscosity 48

3.1.1.5. Adhesion 50

3.1.1.6. *In vitro* release test 56

3.1.2. Spray performance 66

3.1.2.1. Spray actuation test 66

3.1.2.2. Pump Delivery/Shot weight 69

3.1.2.3. Priming ... 72
3.1.2.4. Number of doses/actuations ... 73

3.2. Pre-Stability studies ... 74

3.2.1. Characterization of the formulation ... 74
3.2.1.1. Definition/Description/Appearance ... 74
3.2.1.2. pH ... 76
3.2.1.3. Viscosity ... 76
3.2.1.4. Adhesion ... 77

3.2.2. Spray performance ... 78
3.2.2.1. Spray actuation test ... 78
3.2.2.2. Weight loss (stability) ... 79
3.2.2.3. Repriming (stability) ... 80

Chapter 4 Conclusion ... 83

Chapter 5 References ... 87

Attachment ... 93

LIST OF FIGURES

Figure 1 Headquarters of Bluepharma Indústria farmacêutica SA, Coimbra. 3

Figure 2 Bluepharma's Certifications.. 4

Figure 3 Schematic representation of the objectives. Objective 1: characterization of the formulation; Objective 2: evaluation of the spray performance. The evaluation of these parameters is made immediately after each formulation preparation and over time (non-formal pre-stability evaluation).. 5

Figure 4 Type of dosage forms. Classification of dosage forms based on route of administration (injection/implantation, gastrointestinal, topical/dermal, mucosal and inhalation) or based on physical form of the dosage form (solid, semi-solid, liquid and gaseous). 7

Figure 5 Schematic representation of the local administration using a spray device. Scheme of the drug product containing formulation and spray device components (actuator, pump and container) (**A**). Scheme of a typical mucosal structure, with surface, epithelium and sub-mucosa regions and the representation of the different steps involved in the systemic delivery of drug substance through a mucosa (spray actuation, release of DS, permeation of DS and finally the systemic delivery) (**B**). 8

Figure 6 Examples of container closure system components. Container, actuator, pump and dip tube (**A**). Different actuators shapes (topical/dermal, oral and nasal spray systems) (**B**). Different pump weights (100, 130 and 190 mg) (**C**). Different closure systems (screw-on and snap-on) (**D**). 16

Figure 7 Two-plates model provides a mathematical description for viscosity. 18

Figure 8 Representation of original and modified methods used for adhesion evaluation..... 19

Figure 9 Graphic representation of the specific adhesion parameters: Adhesiveness peak (g), work of adhesion (g.sec) 20

Figure 10 Schematic representation of Franz cell (Model C of USP Chapter 1724 [22]) used for in vitro release test. 21

Figure 11 Schematic representation of the specific gravity procedure. Check that the pycnometer is properly cleaned and dried before weighing (**A**). Fill the pycnometer with the sample to about halfway up to neck and insert the capillary stopper (**B**). Check that the sample fills all the capillary, clean the pycnometer and weight (**C**)............................... 32

Figure 12 Schematic representation of pH measurement using spray device........................ 33

Figure 13 Schematic representation of viscosity measurement using an adapter for small sample volumes.. 34

Figure 14 Schematic representation of adhesion method. Step 1 corresponds to the hydration process. Step 2 corresponds to the equipment preparation and, finally, 2 steps for sample test can be distinguished: with bottle (step 3a) and with spray device (step 3b)............ 36

Figure 15 Schematic representation of the in vitro release test procedure. Place a specific amount of receptor medium on the receptor chamber and put the artificial membrane above the positive meniscus (**A**). Finally, put the sample on the donor chamber (**B**)................ 37

Figure 16 Representation of spray performance procedure using automated actuations on Texture Analyser.. 38

Figure 17 Appearance classification through visual examination. The appearance score starts at “1.00” and ends at “0.00”. The desired score is characterized by clear formulation, without foreign particles. On the other hand, if there is visible jellification, sedimentation or precipitation, the classification is lower. 43

Figure 18 Specific gravity of different formulations. Placebo A (blue color) and Placebo B (orange color) only differ on excipients composition and these placebos have different specific gravities. Prototype B (orange color) has the same excipients as the Placebo B but with drug substance incorporated and the specific gravities obtained are similar. Reference Product (grey color) represent a commercialized product with features similar to the DP under development and the specific gravity is similar to placebo B and prototype B..... 44

Figure 19 Adaptation of pH measurement for spray devices. pH measurement on bottle is compared with pH measurement with spray device through 8, 10, 12, 15 and 20 SPR (actuations using the same pump that releases 150mg/SPR). Statistical analysis using 2way ANOVA, 95% confidence level demonstrate that all the volumes tested were not significantly different from the bottle pH. 45

Figure 20 pH range of two placebo technologies developed. Range of placebo technology A is within 2.2 and 7.2 and within 2.2 and 6.5 to placebo technology B............................... 46

Figure 21 pH measurement on different reference products with similar characteristics to the DP under development (similar dosage form and same mucosal application)............... 47

Figure 22 pH range obtained with reference product and placebo technologies. Range of placebo technology A is within 2.2 and 7.2 and within 2.2 and 6.5 to placebo technology B. Reference product pH range is within 2.1 and 6.1.. 48

Figure 23 Influence of the presence of viscosity-increasing agents at different concentrations (0.3% and 3.0%) on viscosity measurement. The increasing of the viscosity agent increases the viscosity. 49
Figure 24 Relationship between sprayability and viscosity. Formulation viscosities within 11 and 1500 mPas were dispensed as a spray. Viscosities between 5200 and 80000 mPas were not sprayable. To well define the range between 1500 and 5200 mPas more studies are needed. 49
Figure 25 Schematic representation of the two stages on mucoadhesion process: contact and consolidation stage. Adapted from Smart [51]. 50
Figure 26 Texture Analyser procedure for adhesion method and schematic representation of each step. The probe initiate the downward movement (**A**) until contact stage (**B**). After 60 seconds, the probe return distance (**C**) until total detachment (**D**). 51
Figure 27 Statistical analysis (two-tailed T-test) to distinguish adhesive and non-adhesive formulations. 52
Figure 28 Impact of the increasing adhesion enhancers (0.00, 0.63 and 1.00%) on the adhesion measurement. The increasing of the adhesion enhancer increases the adhesiveness, work of adhesion and detachment distance. 53
Figure 29 Adhesion measurements on different analyzed placebos. Adhesion range is within -5.77 and 24.0 g. 54
Figure 30 Adhesiveness measurement on 7 different reference products (RP) with similar properties to the ones under development. Adhesion range is within -3.97 and 56.8 g. . 55
Figure 31 Adhesion ranges of reference products and developed formulations obtained with the developed adhesion method. The adhesion range of reference products is within 1.9 and 78 and within 1.9 and 108 for the developed formulations, assuming adhesive formulations. 55
Figure 32 In vitro release test sampling procedure. Sampling tool used to collect samples and replace medium (**A**) and sampling procedure in the Franz cell (**B**). 56
Figure 33 Membrane influence on the release profile. The study was conducted with 41 μm pore size and without membrane. Statistical analysis demonstrates statistical differences at early time points ($P_{value}<0.0001$ and $P_{value}=0.0101$ for 0.5 and 1 min, respectively) for 95% of confidence level, according to sidak's multiple comparisons tests. 57
Figure 34 Pore size influence on the release profile. The study was conducted with 41μm pore size and 80 μm pore size membranes. Statistical analysis only demonstrates statistical

differences up to 30 seconds (P_{value}=0.0328) for 95% of confidence level and according to Sidak's multiple comparisons tests. 58

Figure 35 Isolation influence on the release profile. The study was conducted with three different isolation conditions: uncovered, with parafilm and with aluminum foil. Statistical analysis demonstrates that the condition uncovered are statistically different from the other two up to 30 seconds (Pvalue=0,0039 with parafilm condition and Pvalue=0,0041 with aluminum foil condition). Furthermore, the cells occluded with parafilm and aluminum foil do not differ statistically (Pvalue=0,9999, for 95% of confidence level). 59

Figure 36 Influence of the receptor medium composition on the release profile. Test to evaluate sink conditions on the medium. The coloring with both concentrations suggests that simulated saliva do not solubilize the DS (**A**). The other study was conducted to compare different medium and the statistical analysis demonstrate that the release only differs on first 30 seconds (P_{value}<0.0001 for 95% of confidence level, sidak's multiple comparisons tests). 61

Figure 37 In vitro release test using a coloring placebo formulation. A visual examination of the formulation diffusion through the membrane was observed when 1, 4 and 8 SPR were applied on the donor chamber (**A**, **B** and **C**, respectively). Membrane before test (left side) and the membrane after in vitro release test (right side) was also inspected by optical microscope: 1 SPR (**D**), 4 SPR (**E**), 8 SPR on center of the membrane (**F**) and 8 SPR on the border of the membrane (**G**). Membrane appearance after in vitro release test was also visual examined (middle panel **D**, **E**, **F**, **G**). 62

Figure 38 Sample amount influence on the release profile. The study was conducted using a finite (1SPR) and an infinite (8SPR) dose of DP and the result demonstrates that the differences only exists at early time points. 63

Figure 39 Comparison between in vitro release test profiles using a 41 μm pore size membrane (**A**) and 80 μm pore size membrane (**B**). Different drug products were studied in both conditions. The results demonstrate that in the two first time point of the study (**A**) (up to 1 min), the membrane with 41 μm pore size statistically discriminated both formulations (0.5 min with Pvalue<0.0001 and 1 min with Pvalue=0.0008). The study (**B**), with 80 μm pore size, shows that the differences are only at 30 seconds (Pvalue<0.0001, for 95% of confidence level, sidak's multiple comparisons tests) 65

Figure 40 Representation of spray characterization using Texture Analyser. Actuation force corresponds to the "start dispending" and the maximum force corresponds to the "stop dispensing". Spray duration is the red zone between the peaks. 66
Figure 41 Viscosity influence on actuation and maximum forces. 67
Figure 42 Actuation force and maximum force of different suppliers and reference product (RP). 68
Figure 43 Pump delivery with 5 g of formulation. Priming actuations, constant doses and tail-off characteristics are represented. 70
Figure 44 Impact of automated and manual settings on spray performance. Automated settings using Texture Analyser (**A**); Manual settings but single person (n=3) (**B**) and manual settings but multiple person (**C**). 71
Figure 45 Priming on different suppliers (**A**, **B**, **C** and **D**). It seems to be necessary to discard 5 to 6 actuations before the first administration to the patient. 72
Figure 46 Priming actuations on different commercialized reference products. It seems to be necessary to discard 5 to 6 actuations before the first administration to the patient 73
Figure 47 Number of actuation on containers with different formulation amounts (containers filled with 5 g and 10 g). 74
Figure 48 Appearance of spray device that fails during non-formal pre-stability study. 75
Figure 49 Appearance of different formulations. Normal appearance (**A**) and Sedimentation/solid jellified (**B**). 75
Figure 50 pH measurement of two placebo formulations over time. Placebo A corresponds to an unstable placebo formulation and Placebo B to a stable placebo formulation. 76
Figure 51 Viscosity measurement on different placebo formulations over time. Placebo technology A (**A**) and placebo technology B (**B**) have stable and unstable formulations. 77
Figure 52 Adhesiveness measurement over time. Unstable and stable formulations are represented. 78
Figure 53 Adhesion measurement during stability studies. 78
Figure 54 Weight loss of placebo formulation and commercialized reference product up to 5 months. 80
Figure 55 Repriming actuation on different spray devices suppliers (**A**,**B** and **C**) with the same formulation and reference product (**D**). 81
Figure 56 Repriming actuations in two spray devices with the same formulation over 4 (**A**) and 6 (**B**) months. 82

LIST OF TABLES

Table 1 Anatomical and physiological parameters on different drug delivery sites [4]........... 10
Table 2 Selected tests recommended to be performed by different authorities for similar DP to the ones under development [11-13].. 13
Table 3 Equipment used both for formulation characterization and for spray performance evaluation... 30
Table 4 Water density at different temperatures. 31
Table 5 Settings for adhesion method. 36

ACRONYMS

ActF: Actuation Force

BSC II: Biopharmaceutics Classification System Class II

CQA: Critical Quality Attributes

CMA: Critical Material Attributes

CPP: Critical Process Parameters

DS: Drug Substance

DoE: Design of Experience

DP: Drug Product

EMA: European Medicines Agency

EMAS: Eco-Management and Audit Scheme

FDA: Food and Drug Administration

GMP: Good Manufacturing Practice

ISO: International Organization for Standardization

IVRT: *In vitro* release test

MaxF: Maximum Force

MeOH: Methanol

M_W: Molecular Weight

OHSAS: Occupational Health and Safety Assessment Series

Ph.Eur.: European Pharmacopoeia

QTPP: Quality Target Product Profile

SPR: Spray actuation

USP: United States Pharmacopoeia

CHAPTER 1

INTRODUCTION

1.1. Contextualization

This master thesis was developed on Bluepharma Indústria Farmacêutica SA on the Research and Innovation department, more precisely on Research and Innovation Technology sector. In general, the work consisted in the development of methods for the characterization and performance evaluation of non-sterile liquid preparation for internal use to be delivered using a spray device.

This dissertation starts with a brief contextualization of the pharmaceutical development of non-sterile liquid preparation and its regulatory environment, in particular regarding the tests required for drug product development, and for release of this type of drug product which corresponds to the drug product specification. Furthermore, Chapter 1 highlight the importance of each method both for formulation characterization and for spray performance evaluation. Following this introductory chapter, on the practical activity, the experimental procedure is described in more detail such as the materials and equipment used during the experimental work. On the results and discussion chapter, the results obtained are presented and discussed. Important to note that certain features of the formulation are undisclosed due to intellectual property constraints. As the DP is a spray device based, the results performed comprised the physical characterization of the formulation and the spray performance evaluation. The results are divided on the tests that are performed immediately after each formulation preparation (day 1 evaluation) and the tests performed over time during the pre-stability. Finally, some conclusions are mentioned.

1.2. Bluepharma Indústria Farmacêutica SA

Bluepharma is a pharmaceutical industry founded in 2001 after the acquisition of the Bayer pharmaceutical industry unit site in Coimbra (**Figure 1**). At the moment, it employs more than 420 employees. The economic group of Bluepharma is constituted by several companies, all related to health, such as BlueClinical where clinical studies are conducted. Bluepharma has an extensive national and international experience and, therefore, it collaborates with large pharmaceutical companies, distributing medicines worldwide, mainly in the United States, Europe, the Middle East, Africa and Asia.

Figure 1 Headquarters of Bluepharma Indústria farmacêutica SA, Coimbra.

Its activity is allocated into distinct areas such as the production of own drugs and for customers, research and development of drugs and, finally, commercialization of generic drugs.

In Bluepharma, different patented technologies have been developed, including BlueOS oral thin film technology; multilayer technology; high potency products; one dose®; hot melt extrusion and complex injectable.

This company can be divided into different departments such as research and innovation, galenical and analytical development, manufacturing, packaging, quality control, among others. Bluepharma performs its activity from product development to the end of the value chain, that is, finished product supply. Accordingly, quality management is critical to enhance the success and assures the safety of all products. Overall, Bluepharma vision is supported by:

- Good Manufacturing Practice (GMP) compliance according to the European (EMA) and Food and Drug Administration (FDA) authorities.
- GMP guidelines.

- Implementation and management of an integrated Quality and Environmental Certification program according to ISO 9001, ISO 14001, OHSAS 18001 and EMAS.

The compliance with GMP requirement and ISO standards leads to a complete and effective quality management system (**Figure 2**) [1].

Figure 2 Bluepharma's Certifications.

Bluepharma mission is to provide pharmaceutical products with the highest quality at competitive prices, actively contributing to the rationalization of the health sector and simultaneously improving the quality of life of the population.

1.3. Objectives

The objectives of my work are listed on the **Figure 3**.

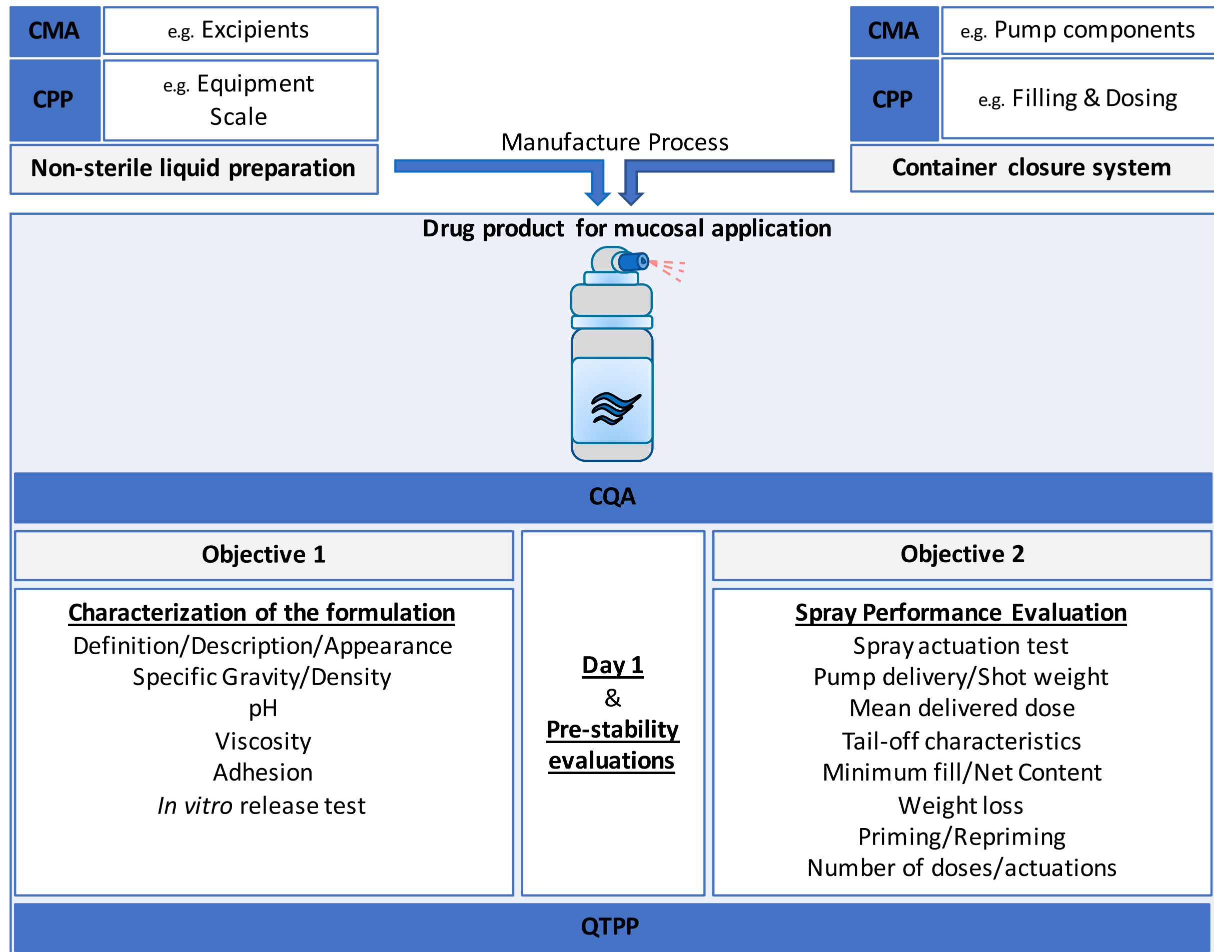


Figure 3 Schematic representation of the objectives. Objective 1: characterization of the formulation; Objective 2: evaluation of the spray performance. The evaluation of these parameters is made immediately after each formulation preparation and over time (non-formal pre-stability evaluation).

The main objective associated with this project was to ensure that the developed drug product met the Quality Target Product Profile (QTPP) established at the beginning of the project. For that, it is important the identification of Critical Quality Attributes (CQA). After defining the CQA of both non-sterile liquid preparation and container closure system, the following objectives were defined:

1) Characterization of the formulation

2) Spray performance evaluation

Both analyses were performed or immediately after each formulation preparation (day 1 evaluation) or over time during a non-formal pre-stability evaluation.

1.4. Pharmaceutical dosage forms

1.4.1. Background

According to the Food and Drug Administration (FDA), drug substance (DS) refers to any substance or mixture of substances intended to be used in the manufacture of a dosage form. Drug substances are formulated into pharmaceutical dosage forms in order to optimize stability, safety and effectiveness of drug substances and to make them suitable for administration. Overall, the dosage forms facilitate dosing, administration and delivery of the medicine (drug substance) to the patients.

The classification of a dosage form can be achieved based on the route of administration, the physical form of the dosage form (**Figure 4**) and through its release pattern. According to the route of administration, it can be classified as injection/implantation, gastrointestinal, topical/dermal, inhalation and finally mucosal. According to the physical form, dosage forms can be classified as solid, semi-solid, liquid or gaseous [2-3].

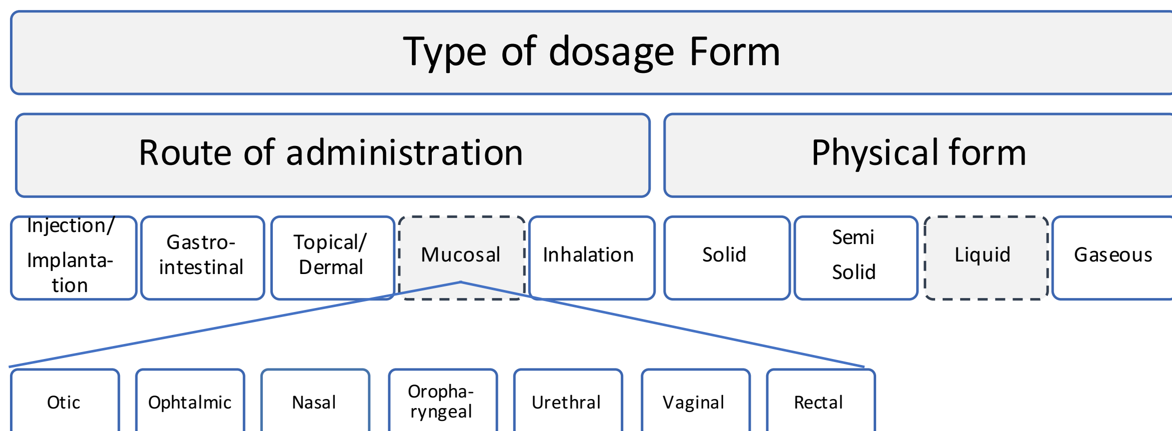


Figure 4 Type of dosage forms. Classification of dosage forms based on route of administration (injection/implantation, gastrointestinal, topical/dermal, mucosal and inhalation) or based on physical form of the dosage form (solid, semi-solid, liquid and gaseous).

Another criterion for the classification of a dosage form is the release pattern of the product. According to the application of the drug product, the United States Pharmacopoeia (USP) identifies three different types of release [2].

- Immediate-release: Describes a dosage form in which no deliberate effort has been made to modify the drug substance release rate.

- Extended-release: Defines the dosage form that is deliberately modified to extend the release rate of the drug substance compared to that observed for an immediate-release dosage form.
- Delayed-release: Corresponds to a type of modified-release dosage form. It is a dosage form deliberately modified to delay the release of the drug substance for some period of time after the initial administration. Normally, it is used when the release of drug substance may occur on the intestinal environment and did not occur on the gastric environment.

For this particular project, and according to the USP classification, the formulations analyzed could be classified as a liquid dosage form intended for mucosal administration. The release pattern could be adjustable to be either immediate or extended release.

The work presented in this dissertation consists in the development of methods for the characterization and the performance evaluation of non-sterile liquid preparations delivered using a spray device. The drug product under development (non-sterile liquid preparation and container closure system) is intended to release drug substance in internal mucosas using a spray device, promoting the systemic permeation of the drug substance into the bloodstream at the site of administration (**Figure 5**).

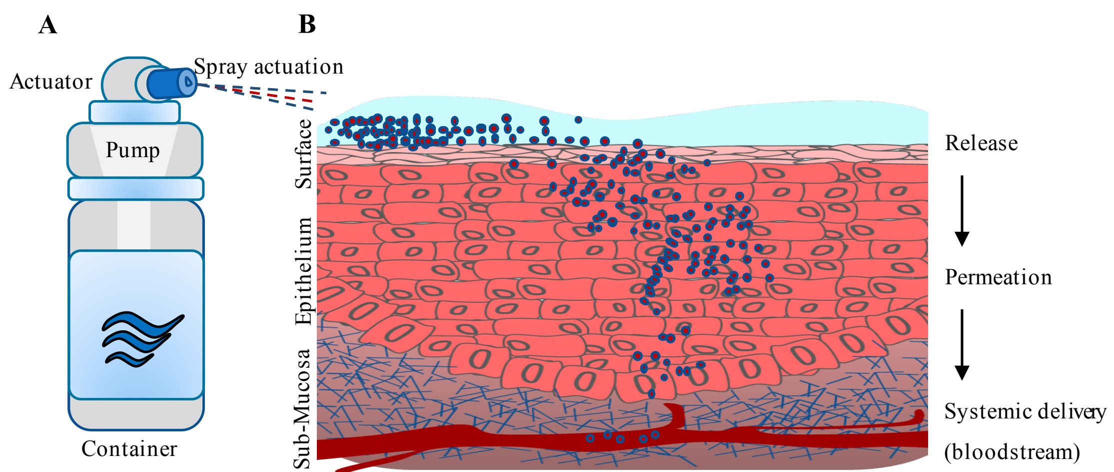


Figure 5 Schematic representation of the local administration using a spray device. Scheme of the drug product containing formulation and spray device components (actuator, pump and container) (**A**). Scheme of a typical mucosal structure, with surface, epithelium and sub-mucosa regions and the representation of the different steps involved in the systemic delivery of drug substance through a mucosa (spray actuation, release of DS, permeation of DS and finally the systemic delivery) (**B**).

In this project, the drug product is composed by a non-sterile liquid preparation (excipients with drug substance) and a container closure system (container and spray device components: dip

tube, spray pump system and actuator). In this case, the drug product is intended to be administered as a spray mist [2].

1.4.2. Formulations for mucosal administration

Overall, solid oral dosage forms remain the most conventional and cost effective way to administer pharmaceuticals. However, these traditional dosage forms present several challenges and limitations associated with oral administration, in particular the gastrointestinal (GI) degradation and first-pass hepatic metabolism, which results in the decrease of drug bioavailability. Other options to improve drug delivery safety and efficiency have emerged such as liquid preparation for mucosal drug delivery.

The development of mucosal drug product refers that the drug (s) substance (s) is/are delivered to the body via mucosal route. Mucosal drug delivery ensures that drugs can be delivered safely and efficiently. The mucosal drug products enable the systemic delivery of drugs on several mucosal surfaces such as otic, ophthalmic, nasal, oropharyngeal, urethral, vaginal and rectal (**Figure 4**) [3]. This type of administration requires that the drug substance, after released from the formulation to the medium that involves the mucosa, permeate the mucosal layers to enter onto the bloodstream (**Figure 5**).

The potential benefits of administering drugs through mucosas are closely related to the local high vascularization of these regions which allows a direct access to systemic circulation via capillaries and venous drainage, and may include [4-8]:

• Enhanced patient compliance and convenience: easy administration, self-administration and painless.

• No need of medical personnel/training.

• Taste masking formulations: Adjust the flavor according to product requirement and target population (for the case of oral mucosa).

• Increase the safety of highly metabolized drugs in the liver by reducing the dose that needs to be administered to achieve the therapeutic effect: Reduced side effects.

• Potential to enhance bioavailability: possible reduction of GI degradation and minimizes hepatic inactivation (first pass metabolism).

• Decrease the variability of GI absorption due to food presence.

• Faster onset of action when compared with solid oral dosage forms.

• Reduce the time needed to achieve the therapeutic effect particularly in emergencies.

• Cost effective manufacturing process.

There are some challenges in mucosal drug delivery, in particular related to drug substance solubility and permeation. Nevertheless, the use of functional excipients, such as surfactants, solubilizes or penetration enhancers could potentially improve drug pharmacokinetic behavior.

Some anatomical and physiological properties of the delivered site are critical for the mucosal drug delivery. It is important to know exactly the composition of the local where the drug product will be applied. Physiological aspects are important to guarantee an efficient transmucosal drug delivery. Physiological aspects such as pH, fluid volume, enzyme activity, permeability of mucosa are important to consider during the drug product development. For example, oral cavity has some differences when compared with the mucosa of the gastrointestinal tract related to the surface area, pH, amount of fluid volume, enzyme activity and capability for drug absorption (**Table 1**).

Table 1 Anatomical and physiological parameters on different drug delivery sites [4].

Drug delivery site	Estimated surface area (m^2)	Local pH	Mean fluid volume (mL)	Relative enzyme activity	Relative drug absorption capacity
Oral cavity	0.01	5.8-7.6	0.9	Moderate	High
Stomach	0.1-0.2	1.0-3.0	118	High	High
Small intestine	100	3.0-4.0	212	High	High
Large intestine	0.5-1.0	4.0-6.0	187	Moderate	Low
Rectum	0.02-0.04	5.0-6.0	-	Low	Low

As represented on the **Table 1**, although the oral cavity has the lower surface area, it has high drug absorption capacity. Oral cavity, when compared to other regions of the gastrointestinal tract, has the higher pH and the lower fluid volume. Another critical parameter is the drug permeability through the mucosa. In order to establish the capability to permeate the mucosa it is important to consider the thickness and surface area of the membrane, the permeability, the residence time and also the blood flow on the delivery site. All these parameters will be taken into consideration for the designing of mucosal drug delivery systems. The application of the

drug product in the oral cavity has advantages when compared, for example, with rectum mucosa mainly due to the high drug absorption capability.

1.5. Product quality and product performance

Pharmaceutical industry is highly regulated and the development of drug products rely on several tests and studies that are advised to be performed in order to assess product quality and performance. The drug product tests can be divided on 1) those that assess general quality tests related with the integrity of the chosen dosage form and 2) tests that ensure product performance quality in order to evaluate *in vivo* drug product, for example, the delivery of the drug substance. Taken together, quality and performance tests ensure the identity, strength, quality, and purity of drug products [9]. Most of these studies will aid the establishment of the dosage form, formulation and manufacturing process features, selection of container closure system and microbiological attributes. Furthermore, the studies during the development of the product will contribute to establish the instructions for the correct use of the drug product, and guarantee that the use is proper and result in suitable product performance.

The product quality and performance tests were selected according to the Critical Quality Attributes (CQA) identified for the technology/product under development and were based on Pharmacopoeias chapters – United states Pharmacopoeia (USP) and European Pharmacopoeia (Ph.Eur.), as well as, on pharmaceutical guidances - European Medicines Agency's (EMA) and Food and Drug Administration (FDA).

The development and implementation of these tests during the drug product development help the formulation team to formulate the product with the quality and performance that is required. Generally, pharmaceutical development follows a systematic approach that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. The main objective is that quality is built into the product. This approach known as Quality by Design (QbD) is highly encouraged by the regional agencies that control drug product development, manufacturing and regulation [10]. QbD approach to drug product development includes the identification of the ideal quality characteristics of a drug product (Quality Target Product Profile – QTPP) and identification of Critical Quality Attributes (CQA). CQA are identified to ensure product quality, safety, purity, and efficacy. CQA can be differentiated on physical, chemical, biological and related with microbiological property. These critical attributes have an impact on product quality and must be studied and controlled. Then, using risk assessment tools and statistical design of

experiments (DoE), the Critical Process Parameters (CPP) and Critical Material Attributes (CMA) that impact CQA can be identified. The defined CQA is influenced by CPP that must ensure the process produces the desired quality product and by CMA that is related with physicochemical properties of active and inactive ingredients. At the end, the objective relies on the establishment of a Design Space that, according to ICH Q8, is defined as a multidimensional combination and interaction of input variables (eg. material attributes) and process parameters that have been demonstrated to provide assurance of quality [10].

In this work, some parameters were identified as CQA of technology/composition under development. The attributes related with formulation properties emphasized were viscosity, adhesion and pH. Furthermore, weight loss upon storage and priming/repriming studies were considered critical attributes for the product performance, and were also investigated.

Table 2 identified a list of selected tests/studies recommended to be performed by different regulatory authorities for similar DP to the ones under development. It is important to notice that, depending on the market region for each product, there are few differences in the regulatory testing requirements for this type of products, which shows the complexity involving pharmaceutical development. In the **Table 2**, the stage of the pharmaceutical development in which each of these tests should be performed is identified. There are tests that are suggested to be performed during the development of the product, whereas other tests are required to be carried out when establishing the finish drug product release specifications for these DP.

Some of the tests are universally applicable to all dosage forms such as definition/description/appearance, identification, assay and impurities. Other tests identified in the table have been selected according to the pharmaceutical dosage form under development, in our case a non-sterile liquid preparation for internal use (mucosa). These specific tests were organized in two main groups of tests for the structural organization of this project: a) the tests related to the characterization of the formulation and b) the tests related to the spray performance.

Table 2 Selected tests recommended to be performed by different authorities for similar DP to the ones under development [11-13].

	Parameter	Description/Objective	DP Development – Authorities			Released finished DP – Authorities		
			Canada	FDA	European	Canada	FDA	European
Universal tests	Definition/ Description	Describes the DP and specifies the DS acceptable range [2,14].	x	x	x	x	x	x
	Identification	Identifies the DS and discriminates compounds [2,9,14].	x	x	x	x	x	x
	Assay	Quantifies the DS [2,9,14].	x	x	x	x	x	x
	Impurities	Identifies and quantifies the impurities [2,9,14].	x	x	x	x	x	x
Characterization of the formulation	pH	Ensures stability profile of the DS and guarantee the safe pH range [9,14-15].				x	x	x
	Microbial limits	Quantifies specific microorganisms [9,14,16-18].				x	x	x
	Antimicrobial preservatives effectiveness	Required only if preservatives are present [9,14,19].	x	x	x	x	x	x
	Viscosity	Useful for formulations containing viscosity enhancers agents [9,20].					x	
	Specific Gravity	Useful for comparative studies between formulations [21].						
	Temperature cycling	Determines the effect after cycling temperature to evaluate the DP stability [14].		x	x			
	In vitro permeation/ release test	Evaluates DS release on specific medium and DS permeation on mucosa [22-23].						
	Adhesion test	Evaluates adhesion capacity of the DP.						

Table 2 (Continuation) Selected tests recommended to be performed by different authorities for similar DP to the ones under development *[11-13]*.

Spray Performance	Uniformity of dosage unit/ Content Uniformity	Evaluates the consistency of dosage unit. Required only for single use spray [24].				**x**		**x**
	Spray content Uniformity/ Delivery dose uniformity	Demonstrates the consistency of the delivered dose. Required for multiple use sprays [9,14,25].	x	x	x	x	x	x
	Mean Delivered dose	Determines the mean of the delivered dose [14].						x
	Pump delivery/Shot weight	Evaluates pump-to-pump reproducibility [26].					x	x
	Droplet size distribution	Controls the delivered plume [14,25].	x	x	x		x	x
	Tail-off characteristic	Determines the doses between the last labeled dose and the last container exhaustion dose.		x	x			
	Spray pattern & Plume Geometry	Evaluates the spray pump performance [9].		x			x	
	Leachables/ Extractables	Evaluates compounds that migrate into a DP and compounds that can migrate from a material, respectively [9,14,16].	x	x	x		x	x
	Minimum fill/Net Content	Ensure that the amount present inside of the product provides the labelled number of actuations [27].	x	x	x	x	x	
	Weight loss	Evaluates the sealing of the container closure system [9].	x	x			x	
	Priming/ Repriming	Determines the number of actuations required before first use and after an unusable time interval, respectively [14].		x	x			
	Number of doses/ actuations	Evaluates the number of doses on each DP [14].					x	x

The selection of tests to evaluate the product quality is not meant to be an all-inclusive list. It is not expected that all the described tests would be conducted on all batches. It is recognized that the wide diversity of non-sterile liquid preparations with specific formulations/delivery device characteristics requires some flexibility on testing methodology. Some commercialized products with characteristics similar to the developed drug product were investigated and the tests that were performed by the pharmaceutical industries were substantially different.

The present study emphasis some of the listed tests on **Table 2**, to support the formulation development, the selection of spray pump device and the drug product specifications. The focus of my work was mainly on the development of definition/description/appearance, specific gravity/density, pH, viscosity, adhesion and *in vitro* release test methods in order to allow formulation characterization. The spray performance of the drug product is evaluated through specific tests as spray actuation test, pump delivery/shot weight, mean delivered dose, tail-off characteristics, minimum fill/net content, weight loss, priming/repriming and the number of doses/actuations. The tests that were performed are theoretically described in the section below.

1.5.1. Characterization of the formulation

1.5.1.1. Definition/Description/Appearance

The appearance of the content (i.e., non-sterile liquid preparation) and the container closure system (container and spray device components) of the DP should be in conformity with their respective descriptions. For this purpose, a visual examination was performed.

The parameters of the non-sterile liquid preparation evaluated were:

- Clarity of the formulation
- Color
- Particles of foreign matter
- Precipitation
- Phase separation
- Deposition

The formulation should be a clear and colorless solution, without any foreign matter visible, with no precipitation, phase separation or deposition.

On the other hand, in what refers to the container closure system, characteristics such as the size and shape of the pump, closure type, composition of the container are carefully evaluated. All parameters can give an indication of the drug product integrity. **Figure 6** shows an example of the components.

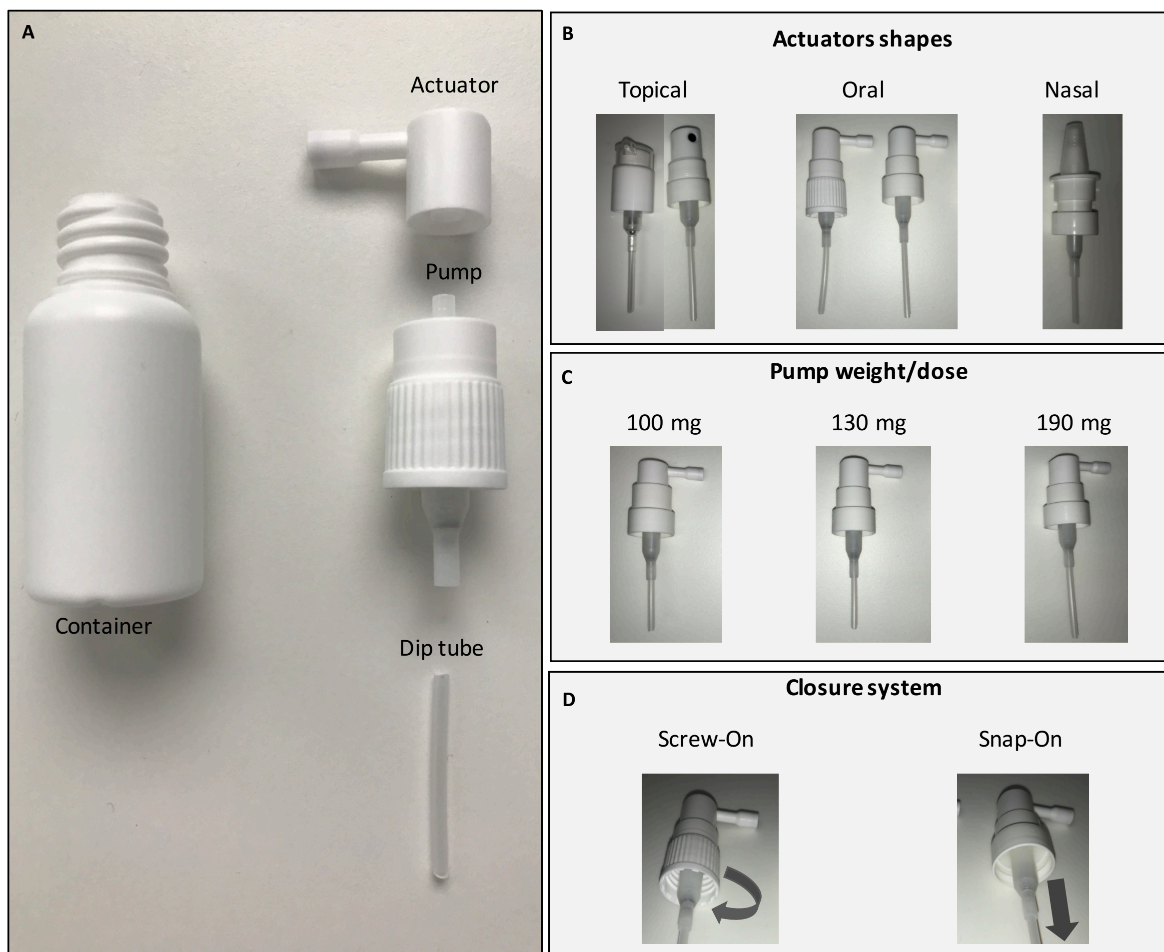


Figure 6 Examples of container closure system components. Container, actuator, pump and dip tube (**A**). Different actuators shapes (topical/dermal, oral and nasal spray systems) (**B**). Different pump weights (100, 130 and 190 mg) (**C**). Different closure systems (screw-on and snap-on) (**D**).

The main components of the container closure system are the container (filled with the formulation), dip tube, spray pump and the actuator (**Figure 6A)**. The actuators can be of different types (topical/dermal, oral and nasal spray systems) according to the desired application (**Figure 6B**). Spray pumps can release different amounts of formulation (e.g., 100, 130 and 190 mg) depending on the therapy needs (**Figure 6C**). Finally, the closure system can be screw-on or snap-on which differ on the insulation capabilities (**Figure 6D)**.

1.5.1.2. Specific Gravity/Density

Density is defined as the mass of a unit volume of a substance at a specific temperature (25 ºC). This parameter is expressed in kilograms per cubic meter or grams per cubic centimeter (kg/m^3 or g/cm^3). Specific gravity is the ratio of the density of the product to the density of a standard substance (usually water). The specific gravity determination is only applicable to liquids and is based on the ratio of the weight of an unknown liquid at 25 ºC and an equal volume of recently boiled water at the same temperature [21]. The described procedure in the USP includes the use of a pycnometer consisting of glass material.

This determination is important to convert the volume of the formulations in weight (mass), in particular to control the filling process. Additionally, allows the conversion of the quantity of mass into volume dispensed by each spray actuation. Finally, in some formulations with moderate viscosities, the compositions could have air bubbles and the determination of density could help identifying this excess of air, which can lead to spoiling and degradation of certain ingredients and affect physical characteristics of the formulation [28].

1.5.1.3. pH

Since the drug product is a non-sterile liquid preparation for mucosal application, monitoring the pH of the composition is crucial for some regulatory authorities for the release of the finished product. This parameter is also tested during the development of the formulation. The pH of the preparation indicates the acidity or basicity of this liquid preparation. The monitoring of the pH over time is crucial for assessing the stability of liquid dosage forms because aqueous-based products are more susceptible to pH modifications from exposure to atmospheric CO_2 [16]. The acceptable limit for the formulation is defined by the stability profile of the drug substance in the formulation and by the pH range of drug product for the intended use. The pH of the formulation is critical for the local tolerability of the DP, as well as, it has an impact on the solubility of drug substance and its systemic permeability in the site of application.

1.5.1.4. Viscosity

The viscometry corresponds to a subject area of a wider knowledge called rheology. Generally, it describes the flow behavior and deformation of the materials. Viscosity is important for the physical characterization of this non-sterile liquid preparation. The viscosity is the quantity that describes a fluid's resistance to flow. The principle of the method is to

measure the force (torque) acting on a rotor when it rotates at a constant rotational speed in a liquid [20].

Fluid's viscosity is supported by shear stress and shear rate. The two-plates model provides a mathematical description for viscosity (**Figure 7**).

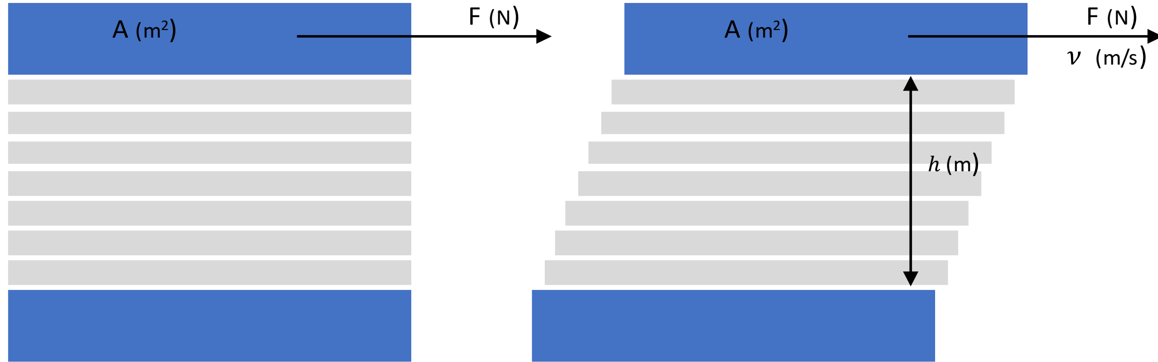


Figure 7 Two-plates model provides a mathematical description for viscosity.

As shown in **Figure 7**, while the upper plate drifts aside (corresponding to the rotational spindle), the lower plate (container used for formulation viscosity measurement) does not move and causing fluid (between the plates) to a stress – shear stress (τ). This component is defined by the force applied to the upper plate (Newton) divided by the plate's area (square meters) (**Equation 1**). This relation between force and area constitute the Pascal unit [29-30].

$$\tau = \frac{F}{A} \qquad \textbf{Equation 1}$$

where F is the force applied to the upper plate and A is the plate's area (m^2).

This mathematic model also allows for the shear rate ($\dot{\gamma}$) determination. This parameter of the viscosity is the velocity of the upper plate (meters per second) divided by the distance of liquid between the two plates (meters) (**Equation 2**).

$$\dot{\gamma} = \frac{\nu}{h} \qquad \textbf{Equation 2}$$

where ν is the velocity and h is the distance between the plates.

Finally, the dynamic viscosity (η) was defined by Newton's Law as shear stress divided by shear rate (**Equation 3**).

$$\tau = \eta \times \dot{\gamma}$$

$$\Leftrightarrow \eta = \frac{\tau}{\dot{\gamma}} \qquad \textbf{Equation 3}$$

Two different types of flow behavior can be differentiated: Newtonian liquids and non-Newtonian liquids. If the fluid's viscosity changes with the shear rate, the fluid is named non-Newtonian. A Newtonian fluid has an internal flow resistance independent of the shear rate. Some factors must be controlled during dynamic viscosity measurement. Temperature and pressure are the most critical parameter and should be kept constant (and controlled) during measurement [29-31].

The non-sterile liquid preparation under development has a shear-independent fluid behavior, thus, it is considered a Newtonian liquid. Experimentally, this parameter (dynamic viscosity) is obtained with a rotational viscometer.

1.5.1.5. Adhesion

Adhesion is a physical attribute identified as critical for the formulation quality under development. Adhesion is measured using Texture Analyser (TA.XT.plus, Stable, UK, 2017) by applying a compression force on the surface of the sample and, as a result, the force needed to pull the probe of the sample is measured. The force of adhesion, cited as adhesiveness, is the force that resists to the separation of two surfaces in contact, between the probe and the sample. In addition to the adhesiveness peak, the work of adhesion and the detachment distance are also identified.

To perform adhesion test using the original method [32], usually gel mucoadhesive probe is coupled on this equipment together with a thermostatically controlled heater to maintain the medium at 37 ºC (**Figure 8**).

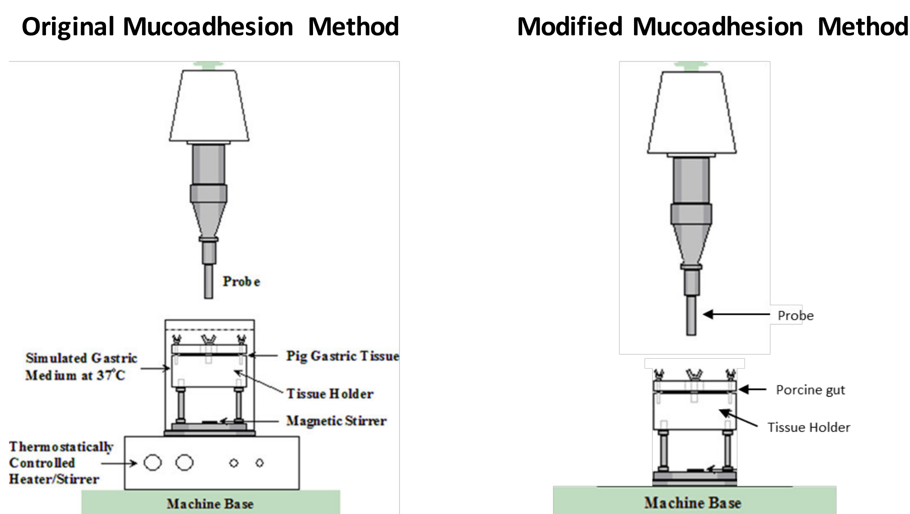


Figure 8 Representation of original and modified methods used for adhesion evaluation.

The original method is specifically used to measure mucoadhesion on the gastrointestinal tract and requires that formulations with higher range of viscosity, to be retained on the probe and resist to gravity force. Since the developed formulation was a liquid preparation and not a gel/semisolid dosage form, the test needed to be slightly modified.

The modified method used the same mucoadhesion rig apparatus to hold the gut mucosa tissue, being the only changes the gel mucoadhesive probe to a cylinder probe and the volume of medium used. In the modified procedure, the formulation to be analyzed was placed over the hydrated mucosa, placed on the tissue holder. Whereas the original method used a stirrer to thermostatically control the simulated gastric medium at 37ºC, in the modified method artificial saliva was used and the temperature controlled with an external thermostatically heater.

As mentioned, Texture Analyser method for adhesion evaluation allows the identification of specific adhesion parameters: Adhesiveness peak (g), Work of adhesion (g.sec) and detachment distance (mm) (**Figure 9**). The adhesiveness peak is the maximum force that the probe has to perform in order to have a total detachment of the formulation, after an established time in contact. This peak value indicates the capability of each composition to be adhesive and the result is higher as the ability for being adhesive increase. The adhesiveness of each formulation is achieved through the difference between the values obtained for the hydrated mucosa after the addition of the formulation and the hydrated mucosa (without formulation). This procedure of analysis allows to exclude the interference of the mucosa used for this study and to determine only the adhesion of the formulation.

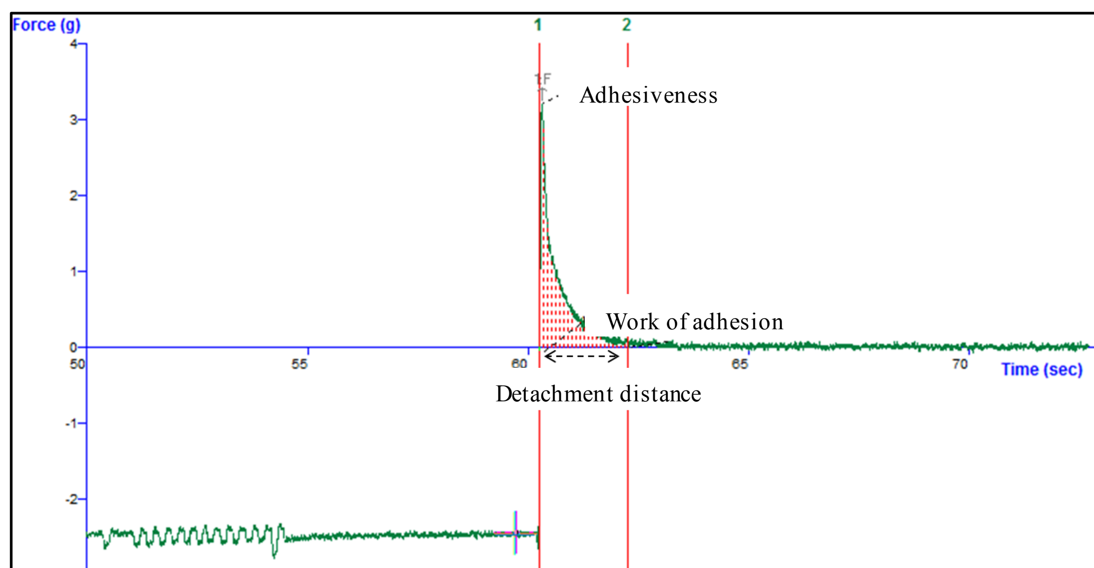


Figure 9 Graphic representation of the specific adhesion parameters: Adhesiveness peak (g), work of adhesion (g.sec) and detachment distance (mm).

The work of adhesion is the area under the curve and it is obtained during the return distance pre-selected in the method settings (10,0 mm). It represents the work required to overcome the attractive forces between the probe and the sample in contact. Finally, the detachment distance represents the prove traveled distance until the total detachment of the formulation after applying a pre-defined compression force for a pre-defined time. This parameter is characterized by the cohesiveness property of each formulation. Similar to the other factors, this also depends on the excipients present in the formulation.

Initially, the tests performed used natural dried porcine gut mucosa. On literature this type of mucosa is the animal mucosa more similar with the human mucosa [33]. Nevertheless, we started to use synthetic gut mucosa due to difficulties inherent to the isolation and acquisition of natural dried gut. Moreover, similarly to the natural dried gut, synthetic gut allowed the discrimination between non-adhesive and adhesive formulations.

1.5.1.6. *In vitro* release test (IVRT)

Product performance quality tests are important for the prediction of the *in vivo* drug product performance. For the non-sterile liquid preparation, the development of i*n vitro* release test is an important tool during the drug product development and for quality control once it guarantees batch to batch consistency and determines some possible deviations during the manufacturing [23]. The IVRT development for this drug product is based on vertical Franz diffusion cell system. Each cell consists of two chambers: a donor chamber and a receptor chamber. This two compartments are separated by a membrane and held together by a clamp. The donor chamber, as the name indicates, has the sample to be analyzed and the receptor chamber has the receptor medium in which the sample will diffuse [23] (**Figure 10**).

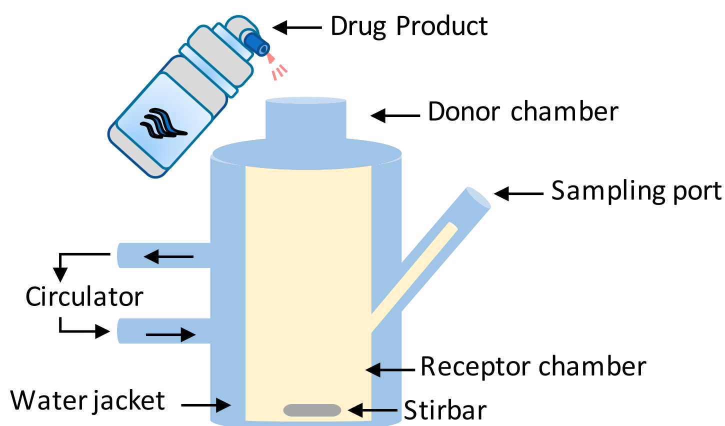


Figure 10 Schematic representation of Franz cell (Model C of USP Chapter 1724 [22]) used for in vitro release test.

Briefly, a specific amount of formulation is dispensed via spray to the donor chamber and by a unidirectional flow, the flux occurs from the donor to the receptor chamber through the membrane. The receptor medium is maintained at a specific temperature by a water jacket. The homogeneity of the medium is achieved with constant agitation through magnetic stirring. Each sample withdrawn through the sampling port is replaced with stock receptor medium in order to maintain the volume. After an appropriate sample preparation, the samples were analyzed by a validated analytical procedure.

For IVRT performance, it is important to consider both physicochemical characteristics of the drug product and the physiological conditions in which the drug substance should be released. According to the literature, the critical experimental parameters that should be studied are the following [22-23,34-35]:

- Receptor medium
- Sample amount and occlusion
- Synthetic membrane
- Number of samples/replicates
- Temperature of the analysis
- Sampling time-point
- Analytical procedure for drug substance quantification

Receptor medium

We have defined that the receptor medium should be bio-relevant to predict the *in vivo* conditions but simultaneously it should have the capability to solubilize the drug substance (s*ink conditions).* This property is commonly mentioned for water-insoluble compounds. The drug released may be controlled by diffusion and for this, the solubility of the drug in a receptor should not influence the response. To achieve *sink conditions*, the saturated solubility of a drug in the receptor medium should be at least 10 times greater than that of the maximum achievable concentration during all the experiment [36]. In the case of low drug solubility, this condition is not accomplished and some components can be added to the receptor medium such as surfactants, alcohol and liposomal preparations. However, in order to maintain the bio-relevance of the receptor medium, it should be noted the limits established by the regulatory authorities. On the literature, some surfactants particularly used for oral mucosa drug delivery are Tween®80 (0,5% v/v) [37] and sodium dodecyl sulfate (0,5% and 1% w/v) [38-39]. In our study,

the receptor medium used was artificial/simulated saliva (Phosphate buffer) at pH 6,8 without any surfactants [40].

Sample amount

The sample amount can be achieved by an infinite or finite dose on the donor chamber. An infinite dose is preferred over a finite dose because reduces the variability due to slight mass variations in finite dosing and simplifies diffusion kinetics. After sample application uniformly on the membrane it must be kept occluded to prevent solvent evaporation and compositional changes.

Synthetic membrane

Since the diffusion occurs across the membrane, it shall be inert toward both receptor medium and formulation and highly permeable with no drug substance binding interaction. Some studies might be performed in order to guarantee that the membrane is not the barrier for the active permeation to provide the diffusion of the drug. A synthetic membrane is preferred over an *in vivo* membrane because features like pore size, thickness and hydrophilicity are controlled.

Number of samples/replicates

The drug substance release rate must be determined by multiple replicates. On the literature six samples are recommended, all of them with the same experimental conditions.

Temperature of the analysis

The temperature of the analysis is chosen considering the physiological application of the drug product. For example, for oral, vaginal and rectal drug delivery system, the temperature is around 37ºC whereas for nasal the temperature employed ranged between 33 and 37ºC [34].

Sampling time-point

At a specific time point, an aliquot is withdrawn and replaced with the same volume of fresh medium. On the literature, it is recommended at least 5 sampling time points. Depending on the formulation's burst effect, and the time for release to reach a *plateau*, the sampling times may be adjusted to each formulation composition.

Analytical procedure for drug substance quantification

Finally, a validated and sensitive analytical procedure should be used to determine the drug concentration and the amount of drug released. In this work, spectroscopy method was used.

1.5.2. Spray performance

1.5.2.1. Spray Actuation test

Spray actuation test is performed during the development of non-sterile liquid preparations as the formulation is released as a mist (or jet, depending on the final viscosity). With this method, we determine whether such formulations can be sprayed when a certain velocity is applied during an established distance at the spray device. The spray actuations are carried out using an automated equipment – Texture Analyser (TA.XT. plus, Stable, UK, 2017) – because it has the capability to guarantee controlled settings and a reproducible method. As a result of this controlled actuations, the force required to initiate the formulation release (actuation force) and the maximum force applied to the spray device during the release of the formulation (maximum force) are determined as well as the spray duration [41]. The physicochemical characteristics of the formulation as well as the pump supplier are considered critical parameters. As such, different formulation composition, different pump suppliers and commercialized spray products were studied.

1.5.2.2. Pump Delivery/Shot weight

Pump delivery test evaluates the shot weight/dose of formulation released at each spray actuation and it is widely used to assess pump-to-pump reproducibility in terms of drug product performance. This test is influenced by the pump supplier, the composition of the formulation (e,g, formulation density) and by the actuations type - manual or automated actuations. Pump delivery test is a requirement for some of the regulatory authorities for release finished product specification and widely used by pharmaceutical industry for assessment of equivalence between different products.

FDA authority mentions that the pump spray weight delivery acceptable criteria should control the weight of the individual sprays to within 15% of the target weight and their mean weight to within 10% of the target weight [12].

1.5.2.3. Mean delivered dose

EMA authority requires this test for release finished product specification. This test is based on the analysis of the amount of drug substance per actuation, using an appropriate analytical method. Afterwards, the mean of the delivered dose was calculated. The regulatory authority also indicates an acceptable criteria of ± 15% of the label claim apply [14]. Since drug substance quantification is not the scope of my work, this test has not been performed.

1.5.2.4. Tail-off characteristics

Tail-off characteristics describe the profiling of sprays near container exhaustion. This profile is observed after the point at which the labeled number of actuations have been dispensed until no more actuations are possible. Factors such as pump design, container geometry and formulation composition can have influence on tail-off characteristics. This characteristic knowledge supports the determination of the target filling of the containers in order to guarantee the labeled doses [12,14].

1.5.2.5. Minimum fill/Net content

Evaluation of the minimum fill is a requirement for all the regulatory authorities. This study demonstrates that the individual container minimum fill is sufficient to provide the labelled number of actuations [14]. FDA authority refers that nasal spray drug products should include acceptable criteria for net content of the formulation in the container and should be in accordance with the release specification [12]. Although it is an important test to perform for release of the drug product, at the stage of my internship it was not performed, being the minimum fill guaranteed only by balance weight control.

1.5.2.6. Weight loss (stability)

Weight loss upon storage is a requirement for liquid dosage forms while they are stored on a semipermeable container. Monitoring weight loss over time assess the moisture-loss protective properties of the overall container-closure system. The selected packaging material must not interact physically or chemically with a packaged compound. FDA authority mentioned that the spray device orientation has a great impact on weight loss upon storage. On guidance for industry related with stability, it is referred that a 5% loss in weight from its initial

value is considered a significant change for a product packaged in a semipermeable container after an equivalent of 3 months [42].

1.5.2.7. Priming and Repriming (stability)

Both FDA and EMA regulatory authorities require priming and repriming performance tests during drug product development only if the product is a multiple-dose spray drug product.

Priming characterization supports the number of actuation recommended in the label of the drug product that should be fired to waste prior to the consumer using the product for the first time.

Repriming of the container supports 1) the length of time that the drug product may be stored without use before the need to repriming again in order to achieve the shot weight claim in the label and 2) the number of repriming actuations required until the shot weight meet the drug product specification claim in the label. In this case, some parameters can be tested such as different orientations of the DP during actuations, different stages through container life and multiple time points studies [12,14].

Both priming and repriming information will be used to support the proposed labelling statements.

1.5.2.8. Number of doses/actuations

The number of actuations throughout the shelf life of the DP should be indicated for each container fill weight. The number of doses shall be recorded taking into account the acceptable criteria defined by FDA wherein the weight of the individual sprays should be within 15% of the target weight [12]. As such, the doses discarded during priming and repriming actuations are not considered [14].

The determination of the number of doses for each container fill weight is important because it should be concordant with the labelled number of doses.

Overall, the drug product label should include the following information:

- Usage and orientations information
- Number of priming actuations
- Number of repriming actuations
- Length of time without use before repriming is needed
- Number of doses/actuations
- Amount of drug substance per dose

CHAPTER 2

PRACTICAL ACTIVITY

2.1. Materials

Synthetic gut used for adhesion test is made of collagen, had a smooth surface and it was supplied from SaborPlus (PT).

Disodium phosphate (Na_2HPO_4, M_w =141.96 g/mol) and monopotassium phosphate (KH_2PO_4, M_w =136.08 g/mol) were purchased from Panreac. Sodium chloride (NaCl, , M_w =58.44 g/mol) from Merck. These three components were used for the preparation of simulated saliva (phosphate buffer, pH=6.8) according to Margareth *et al.* [40]. The composition of the formulations under development is confidential. Ultrapure water was obtained by an internal water purification system using a Merck Millipore Milli-Q® equipment. The spray pump devices were acquired from different suppliers including AeroPump, Aptar and Nemera.

2.2. Equipments

Table 3 described the equipment's used throughout the project, indicating the names, the manufactures and models.

Table 3 Equipment used both for formulation characterization and for spray performance evaluation.

Equipments		
Name	**Manufacturer**	**Model**
Analytical Balance	Mettler Toledo (USA)	X205DU
Precision Balance	Mettler Toledo AG (USA)	MS3002S/01
Viscometer	Viscotech (Spain)	VR3000 V1L
Texture Analyser	Stable (UK)	TA. XT Plus
pH Electrode	WTW (Germany)	InoLab pH 7310P
Spectrophotometer	Analytikjena (Germany)	Special 210
Franz cell	PermeGear (USA)	V6A-02
Water circulator	ThermoScientific (USA)	SC100
Heating and stirring plate	VWR (USA)	VMS C7 Advanced
Microscope	Motic (Spain)	BA 310 Met-T
Pycnometer	Not defined	Not defined

2.3. Procedures

2.3.1. Characterization of the formulation

2.3.1.1. Definition/Description/Appearance

Appearance evaluation was made by visual inspection of the formulation. Whereas the formulation appearance, the evaluation of the integrity of the container closure system was also made when the final DP was analyzed.

2.3.1.2. Specific Gravity/Density

Select a clean and dry pycnometer and determine its weight. Then, fill the same pycnometer with recently boiled water, at 25 °C, and weight [21] (**Figure 11**). According to the **Equation 4**, the volume of water that is filling the pycnometer and the stopper can be determined as follows:

$$V = \frac{m_{H_2O}}{\rho_{H_2O}} \quad \textbf{Equation 4}$$

where m_{H_2O} is the experimentally determined weight of water (empty pycnometer weight subtracted) and ρ_{H_2O} is water density (**Table 4**). With this equation, the volume (V) is calculated, according to the density of water at 25 ºC (0.99705 g/cm^3).

Table 4 Water density at different temperatures.

T (ºC)	ρ_{H_2O} (g/cm^3)
15	0.99996
16	0.99994
17	0.99990
18	0.99985
19	0.99978
20	0.99820
21	0.99799
22	0.99777
23	0.99754
24	0.99730
25	0,99705

Clean the pycnometer properly and place the formulation, at 20 ºC, inside. Then adjust the temperature of the filled pycnometer to 25 °C and weight again. Empty pycnometer weight was deducted. The determination of the density of the formulation is based on **Equation 5**:

$$V = \frac{m_{formulation}}{\rho_{formulation}} \quad \textbf{Equation 5}$$

where volume (V) was determined previously on **Equation 4** and weight of formulation is determinate as referred above.

Combining **Equation 4** and **Equation 5** it is obtained a relation that directly provides the density calculation of the formulation ($\rho_{formulation}$) (**Equation 6**).

$$\rho_{formulation} = \frac{m_{formulation}}{m_{H_2O}} \times \rho_{H_2O} \quad \textbf{Equation 6}$$

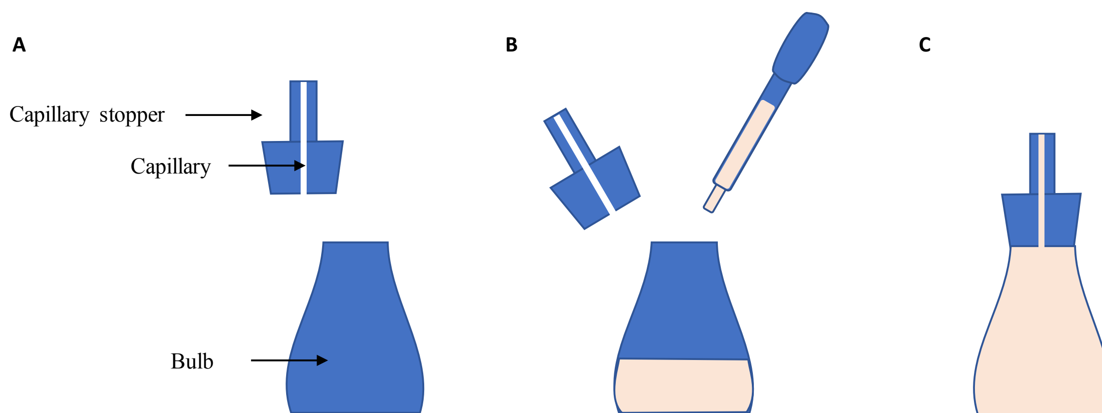


Figure 11 Schematic representation of the specific gravity procedure. Check that the pycnometer is properly cleaned and dried before weighing (**A**). Fill the pycnometer with the sample to about halfway up to neck and insert the capillary stopper (**B**). Check that the sample fills all the capillary, clean the pycnometer and weight (**C**).

This procedure requires certain important consideration including the following:

- The pycnometer must be perfectly clean and dry before the initial weighing.
- To fill the pycnometer with liquid, use a Pasteur pipet to fill the bulb to about halfway up the neck. Then slowly insert the capillary stopper.
- When full, there should be no bubbles in the bulb or capillary of the pycnometer, and no air space at the top of the capillary.
- Before each weighing, the outside of the pycnometer should be perfectly dry.

2.3.1.3. pH

pH determination is carried out using two different procedures: directly from the bottle and through the final drug product. Both procedures use a surface electrode (InoLab pH 7310P, WTW, Germany, 2017). Before reading the pH of the formulations, calibration of the electrode with buffer solutions is necessary [15]. And, if the pH measurement is through bottle, placing the electrode dipped into the formulation and cap the bottle with silver paper to minimize the evaporation of volatile excipients that are present on the formulations. Between measurements, cleaning the electrode with purified water and then remove the excess of water with cleaning paper.

On the other hand, the pH of the formulation can be measured across the spray device as represented in **Figure 12**. After electrode calibration, put 10 SPR (~1.5 mL) with spray device on a glass cell (h=30 mm; d= 25 mm; v~15 mL). Finished this step, measure the pH with the surface electrode touching into the formulation. Both procedures for pH measurement should be made in triplicate.

If the surface electrode does not incorporate temperature sensor, it is necessary external measurement and the consideration of this value in the pH measurement.

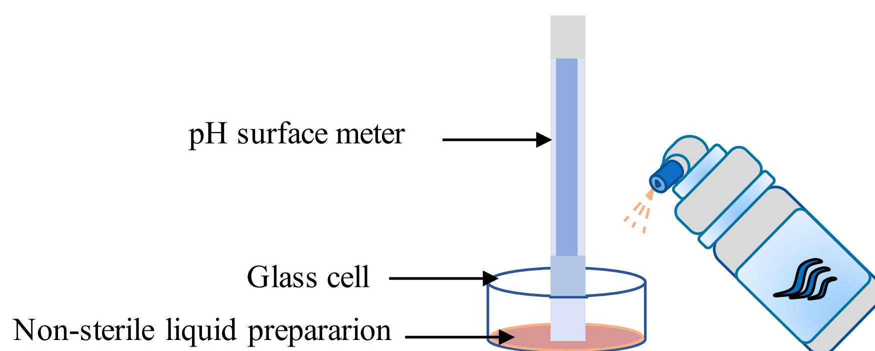


Figure 12 Schematic representation of pH measurement using spray device.

2.3.1.4. Viscosity

Viscosity measurement of the formulations is carried out using a rotational viscometer VR3000 V1L (Viscotech, SPAIN, 2014) (**Figure 13**). If the amount of formulation is reduced it can be measured with an adapter for small sample volumes. In this case, a fixed volume of formulation is placed in a container coupled to the apparatus. The temperature during the

analysis should be kept constant (21.0 ± 0.5 °C) that, with this accessory, it is possible with a water circulator.

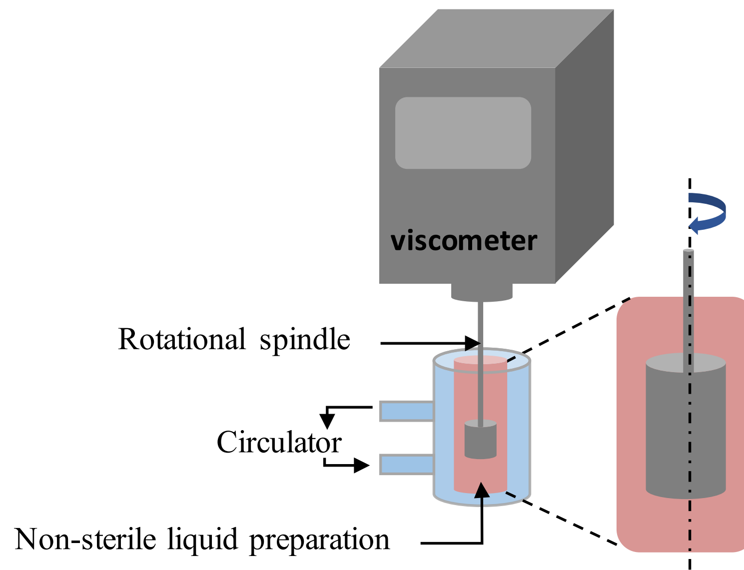


Figure 13 Schematic representation of viscosity measurement using an adapter for small sample volumes.

The choice of spindle (TL5, TL6 or TL7) should be made based on the viscosity of each formulation as shown in the **Table A1** on attachment.

However, if the volume is not reduced, the viscosities can be read directly from the bottle where they are usually stored. To do this, immerse the respective spindle in the sample until the spindle mark. In this case, the choice of the spindle is based on the information present in the **Table A2** on attachment.

The instruction guide refers that measurements obtained from the limits of the torque range (less than 10% and more than 90%) have an inaccurate error associated. Accordingly, we choose that values at the middle of the torque range (~50%) provide flexibility for possible variance in measurements that can occur during production. Thus, viscosity is determined based on the average of three viscosity values achieved at the middle of the torque range.

2.3.1.5. Adhesion

Adhesion is measured with Texture Analyser (TA.XT.plus, Stable, UK, 2017) equipped with a 5 kg load cell and a P10 probe (10.0 cm diameter). The exponent software, coupled to the equipment, generates force versus time curves.

The protocol is divided into three phases: hydration process, equipment preparation and sample test.

Hydration Process

On the first step, designated as hydration, it is important to guarantee that the chosen gut mucosa is well pre-hydrated in order to allow a correct assessment of the adhesiveness peak. For that, the mucosa is placed over 4 mL simulated saliva (Phosphate buffer, pH 6.8) at 37 ºC, for 2 minutes. The temperature was controlled to 37ºC with a heating and stirring plate (VMS C7 Advanced, VWR , USA, 2013). Afterwards, the mucosa became softer and less opaque (step 1 on **Figure 14**).

Equipment preparation

Hereafter, the hydrated mucosa is placed on mucoadhesion rig, between the two pieces of the tissue holder and then the measurement is made on Texture Analyser. The obtained adhesiveness of this mucosa is considered the baseline and, subsequently, this influence will be removed from the final result (step 2 on **Figure 14**).

Sample test

Two procedures for sample test can be distinguished: one refers to formulation packaged on amber bottles and the other refers to formulation packaged on spray device. To the humidified mucosa already analyzed, add 2 drops of the formulation if it is stored in a bottle (step 3a on **Figure 14**), or 2 SPR if it is stored in a spray device (step 3b on **Figure 14**). After 2 minutes of contact, starts measurement.

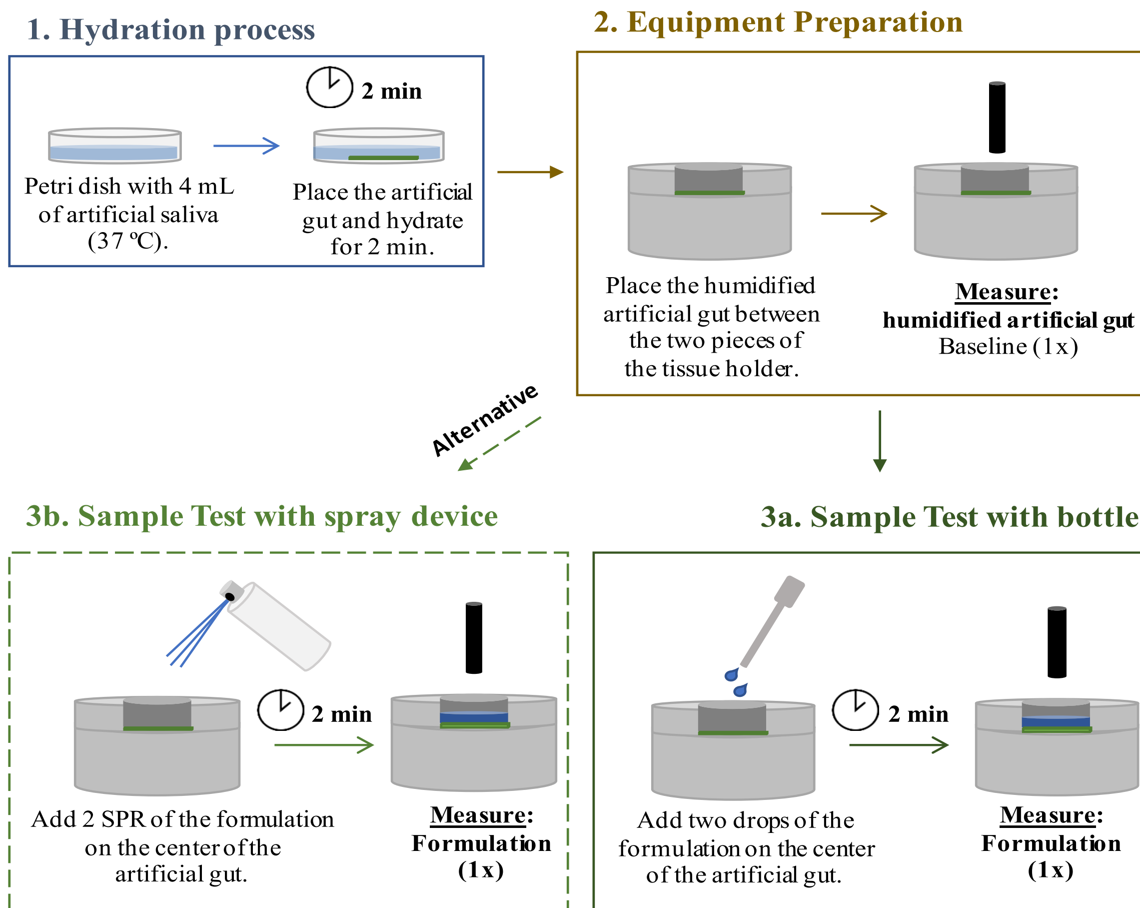


Figure 14 Schematic representation of adhesion method. Step 1 corresponds to the hydration process. Step 2 corresponds to the equipment preparation and, finally, 2 steps for sample test can be distinguished: with bottle (step 3a) and with spray device (step 3b).

Adhesion measurement using Texture Analyser requires the optimization of some settings. **Table 5** shows the well-defined settings for the developed adhesion method.

Table 5 Settings for adhesion method.

Settings	Values
Pre-test Speed	0.80 mm/Sec
Test Speed	0.10 mm/Sec
Post Test Speed	0.80 mm/Sec
Applied Force	2.50 g
Return Distance	10.0 mm
Contact Time	60.0 Sec
Trigger Type	Auto
Trigger Force	5.0 g
Advanced Options	Off

2.3.1.6. *In vitro* release test

This test is carried out using Franz diffusion cell - one type of vertical diffusion cell. The inferior compartment is filled with 12.0 ± 0.2 mL (volume exactly determined for each cell by the manufacturer) of simulated saliva (Phosphate buffer, pH 6.8 at 37 ºC) while the donor chamber is placed with one actuation (~200 mg) of drug product. A nylon artificial membrane with pore size of 41 µm was placed between chambers. The receptor medium has a temperature controlled of 37.0 ± 0.5 ˚C by the water jacket that is coupled to the Franz diffusion cell. The medium was also continuously stirred at ~600 rpm using a magnetic stirrer to uniform the medium and also to simulate the mechanical movements of the mouth (**Figure 15**).

The test initiate when the sample was dispensed for the donor chamber. At pre-selected time points (0.5, 1, 2, 5, 10 and 20 min), with the apparatus properly occluded, removed 400 µL aliquots with a 1 mL graduated syringe (Omnifix 100 Solo, Braun) and replace with the same volume of fresh medium at 37.0 ± 0.5 ˚C. The samples withdrawn were treated according to a validated analytical procedure using spectroscopy. The release profile was constructed plotting the cumulative mass of diffusant versus time.

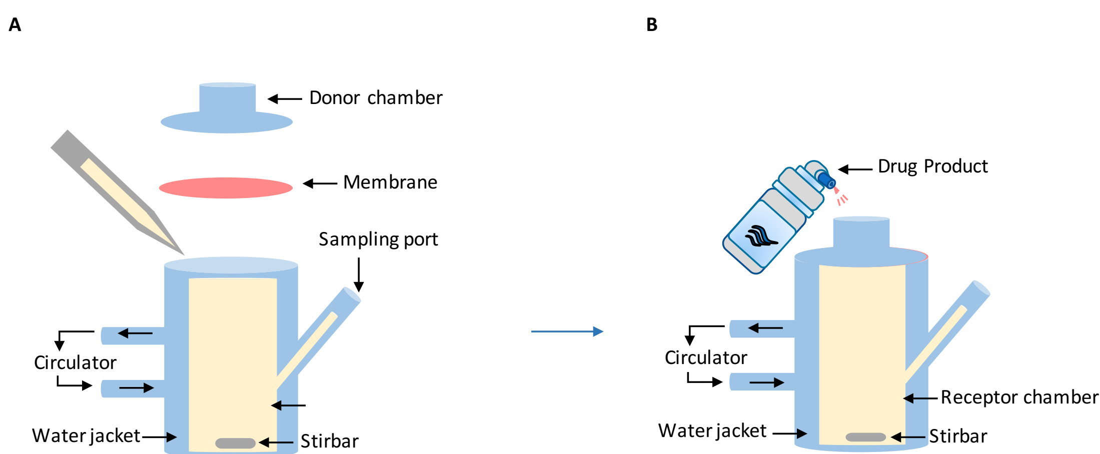


Figure 15 Schematic representation of the *in vitro* release test procedure. Place a specific amount of receptor medium on the receptor chamber and put the artificial membrane above the positive meniscus (**A**). Finally, put the sample on the donor chamber (**B**).

2.3.2. Spray Performance

2.3.2.1. Spray actuation test

To measure actuation force, maximum force and spray duration, Texture Analyser (TA.XT.plus, Stable, UK, 2017) equipped with a 30 kg load cell and a P25 probe (25.0 cm diameter) was used (**Figure 16**). The exponent software, coupled to the equipment, generates force versus time curves [43].

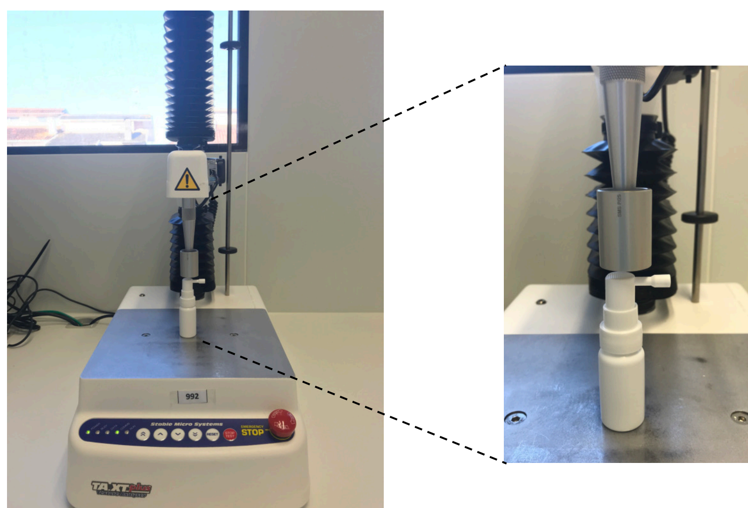


Figure 16 Representation of spray performance procedure using automated actuations on Texture Analyser.

2.3.2.2.Pump delivery/Shot weight

To quantify each pump spray weight delivery an analytical balance (X205DU, Mettler Toledo, USA, 2007) was used. Each actuation was performed on Texture Analyser (TA.XT.plus, Stable, UK, 2017) equipped with a 30 kg load cell and a P25 probe (25.0 cm diameter) (**Figure 16**).

2.3.2.3. Mean delivered dose

Drug substance amounts in each actuation were achieved by weighing accurately each pump spray delivery on an analytical balance (X205DU, Mettler Toledo, USA, 2007). Each actuation was performed on Texture Analyser (TA.XT.plus, Stable, UK, 2017) equipped with a 30 kg load cell and a P25 probe (25.0 cm diameter) and collected in a Falcon tube. After the sample preparation, assess the relevant analytical method to determine the amount of drug substance.

2.3.2.4. Tail-off characteristics

An analytical balance (X205DU, Mettler Toledo, USA, 2007) was used to weigh the pump spray weight delivery. Each actuation is performed on Texture Analyser (TA.XT.plus, Stable, UK, 2017) equipped with a 30 kg load cell and a P25 probe (25.0 cm diameter) (**Figure 16**).

2.3.2.5. Minimum fill/Net content

Fill the container with the target formulation volume and close it. Weight the spray device without any labelling that might be altered in weight during the removal of the container contents ($Weight_{FilledContainer}$). Remove all the formulation container on the spray device. Washing with a suitable solvent (e.g., ethanol) and, if necessary, put the spray device on fume hood for solvent evaporation (at 100 ºC). Afterward, dry and weigh each empty container together with its corresponding parts ($Weight_{CleanContainer}$). The result is obtained through the weights subtraction. Repeat the procedure for 10 containers [27]. Considering the stage of my internship, minimum fill was guaranteed only by balance weight control.

2.3.2.6. Weight loss (stability)

Spray devices acquired from different suppliers are stored in upright position in the dark and at an uncontrolled temperature. Weekly, the spray devices were weighed on an analytical balance (X205DU, Mettler Toledo, USA, 2007).

2.3.2.7. Priming and Repriming (stability)

To quantify each pump spray weight delivery an analytical balance (X205DU, Mettler Toledo, USA, 2007) was used. Each actuation is performed on Texture Analyser (TA.XT.plus, Stable, UK, 2017) equipped with a 30 kg load cell and a P25 probe (25.0 cm diameter) (**Figure 16**).

2.3.2.8. Number of doses/actuations

To record the number of actuations in a specific amount of non-sterile liquid preparation, fill the container with a specific amount of sample and, using Texture Analyser (TA.XT.plus, Stable, UK, 2017) equipped with a 30 kg load cell and a P25 probe (25.0 cm diameter) to perform each actuation, build the graphic of the shot weight as function of number of actuations. To weight the spray device we used an analytical balance (X205DU, Mettler Toledo, USA, 2007). Assuming the acceptable criteria of pump delivery defined by FDA authority, record the number of constant doses released (**Figure 16**).

CHAPTER 3

RESULTS AND DISCUSSION

The work performed during my internship supported the development of non-sterile liquid preparations through the characterization and evaluation of drug product performance either immediately after preparation (day 1 evaluations) and also during non-formal pre-stability studies.

During the development of new dosage forms based on Design of Experiments (task performed by an internal team from Research and Innovation department at Bluepharma), several formulations without drug substance (referred as "placebo" formulations) were physically characterized, which provide the support for the development of "prototypes" (formulations with drug substance incorporated). Some of these formulations were also characterized over time – pre-stability studies. The pre-stability studies offered evidence on how the quality of a drug substance or drug product could vary within time under the influence of environmental factors such as temperature, humidity and light.

Several commercialized reference products/technologies were identified due to their similarities with the drug product under development (site of application, main features, spray pump, among others) and have been characterized *in-house*. These reference products were studied using the same methodologies implemented to evaluate the drug products under development.

In order to structure the results, Chapter 3 is divided into two subchapters. The first subchapter (section 3.1) concerns to the evaluations performed immediately after formulation preparation (day 1 evaluation). The second subchapter (section 3.2) expose the results obtained during pre-stability studies, performed at different time points. In both subchapters, some comparisons between DP under development and reference technologies were made.

3.1. Day 1 Evaluation

3.1.1. Characterization of the formulation

3.1.1.1. Definition/Description/Appearance

Immediately after the formulation preparation, we have analyzed the appearance by visual inspection and classified it according to the **Figure 17**.

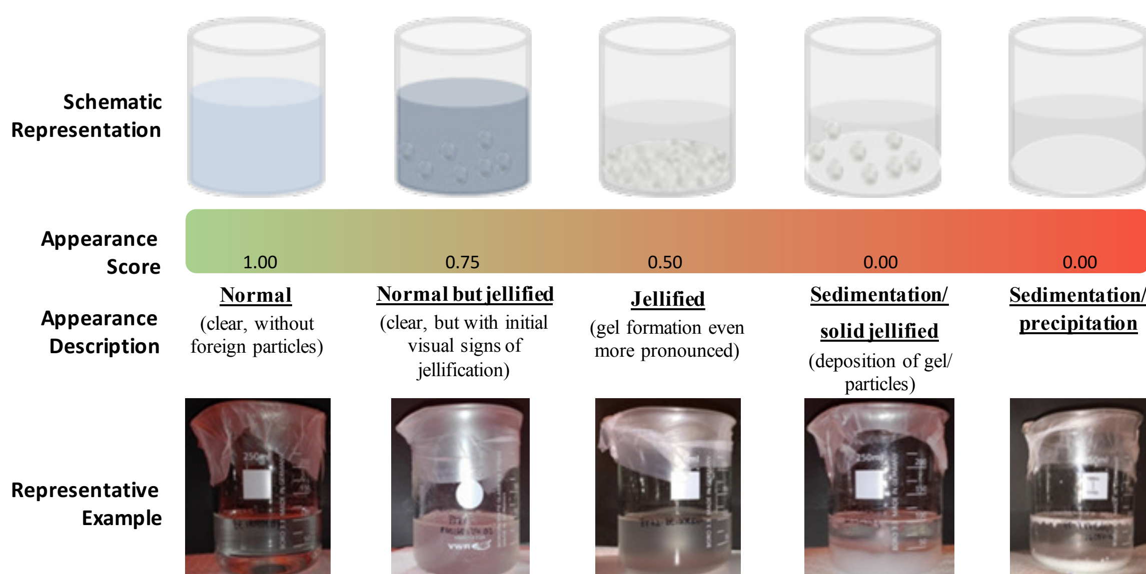


Figure 17 Appearance classification through visual examination. The appearance score starts at "1.00" and ends at "0.00". The desired score is characterized by clear formulation, without foreign particles. On the other hand, if there is visible jellification, sedimentation or precipitation, the classification is lower.

The qualitative classification was established according to the CQA defined for the DP under development and is divided into 5 categories (**Figure 17**): Normal (the "desired" scenario), normal but jellified, jellified, sedimentation/solid jellified and sedimentation/precipitation. Each category was semi-quantified using a scale ranging from 0 (undesired) to 1 (desired). Formulations trials rated with "1.00" exhibit clarity and translucent aspect without signs of gel formation or presence of sediment. On the other hand, the formulations that revealed this appearance however with signs of jellification but not prominent, were scored with "0.75". These formulations are still accepted but have a risk of cannot be dispensed via spray. If gel features start to be more prominent, the formulations were scored with "0.50" and the risk of not being *sprayable* was even higher. Finally, if formulation trials appear to be too jellified (visible solid gel structures), or with sedimentation or if precipitation occurred, we have rated it with "0.00".

Some factors during the manufacturing procedure like the mixing apparatus, stirring speed and time, could affect formulation appearance. Furthermore, some intrinsic properties of the formulation such as the type and quantities of excipients, solvents or the pH of the mixture could also contribute to modify the appearance of the formulation. Importantly, the main cause identified for the lower classification of appearance (lower than 0.50) was the incompatibility between some excipients used during the manufacture process.

Appearance evaluation must be performed over time to verify any alteration of the formulation, which could be a stability indicator of the composition. Ideally, the formulations must maintain their slightly opaque to clear appearance (as defined in the DP specification).

3.1.1.2. Specific Gravity/Density

Specific Gravity/Density was monitored during the drug product development. This parameter is mainly influenced by the type of excipients used in the formulation. Results demonstrate that the addition of certain components (undisclosed information) could significantly modify the density of the formulation, as represented in figure below (**Figure 18**).

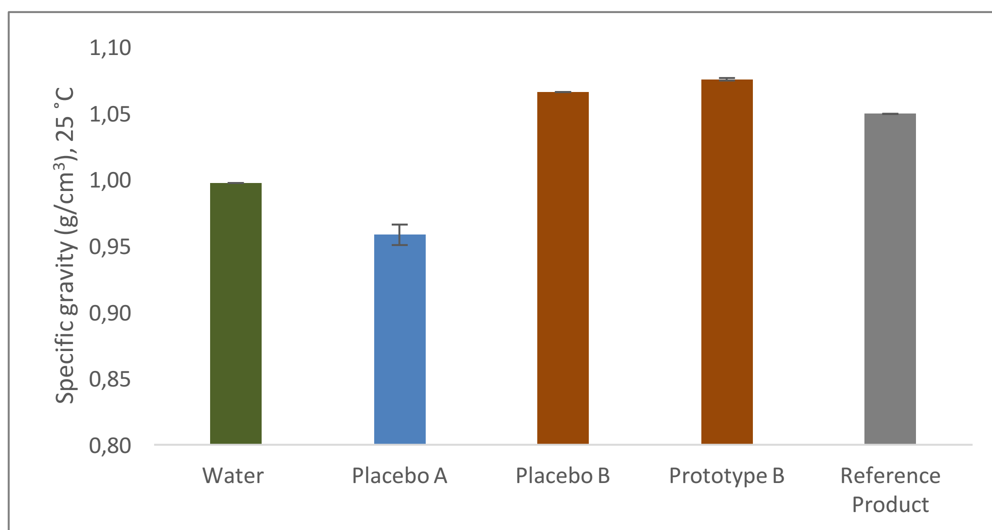


Figure 18 Specific gravity of different formulations. Placebo A (blue color) and Placebo B (orange color) only differ on excipients composition and these placebos have different specific gravities. Prototype B (orange color) has the same excipients as the Placebo B but with drug substance incorporated and the specific gravities obtained are similar. Reference Product (grey color) represent a commercialized product with features similar to the DP under development and the specific gravity is similar to placebo B and prototype B.

In **Figure 18** is represented the quantification of specific gravity for different formulations. The water was used as a control and is represented here for comparison with our formulations. Two different formulations were tested: placebo A and placebo B. The results show that the specific gravity of placebo A (0.958 ± 0.008 g/cm^3) is lower than water (0.997 g/cm^3 [44]) and placebo B (1.066 g/cm^3) is higher than water. The only difference between the placebos is the presence of a specific excipient in the composition placebo B, which increase the specific gravity of the formulation. The presence of the drug substance, on prototype B, does not vary too much the specific gravity when compared with placebo B. Reference product was also evaluated as a reference for the DP under development. The results show that the reference product is more similar to placebo and prototype B than placebo A.

Specific gravity parameter has a high influence on the type of spray dispensed: jet or mist form [45]. Additionally, it can be used as a manufacture process control because it can detect the presence of air bubbles in the formulation by smaller specific gravity. Overall, specific gravity determination will help on the conversion of the units from weight to volume, information that could be important to handle the product during manufacture and also to introduce later on the label claim of the drug product.

3.1.1.3. pH

pH measurement is a stability indicator parameter that could be performed either directly from the bottle or following spray actuations.

During initial formulation development, the formulations were stored on bottles in order to facilitate the visual inspection and handling of the formulations at early development stages. At this point, the pH measurement was performed directly exposing the composition from the bottle. Later, for pre-stability studies, the compositions were stored in the final container - spray device - to mimic the future stability studies of the DP. At this later stage, the pH measurements were performed following spray actuations. In this work, we have performed some tests to provide evidence that the pH measurement is not influenced by packaging type, i.e. measurements directly from a container (destructive method), or after spray actuation (non-destructive method).

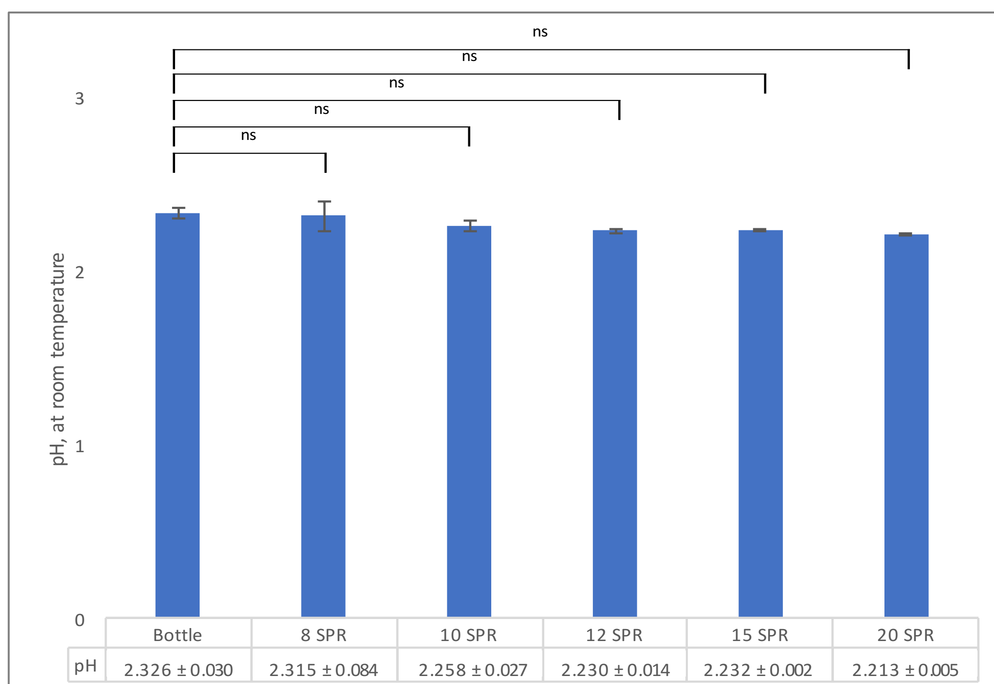


Figure 19 Adaptation of pH measurement for spray devices. pH measurement on bottle is compared with pH measurement with spray device through 8, 10, 12, 15 and 20 SPR (actuations using the same pump that releases 150mg/SPR). Statistical analysis using 2way ANOVA, 95% confidence level demonstrate that all the volumes tested were not significantly different from the bottle pH.

The most important consideration related to pH measurement following spray actuation is the fact that must be performed in a way that maintains the integrity of the spray device. To do this, different volumes of actuations (designed as SPR) were released on a glass cell and pH was measured. The smaller volume used was 8 SPR since it is the minimum volume required to coat the glass cell and allow the full contact between the surface electrode and the sample. The pH measurement following spray actuations demonstrates that all the volumes tested were not significantly different from the bottle pH measurement (2way ANOVA, 95% of confidence level) (**Figure 19**). The main purpose of this study was the identification of the minimal number of spray actuations enough to obtain a comparable pH measurement to a direct evaluation from bottle container. Although the average of the pH measurement on glass cell with 8 SPR (2.32 ± 0.08) was not significantly different from the bottle pH measurement (2.33 ± 0.03), the standard deviation of this measurement was higher (±0.08) when compared to the other measurements and, therefore, was discarded. As shown in **Figure 19** the standard deviation decreases with higher volumes of sample, which indicates that the analyses become more precise. Since the pH measurement with 10 SPR was not significantly different from the pH measured directly from the bottle, and the standard deviation was reduced, we have selected this number of actuations to future evaluations when a nondestructive evaluation is required (mainly during pre-stability studies).

pH is a stability indicator parameter of liquid preparations and it is an important feature to be considered during DP development as it is expected to have an impact on the local tolerability. Additionally, the pH could influence the solubility and systemic permeability of drug substance and therefore require to be investigated since early development stages. The absorption of the drug substance across a membrane depends upon its lipophilicity, which in turn depends on its degree of ionization and partition coefficient. Where the higher the unionized fraction of a drug, the greater is its lipid solubility. The pH of the mucosal membrane and the pKa of the drug has an impact on the degree of ionization [46-48].

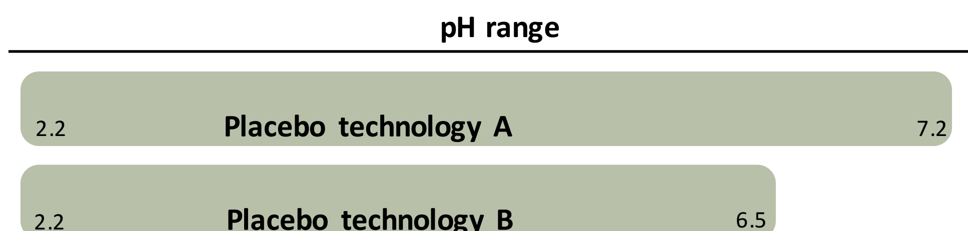


Figure 20 pH range of two placebo technologies developed. Range of placebo technology A is within 2.2 and 7.2 and within 2.2 and 6.5 to placebo technology B.

In this work, the research and innovation team have developed two different formulations types, identified in this section as Placebo technology A and B (undisclosed information). Each of these compositions was prepared with specific excipients each, and a series of experiments (under DoE) were performed to investigate the versatility of each composition regarding different physical characteristics, including pH. **Figure 20** depicts the range of pH where each composition maintained the optimal responses identified in the QTPP of this technology (i.e. prove the concept established). The results demonstrate that the pH values are within 2.2 and 7.2 for Placebo technology A and within 2.2 and 6.5 for Placebo technology B.

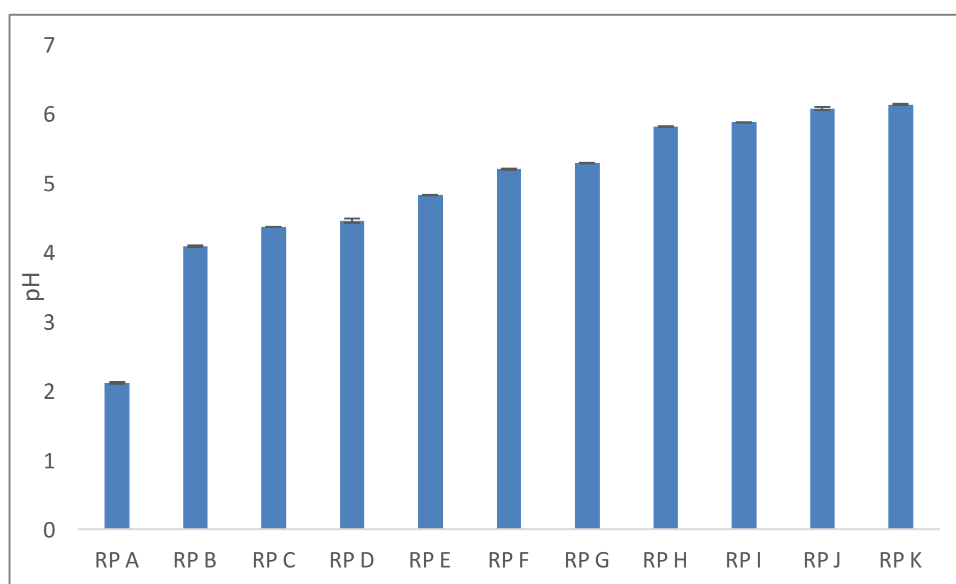


Figure 21 pH measurement on different reference products with similar characteristics to the DP under development (similar dosage form and same mucosal application).

In addition, we have also compared the pH of the placebo technologies to the pH of some commercialized products with similar features to the DP under development. For the selection of these reference products, we considered the dosage form and a specific site of application (undisclosed information) and this analysis resulted on 11 reference products (**Figure 21**). The range of pH of these commercialized products is within 2.101 and 6.132. When comparing the placebo technologies with these reference products (**Figure 22**), one could notice that the range of values for the references is within the acceptance range of the placebo technologies developed. The versatility of the technology developed will allow the formulation team to modulate the composition to a specific site of application, promoting local tolerability or enhancing drug substance solubilization/permeation.

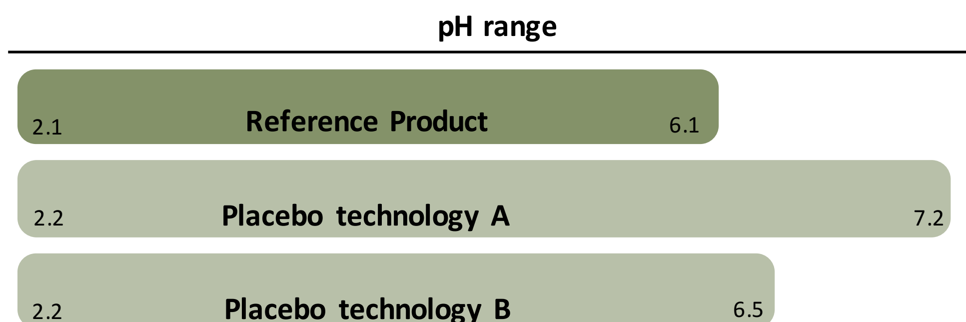


Figure 22 pH range obtained with reference product and placebo technologies. Range of placebo technology A is within 2.2 and 7.2 and within 2.2 and 6.5 to placebo technology B. Reference product pH range is within 2.1 and 6.1.

3.1.1.4.Viscosity

Viscosity is a stability indicating parameter, that is commonly evaluated immediately after preparation of the non-sterile liquid preparation. This parameter is highly influenced by the type and quantity of excipients used and its concentration on the mixture. Certain viscosity-increasing agents were used as necessary, with an appropriate concentration. The choice of the right excipient with the suitable viscosity has an important impact on the DP performance. Those agents are used in pharmaceutical formulations to stabilize disperse systems, to reduce the rate of solute or particulate transport, or to decrease the fluidity of liquid formulations. Most of the agents are hydrophilic carbohydrate molecules and non-carbohydrate hydrophilic macromolecules [49]. Some commercialized products, FDA-approved, used this kind of agents in order to produce three-dimensional structures with certain polymers for gel formation and stability [28].

The specific excipients of the developed formulations, including viscosity-increasing agents, cannot be mentioned in this work because of concerns regarding confidentiality. Nevertheless, some obtained results with different concentration of viscosity-increasing agents will be discussed below. For example, in **Figure 23**, the selected formulations just differ on the concentration of this agents, while the other excipients are comparable and are within similar quantitative percentage.

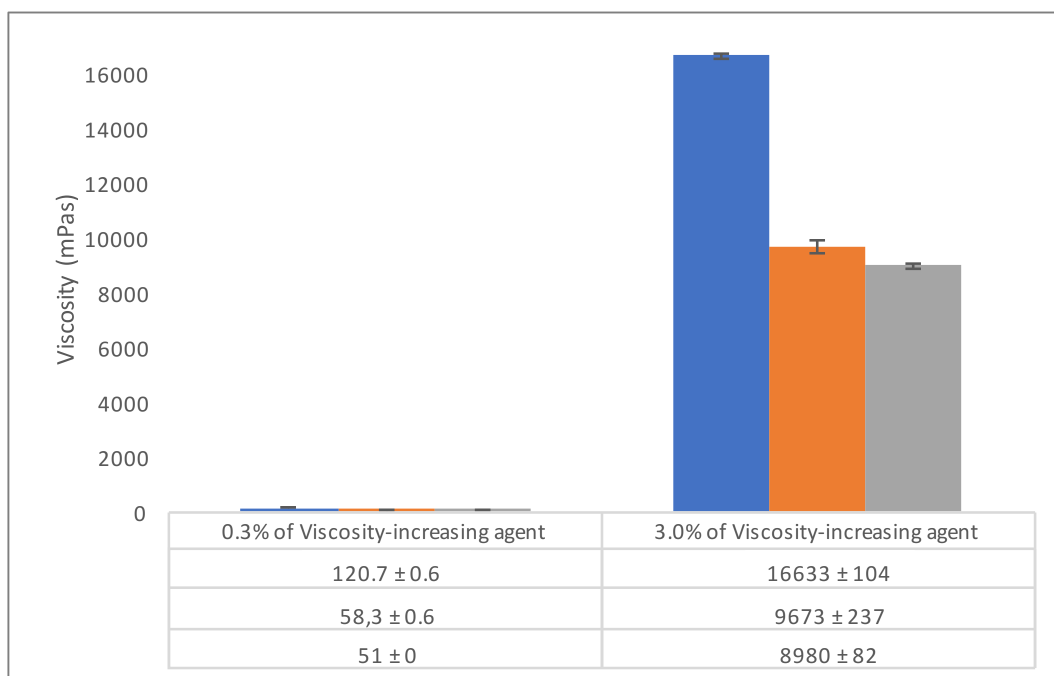


Figure 23 Influence of the presence of viscosity-increasing agents at different concentrations (0.3% and 3.0%) on viscosity measurement. The increasing of the viscosity agent increases the viscosity.

As shown in **Figure 23**, increasing the concentration of the viscosity agent, the viscosity of the formulation increases abruptly. A remarkable influence of the viscosity-increasing agent supports the formulation development team during the initial stage.

The target viscosity is dependent on the drug product application. In this case, the product will be dispensed via spray, and therefore viscosity parameter is critical as will influence the choice of the spray pump type.

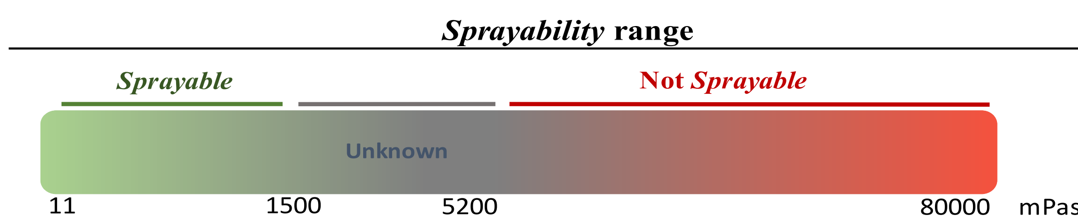


Figure 24 Relationship between sprayability and viscosity. Formulation viscosities within 11 and 1500 mPas were dispensed as a spray. Viscosities between 5200 and 80000 mPas were not *sprayable*. To well define the range between 1500 and 5200 mPas more studies are needed.

During formulation development, the viscosity of several compositions was investigated and, as demonstrated in **Figure 24**, the accepted viscosity values to be dispensed via spray were found to range within 11 and 1500 mPas. This range is obtained with conventional spray devices for dispensed liquid formulation as desired in this work. Alternatively, if the formulation has a viscosity range higher than 1500 mPas, other kinds of devices could be used.

3.1.1.5. Adhesion

Adhesion evaluation is made immediately after formulation development to guarantee an accurate result. Adhesion method was developed *in-house* for the intended use of this specific drug product since it is not described on pharmacopoeias or guidances. Adhesion measurement could be performed from either an open bottle (using few drops) or following spray actuations, depending in the way the formulation was packaged.

In order to obtain an efficient mucosal drug delivery, the release of the DS must happen together with an increase of the residence time of the formulation within the site of application, preventing the local clearance of the DS from the region. Then, the retention time of the DS at the site of absorption will promote the effective drug delivery. As results, various surface interactions between certain formulation components and mucosa surface were formed [50-51]. The interaction process is very complex and two phases can be distinguished: contact stage and consolidation stage (**Figure 25**).

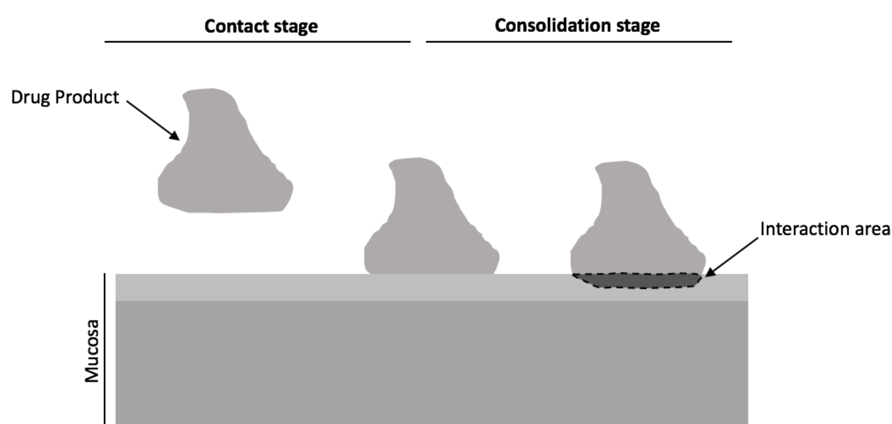


Figure 25 Schematic representation of the two stages on mucoadhesion process: contact and consolidation stage. Adapted from Smart [51].

In the first stage – **contact stage** – an intimate contact occurs between the mucosa and DP. This stage is characterized by the initial approximation of the drug product to the mucosa where it will interact. In cases where formulation is released as a spray, these two surfaces are mechanically brought together because the drug product is placed exactly on the absorption site. This stage is relatively fast and mostly dependent on the formulation composition. After this stage, both surfaces in contact initiate various physicochemical interactions to strengthen their connection leading to adhesion – **consolidation stage** [51].

The development of the adhesion method is based on the determination of the force that is required for the detachment of the surfaces, this is between probe surface (of the equipment) and formulation (that was placed above the pre-humidified artificial membrane). Furthermore, the work of adhesion and detachment distance were also determined through the developed method using Texture Analyser instrument (**Figure 26**).

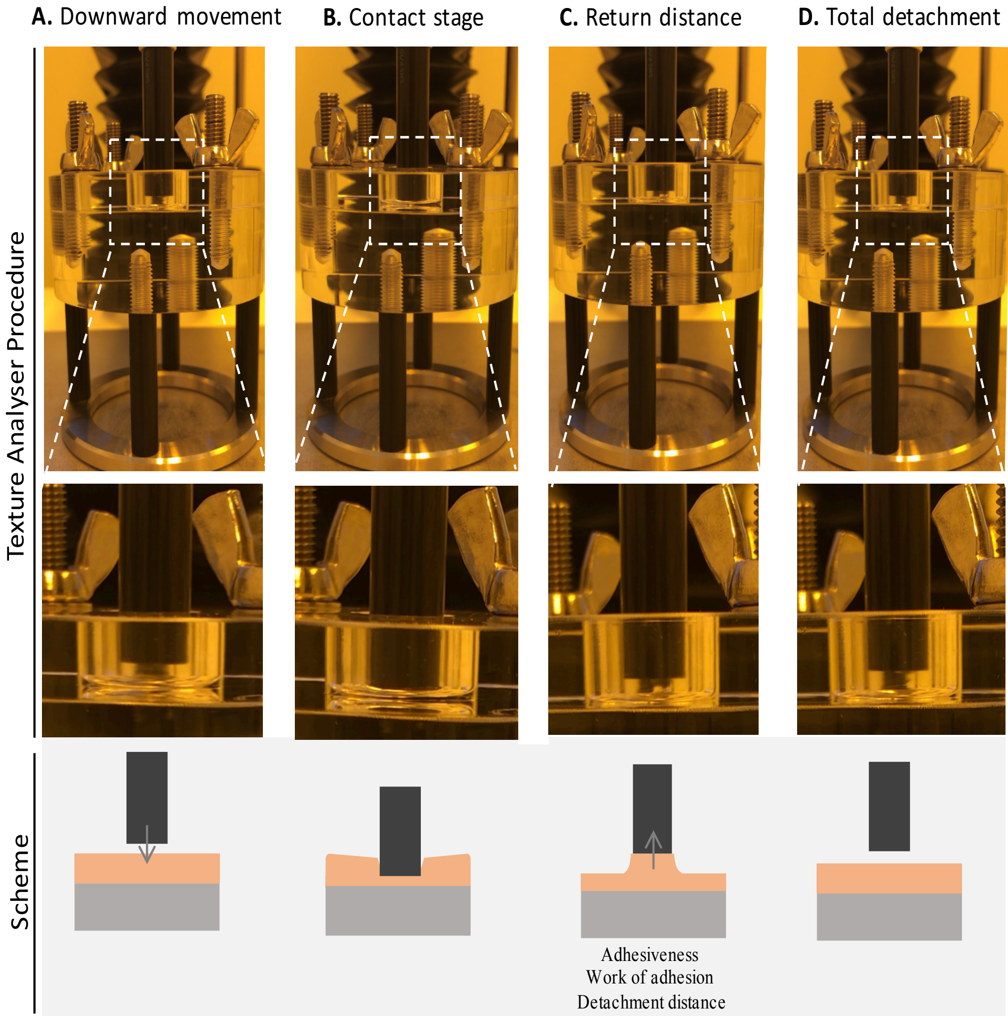


Figure 26 Texture Analyser procedure for adhesion method and schematic representation of each step. The probe initiate the downward movement (**A**) until contact stage (**B**). After 60 seconds, the probe return distance (**C**) until total detachment (**D**).

Figure 26 shows the procedure performed in the Texture Analyser for adhesion measurement accompanied by the respective schematic representation. In the first step, the probe initiates the downward movement (**Figure26A**) until the contact and consolidation stage (**Figure26B**) where the two surfaces are mechanically brought together. After a well-established time, the probe initiates the upward movement (return distance) and, as exhibited in **Figure 26C,** the formulation remained adhered to the probe during the upward movement of the probe. In this

stage, the three parameters were determined. The last step refers to the total detachment (**Figure26D)**.

The adhesion is mainly influenced by the composition of the formulation. To improve the capability of being adhesive, some adhesion enhancers could be added to the composition. This test only distinguishes the adhesion into two classes: adhesive and non-adhesive. This difference was statistically demonstrated using two-tailed T-test (95% of confidence level). Non-adhesive formulations were found when the difference between the adhesiveness of the formulation and the adhesiveness of humidified gut (no formulation presence) is below 0 g. When this difference is higher than 0 g, the formulations are classified as adhesive. In **Figure 27**, it is graphically exemplified the statistical difference between some non-adhesive formulations and adhesive formulations (P_{value}<0.001).

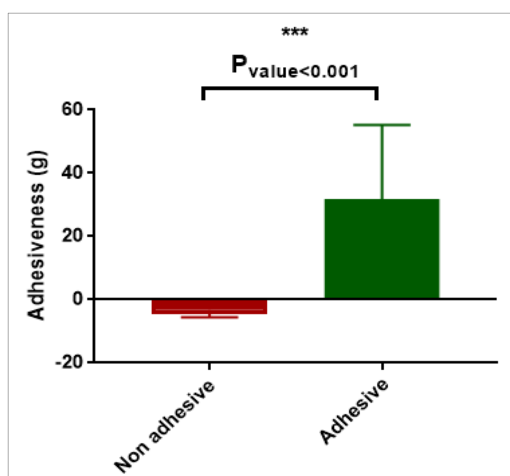


Figure 27 Statistical analysis (two-tailed T-test) to distinguish adhesive and non-adhesive formulations.

The results of this section rely on a series of experiments using specific excipients (and different percentages) known to promote adhesiveness. Overall, these experiments demonstrate which compositions, and which excipients could impact the adhesion of placebo technologies developed.

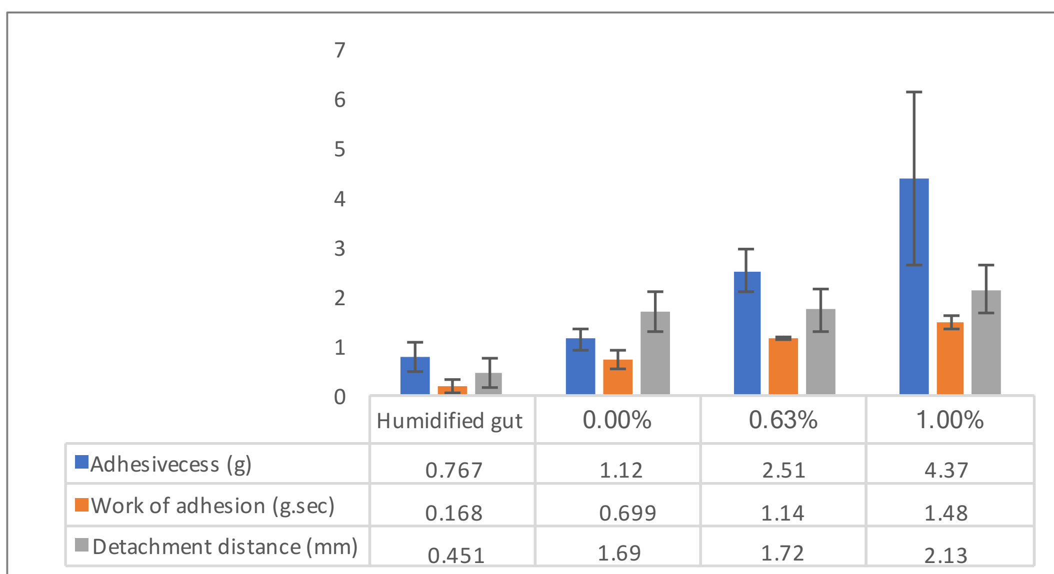


Figure 28 Impact of the increasing adhesion enhancers (0.00, 0.63 and 1.00%) on the adhesion measurement. The increasing of the adhesion enhancer increases the adhesiveness, work of adhesion and detachment distance.

Figure 28 shows the results of a study where a specific adhesion enhancer was used at different percentages. As expected, increasing amounts of this particular adhesion enhancer (undisclosed information), lead to considerable increase of the adhesiveness of the formulation. This indicates that as higher the percentage of adhesion promoters, higher the force that is required for the detachment between probe and formulation. On the literature, there are referred some attributes that could increase retention time and support the occurrence of mucoadhesive bonds at target sites, for example: ionic bonds, covalent bonds, hydrogen bonds, van-der-waals bonds and hydrophobic bonds [51].

The similar trend was observed for work of adhesion and debonding distance, highlighting the effect of such excipient in the adhesion performance of the formulations.

The selection of the adhesion enhancer agent and its amount define the adhesion of the formulation to the mucosa. Target adhesion is dependent on the type of DS release rate: the quicker the release rate, the slower the necessity of adhesion enhancers enrichment.

In **Figure 29** it is demonstrated some of the placebo formulations analyzed in this work.

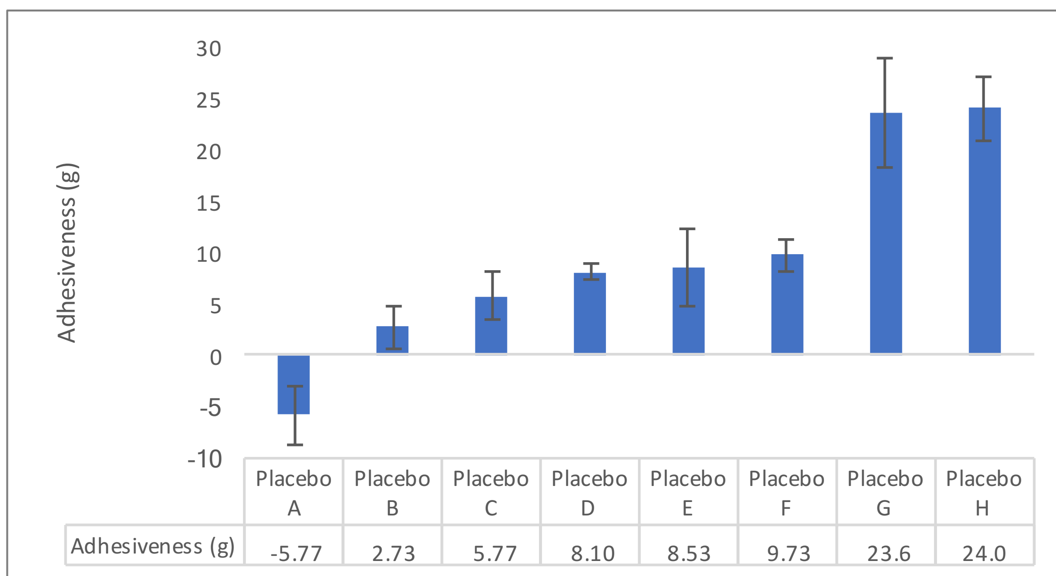


Figure 29 Adhesion measurements on different analyzed placebos. Adhesion range is within -5.77 and 24.0 g.

The adhesion measurement of this preliminary study, using different excipients, shows non-adhesive and adhesive formulations. Although the amount of adhesion enhancers being the most important factor in adhesion measurement, the compatibility between the remaining excipients also plays an important role, as demonstrated with these results. Since the results of work of adhesion and debonding distance have not shown a relationship, further studies are needed to understand them. At this time, we will only focus on the adhesiveness of the formulations.

The obtained results with the developed formulations were compared with some commercialized reference products which were selected according to the label information's related to their adhesiveness properties. For this study, 7 reference products (RP) were selected and analyzed with the adhesion method developed, as shown in **Figure 30.**

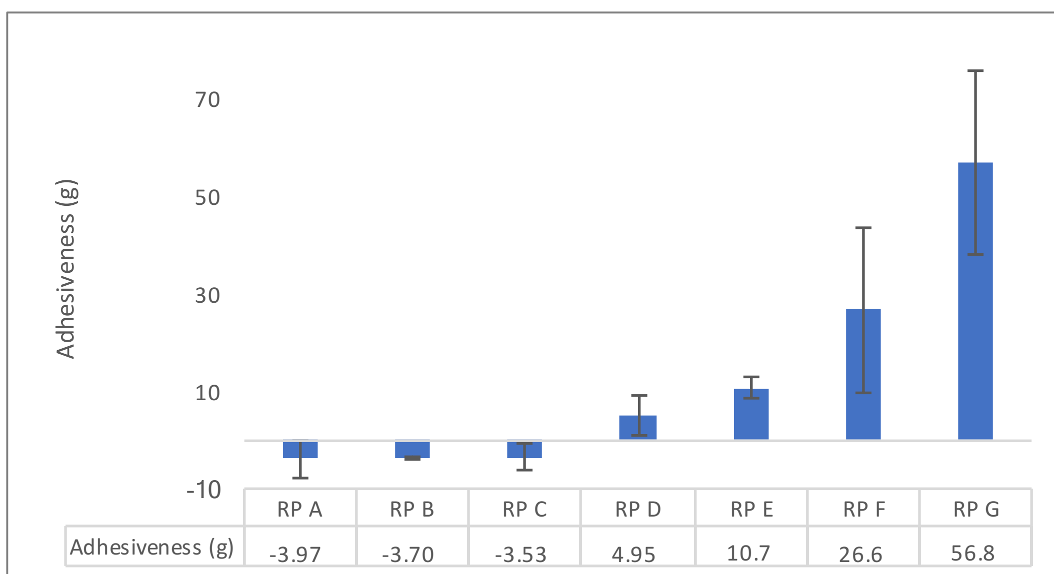


Figure 30 Adhesiveness measurement on 7 different reference products (RP) with similar properties to the ones under development. Adhesion range is within -3.97 and 56.8 g.

Figure 30 shows that only 4 reference products are classified as adhesive, according to adhesion method developed. Even though, the other 3 reference products that are described as adhesive, do not emphasize this property since it presents negative values of adhesion. The possible justifications for these measurements could be due to the low sensitivity of the developed method or due to different adhesion evaluation method used by the pharmaceutical companies.

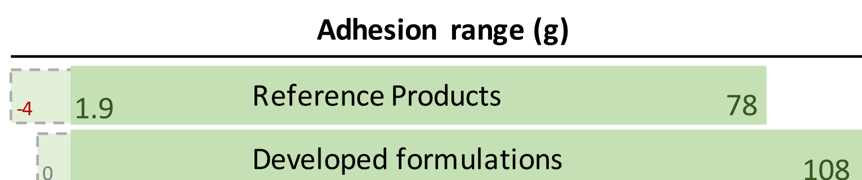


Figure 31 Adhesion ranges of reference products and developed formulations obtained with the developed adhesion method. The adhesion range of reference products is within 1.9 and 78 and within 1.9 and 108 for the developed formulations, assuming adhesive formulations.

Figure 31 demonstrates the adhesion ranges of reference products and developed formulations. The obtained ranges show that it was possible to develop adhesive formulations and, in general, it was possible to modulate adhesion to values even higher than those observed in the analyzed reference products.

3.1.1.6. *In vitro* release test

For the development of *in vitro* release test, some of the parameters were evaluated and optimized during the method development, in particular: the sampling procedure, the influence of the membrane, the pore size of membrane, the type of isolation used to prevent evaporation of solvent, the composition of the receptor medium and the amount of sample applied. The determination of these settings will support the development of an *in vitro* release test that could be used for 1) quality control of the drug product; 2) predict the performance of the drug substance once in contact with the mucosa and 3) comparison studies between different drug products.

Sampling procedure

Firstly, it was necessary to establish the practical procedures to follow. The method must be well structured in order to avoid variations in the results obtained. To decrease some variability, the sampling procedure was carefully evaluated and tested. To this, different syringes, tubes composition and length were screened and tested. **Figure 32** shows the final chosen sampling tools.

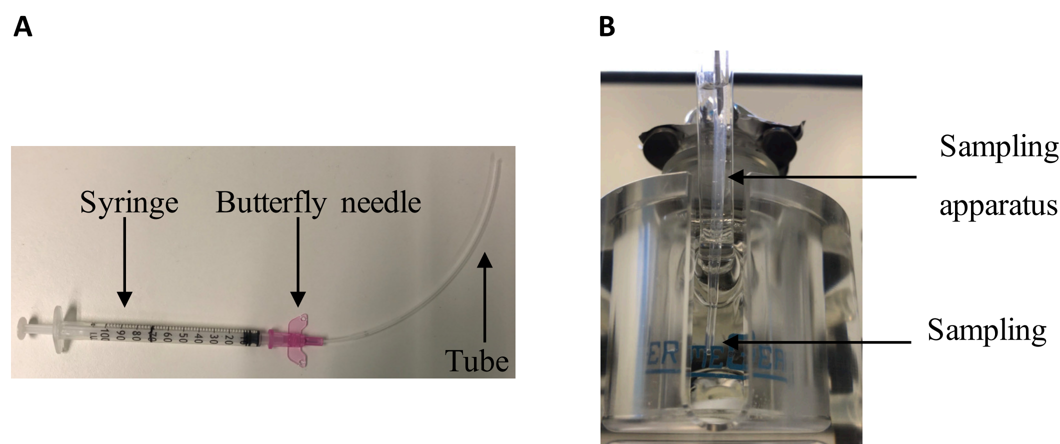


Figure 32 *In vitro* release test sampling procedure. Sampling tool used to collect samples and replace medium (**A**) and sampling procedure in the Franz cell (**B**).

This sampling tool is comprised by 1 mL graduated syringe (Omnifix 100 Solo, Braun), a butterfly needle and a dip tube with a specific length.

Concerning the sampling procedure, it is important guarantee that the sampling occurs always in the same point in the middle of the receptor chamber and at a pre-defined specific time in all the cells in order to ensure the homogeneity of the samples and reduce the variability

between the Franz cells. In addition, as the volume of the DS is smaller, any volume losses were significant for DS quantification. In order to minimize losses, we used a sampling tool, as shown in **Figure 32A**, for sample collection and another to replace the medium in each Franz cell. With this strategy, it was possible to reduce the successive dilutions that occurred during sampling and, therefore, reduce sampling errors during the experimental procedure.

Replacement of the receptor chamber is important to allow the diffusion of the sample present in the donor chamber since the artificial membrane, which separates both compartments, must always be in contact with artificial saliva.

Membrane influence

After the procedure optimization, some parameters that were identified as critical for the drug substance release were studied. Firstly, we evaluate the influence of the membrane on the release profile. Commonly, the membrane used in these tests must not act as a barrier to diffusion [36]. In order to evaluate this influence, we studied two different conditions: no membrane and with a membrane with 41 µm pore size (**Figure 33**). The selected receptor medium was MeOH/H_20 (50:50) because it has the capability to solubilize the drug substance (*sink conditions*).

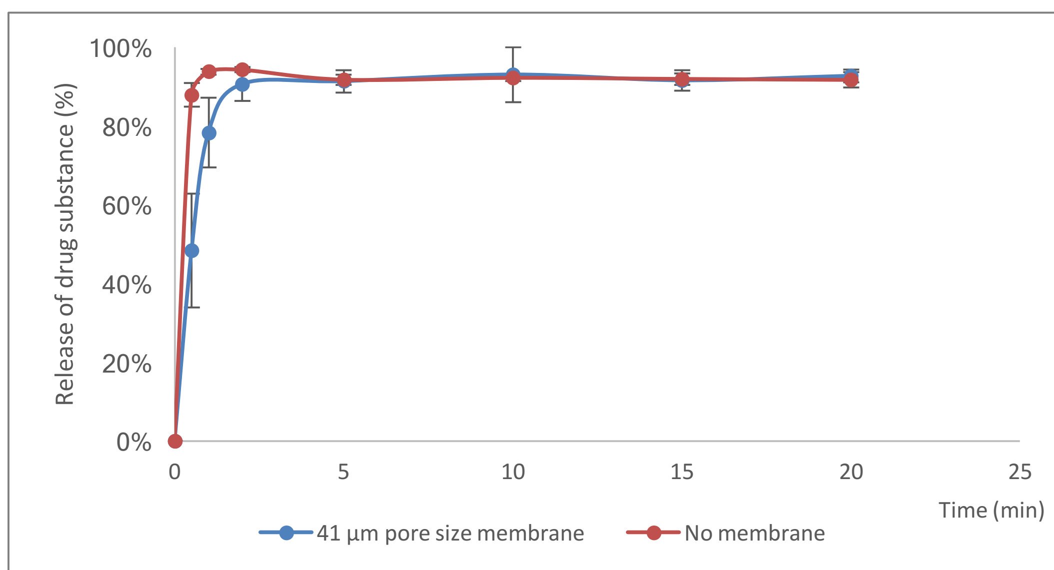


Figure 33 Membrane influence on the release profile. The study was conducted with 41 µm pore size and without membrane. Statistical analysis demonstrates statistical differences at early time points ($P_{value}<0.0001$ and $P_{value}=0.0101$ for 0.5 and 1 min, respectively) for 95% of confidence level, according to sidak's multiple comparisons tests.

The results demonstrate that in the two first time points (up to 1 minute), the drug released is significantly different (P_{value}<0.0001 and P_{value}=0.0101 for 0.5 and 1 min, respectively) for 95% of confidence level, according to sidak's multiple comparisons tests. However, in the followed time points the drug release does not show any statistical differences. The results showed that the presence of the membrane has an influence on the profile during the exponential drug release - the presence of the membrane seems to delays the release. For the intended use of this method, the presence of the membrane is important as it acts as a mechanism for distinguishing different formulations compositions.

Pore size of the membrane

The effect of membrane pore size was evaluated using different pore size membranes: 41 and 80 μm pore size. (**Figure 34**).

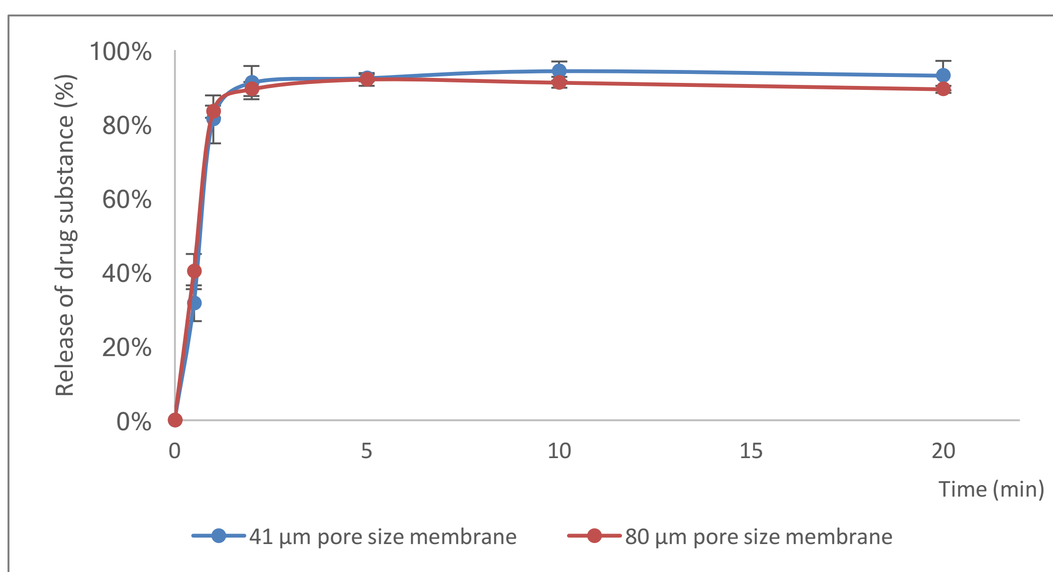


Figure 34 Pore size influence on the release profile. The study was conducted with 41μm pore size and 80 μm pore size membranes. Statistical analysis only demonstrates statistical differences up to 30 seconds (P_{value}=0.0328) for 95% of confidence level and according to Sidak's multiple comparisons tests.

The results showed that the release of drug substance is only significantly different up to 30 seconds of the analysis (P_{value}=0.0328) for 95% of confidence level and according to Sidak's multiple comparisons tests. Since this test was performed to select the ideal membrane, the choice was the lower pore size membrane since it allows a discriminatory response. This capability is crucial for formulation comparison studies. If the study is performed without any

membrane, (or with higher size pore membrane), there is a risk that the method will lose the capability to differentiate different release rate profiles.

Type of isolation

During the procedure, it is important to ensure that the system is correctly occluded, more specifically the donor chamber. In this way we could minimize possible evaporation of the volatile excipients that are present in the formulation, avoiding misleading results (**Figure 35**).

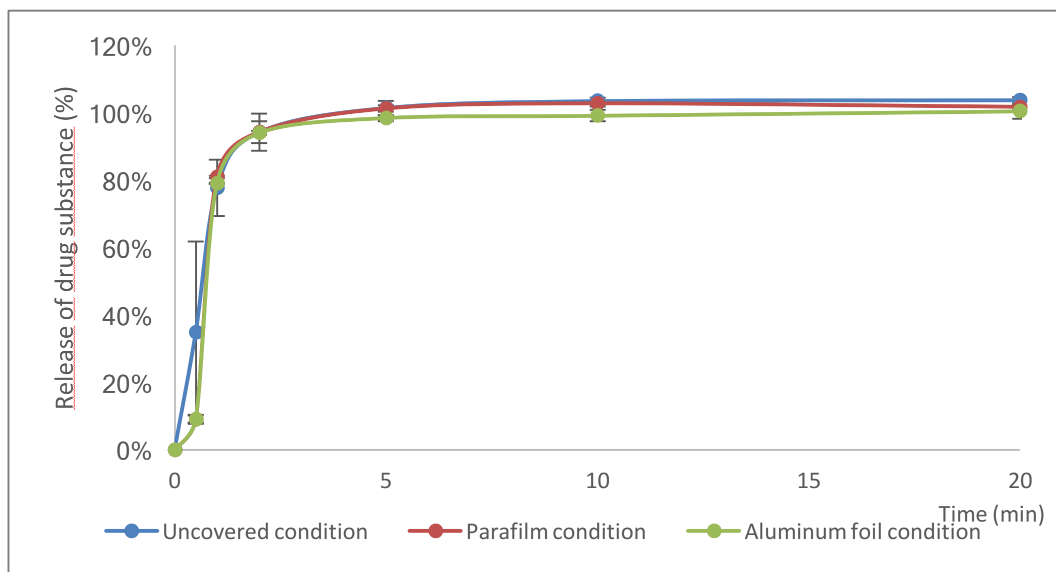


Figure 35 Isolation influence on the release profile. The study was conducted with three different isolation conditions: uncovered, with parafilm and with aluminum foil. Statistical analysis demonstrates that the condition uncovered are statistically different from the other two up to 30 seconds (Pvalue=0,0039 with parafilm condition and Pvalue=0,0041 with aluminum foil condition). Furthermore, the cells occluded with parafilm and aluminum foil do not differ statistically (Pvalue=0,9999, for 95% of confidence level).

We have tested three different isolation conditions: uncovered, covered with parafilm and covered with aluminum foil. The cells that are with the uncovered condition have a higher standard deviation at 30 seconds of analyses due to the variability of the results. The other 2 conditions demonstrate small standard deviations with similar responses, confirming that both isolations were efficient. Statistical analysis, using Tukey's multiple comparisons tests for 95% confidence level, confirm that the condition uncovered are only statistically different from the other two up to 30 seconds (P_{value}=0.0039 with parafilm condition and P_{value}=0.0041 with aluminum foil condition). The cells occluded with parafilm and aluminum foil do not differ statistically (P_{value}=0.9999, for 95% of confidence level). Importantly, we have noticed the accumulation of air bubbles below the membrane when parafilm isolation was used. This could compromise the release test (influence of further dilution/concentration or lack of homogeneity

of the sample). Therefore, for further analysis, we have used only aluminum foil and an elastic to perfectly occlude the cell.

Receptor medium composition

The medium has an important influence in the drug substance release profile as it interferes with the DS solubility. Considering the case where the drug product is for buccal application, it would be important to use the buccal environment as a model to study *in vitro* release of drug substance. Anatomical and physiological conditions of the mouth have an important role on the DS release profile. The fluid in the mouth is called saliva. The main functions of saliva are to lubricate the oral cavity, facilitate swallowing, allows carbohydrate digestion and to prevent demineralization of the teeth [52-53]. In addition to the fluid amount (0.5 – 2.0 L), its composition may also interfere with the DS solubility. In cases where the DP is water soluble, the composition of the saliva provides a favorable environment for the release of the drug due to the water rich environment [4].

Regardless of the medium selection, *sink conditions* must be ensured. To this, a wide variety of medium was evaluated in order to ensure the DP solubility. The evaluation of the *sink conditions* of the medium is based on test three and five times the work concentration of the DS. If the DS solubilize, the medium follows *sink conditions*.

The first study performed was the evaluation of the *sink conditions* with the simulated saliva (Phosphate buffer, pH 6.8). The results demonstrate that with both concentrations, the DS, as it is classified as poorly soluble (BSC II), does not solubilize in this medium (**Figure 36A**). Because of this, it was decided to test a medium in which the drug substance is soluble – Methanol:H_2O (80:20, v/v). To this, one test was performed in order to compare the release profile of the drug substance when *sink conditions* are achieved (**Figure 36B**).

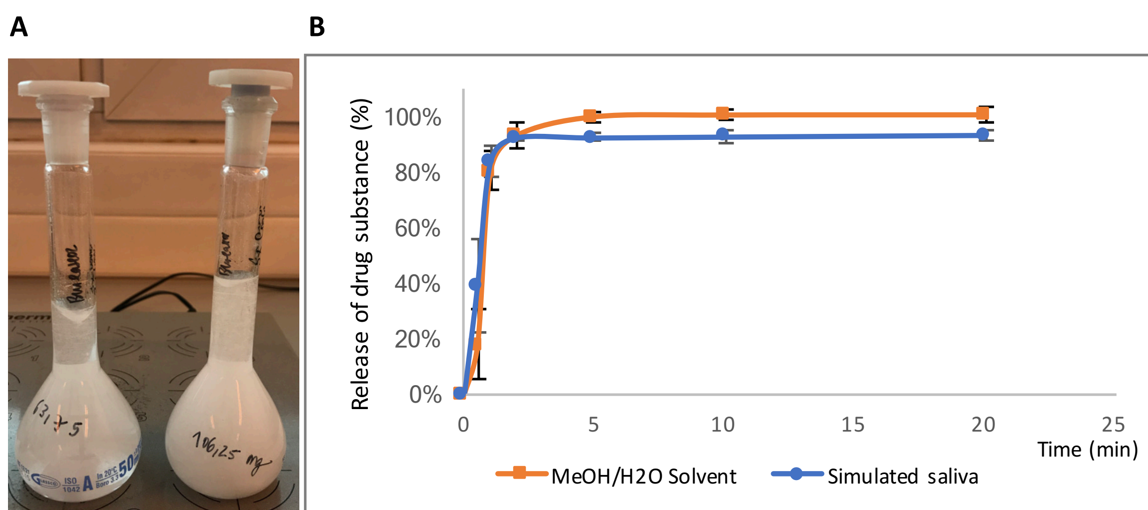


Figure 36 Influence of the receptor medium composition on the release profile. Test to evaluate *sink conditions* on the medium. The coloring with both concentrations suggests that simulated saliva do not solubilize the DS (**A**). The other study was conducted to compare different medium and the statistical analysis demonstrate that the release only differs on first 30 seconds (P_{value}<0.0001 for 95% of confidence level, sidak's multiple comparisons tests).

The results demonstrate that both release profiles of the drug substance are similar. It was initially verified a "burst" increase of the release rate followed by a constant release at 2 minutes. The statistical analysis of the results, using sidak's multiple comparisons tests, demonstrate that the responses only differ in the first 30 seconds of the analysis (P_{value}<0.0001 for 95% of confidence level). Since the differences are only at the beginning of the test, the medium selection for the following tests was the bio-relevant medium – simulated saliva.

Amount of sample

The amount of sample applied is an important parameter for the release rate profile because has an impact on the kinetics of the DS release. As previously referred, an infinite dose is preferred over a finite dose as it simplifies the diffusion kinetics. If the amount is finite, the graphic of the amount of drug substance released as function of the time, will be constant after certain time interval (slope equal zero) contrary to the other infinite dose. United States Pharmacopoeia mentioned that the amount of sample recommended is approximately 1.0 mL/cm^2 or 1.0 g/cm^2 (in which the area of the cells used is 1.77 cm^2). The addition of a small excess of sample to a donor chamber - infinitive dose - assume that the release is not rate-limiting so the permeant leaves the donor chamber and it is replaced by molecules entering the solution from the formulation excess [54].

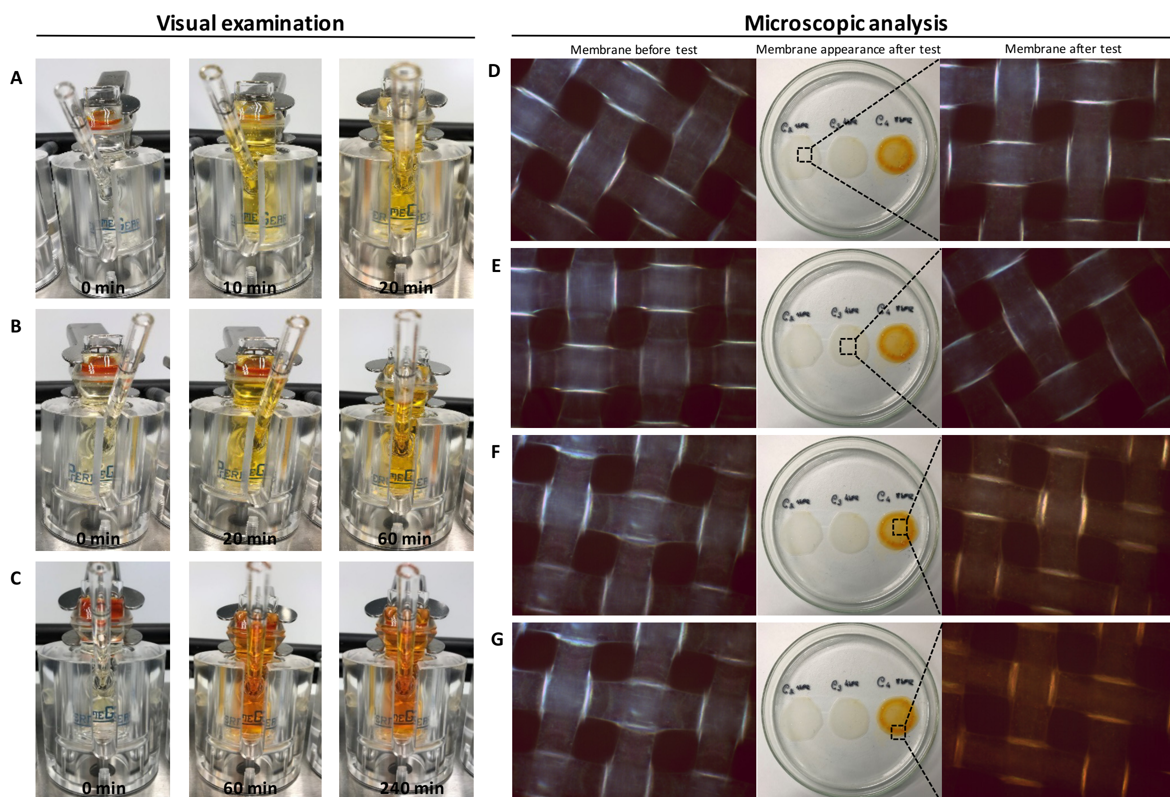


Figure 37 *In vitro* release test using a coloring placebo formulation. A visual examination of the formulation diffusion through the membrane was observed when 1, 4 and 8 SPR were applied on the donor chamber (**A**, **B** and **C**, respectively). Membrane before test (left side) and the membrane after *in vitro* release test (right side) was also inspected by optical microscope: 1 SPR (**D**), 4 SPR (**E**), 8 SPR on center of the membrane (**F**) and 8 SPR on the border of the membrane (**G**). Membrane appearance after *in vitro* release test was also visual examined (middle panel **D**, **E**, **F**, **G**).

In order to visually analyze the diffusion of the donor chamber, a study was performed with a placebo formulation containing a yellow dye. For such, different amounts of formulation (1, 4 and 8 SPR) are applied in donor chamber and a membrane with 41 µm pore size was used**.** The results show that when 1 SPR (pump that releases 180mg/SPR) is applied, all drug substance seems to have been released after 20 minutes of the analysis because visually there is no evidence of formulation on the donor chamber **(Figure 37A).** The results with 4 SPR demonstrated that all drug substance seems to have been released after 60 minutes of the analysis because visually there is no evidence of formulation on the donor chamber (**Figure 37B**). Finally, when it was applied 8 SPR, the yellow color never disappears totally from the donor chamber until 240 minutes (**Figure 37C**).

Following each test, we made a visual inspection of the membrane and the lack of visible coloring in the membranes of the tests with 1 and 4 SPR seems to demonstrate that the diffusion of the placebo formulation occurred entirely (middle panel of **Figure 37D and Figure 37E**). Besides that, the color photograph with 8 SPR revealed that the membrane remains with

visible yellow color which indicates that part of the formulation was still retained within the membrane (middle panel of **Figure 37F**).

These results were proved when, after each test, the membrane was inspected by an optical microscope and compared with the membrane before test (humidified membrane - without sample applied). The results obtained with 1 and 4 SPR showed that the formulation diffuse through the membrane and do not stay arrested on the membrane (**Figure 37D and Figure 37E**). Otherwise, when we used an infinite dose (8 SPR) of placebo formulation, it seems that the formulation stay arrested on the membrane because it had some coloration that did not exist on the humidified membrane (**Figure 37F**). Despite this, this coloration is more intense on the border when compared with the center of the membrane which indicates that the sample is diffused in the middle of the membrane (**Figure 37G**). Sample deposition on the membrane periphery could be justified by the positive meniscus of the receptor chamber medium.

Beyond these qualitative tests, a quantitative test was also performed using a finite (1SPR) and infinite (8SPR) dose of DP. **Figure 38** demonstrates the obtained release profiles for both volumes.

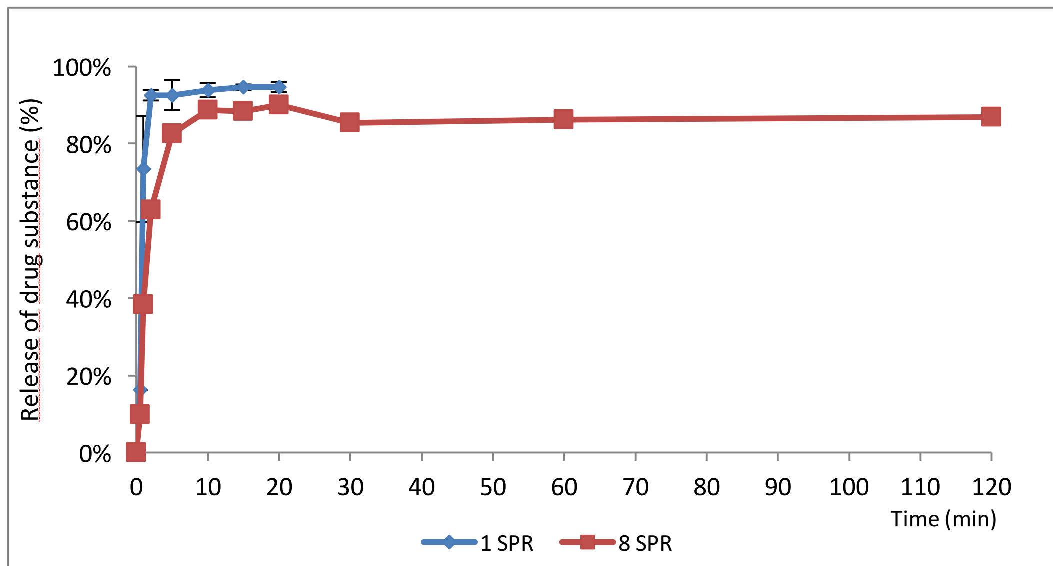


Figure 38 Sample amount influence on the release profile. The study was conducted using a finite (1SPR) and an infinite (8SPR) dose of DP and the result demonstrates that the differences only exists at early time points.

The results demonstrate that the responses are statistically different (**Figure 38)** at early time points. When the amount of formulation is infinite (8 SPR) the drug substance released is slower when compared with 1 SPR of formulation (finite doses). With the infinite dose, the release

rate at the beginning of the test is more discriminatory. Nevertheless, the higher amounts appeared to lead to sample losses due to sample evaporation and visible adhesion of the cell wall of donor chamber. This phenomenon was observed at longer time points (after 20 minutes), where a decrease of drug release was also observed.

This test allowed to conclude that using only 1 SPR the diffusion totally occurs without formulations losses by evaporation and retention on the membrane. However, the use of higher sample amounts is not totally discarded for further studies when, for example, the kinetics of the reaction is studied. At this early stage of the study, it was only intended that the method be able to distinguish between different formulations compositions and the use of 1 SPR seems to be sufficient.

In vitro release test for comparison studies

One of the applications of this study was the capability to distinguish different formulation compositions. Thus, initially we define the parameters for this test as follows:

- Receptor medium: simulated saliva
- Membrane: Nylon 41 µm pore size
- Amount of sample: 1 SPR
- Type of isolation: Aluminum foil

After method development, the performance of this method was tested with the defined parameters above, to distinguished the different formulation compositions. Each formulation corresponded to different prototype developed internally and for a confidential reason, the composition cannot be disclosed.

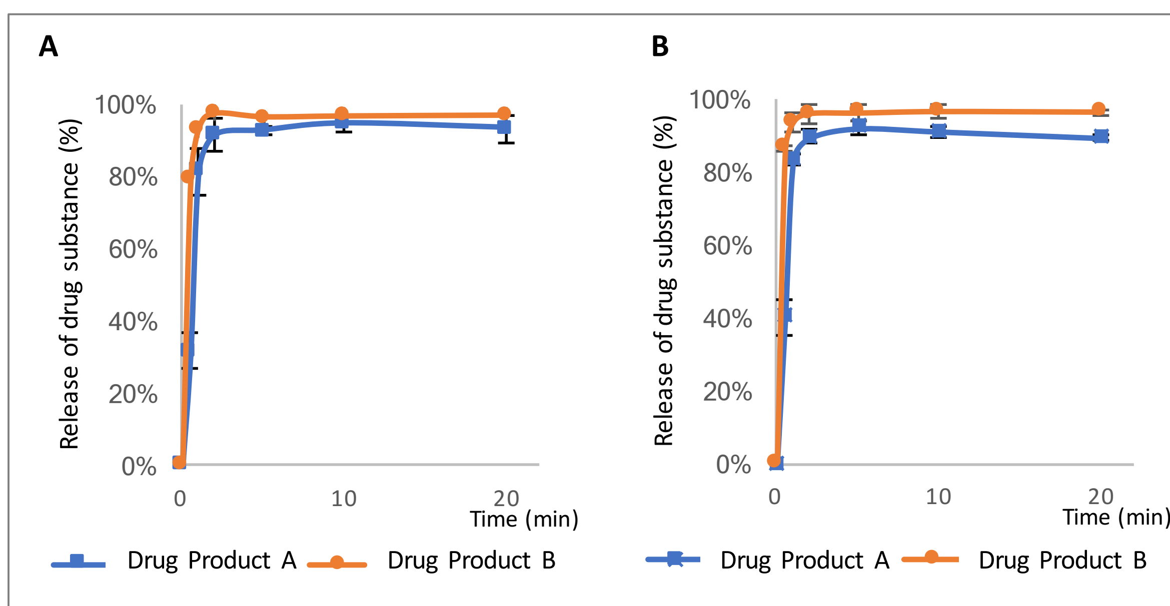


Figure 39 Comparison between *in vitro* release test profiles using a 41 µm pore size membrane (**A**) and 80 µm pore size membrane (**B**). Different drug products were studied in both conditions. The results demonstrate that in the two first time point of the study (**A**) (up to 1 min), the membrane with 41 µm pore size statistically discriminated both formulations (0.5 min with Pvalue<0.0001 and 1 min with Pvalue=0.0008). The study (**B**), with 80 µm pore size, shows that the differences are only at 30 seconds (Pvalue<0.0001, for 95% of confidence level, sidak's multiple comparisons tests)

We have tested both drug products with membranes with 41 µm pore size (**Figure 39A**) and 80 µm pore size (**Figure 39B**) to evaluate the discriminatory property of the developed method. As shown in **Figure 39**, the drug product A demonstrate, in both pore size membranes, less drug substance released when compared with drug product B. This difference was likely related to the formulation compositions of each DP. As expected, the use of a membrane with a lower pore size allows a more discriminatory response. It was found that in the two first time point of the study (up to 1 min), the membrane with 41 µm pore size statistically discriminated both formulations (0.5 min with $P_{value}<0.0001$ and 1 min with $P_{value}=0.0008$, 95% confidence level). The same conclusion is also observed with 80 µm pore size but only up to 30 seconds ($P_{value}<0.0001$, for 95% of confidence level and sidak's multiple comparisons tests). Accordingly, these results supported our previous findings that the 41 µm pore size has the capability to better distinguish different formulation compositions.

Considering the purpose of *in vitro* release test, the developed method meets custom requirements as we could discriminate formulations with different compositions. In addition, it was also observed a rapid release of the DS into the medium in both DP analyzed.

3.1.2. Spray performance

3.1.2.1. Spray actuation test

The evaluation of the spray actuation is based on the registration of the forces that are required for the release of the formulation through the spray nozzle. There are two main resistance forces that can be identified during the actuation of the device: actuation force (ActF) and maximum force (MaxF) [41]. Actuation force is the force required to initiate the formulation release, i.e., the spray device starts dispensing. Maximum intensity force represents the maximum force applied to the spray device during the release of the formulation (near the end of actuation). Both forces are related not only to physical characteristics of the formulation to be dispensed (viscosity for example) but also to intrinsic features of each type of spray device (different suppliers use different material composition and possess different physical features), requiring different settings to establish the method.

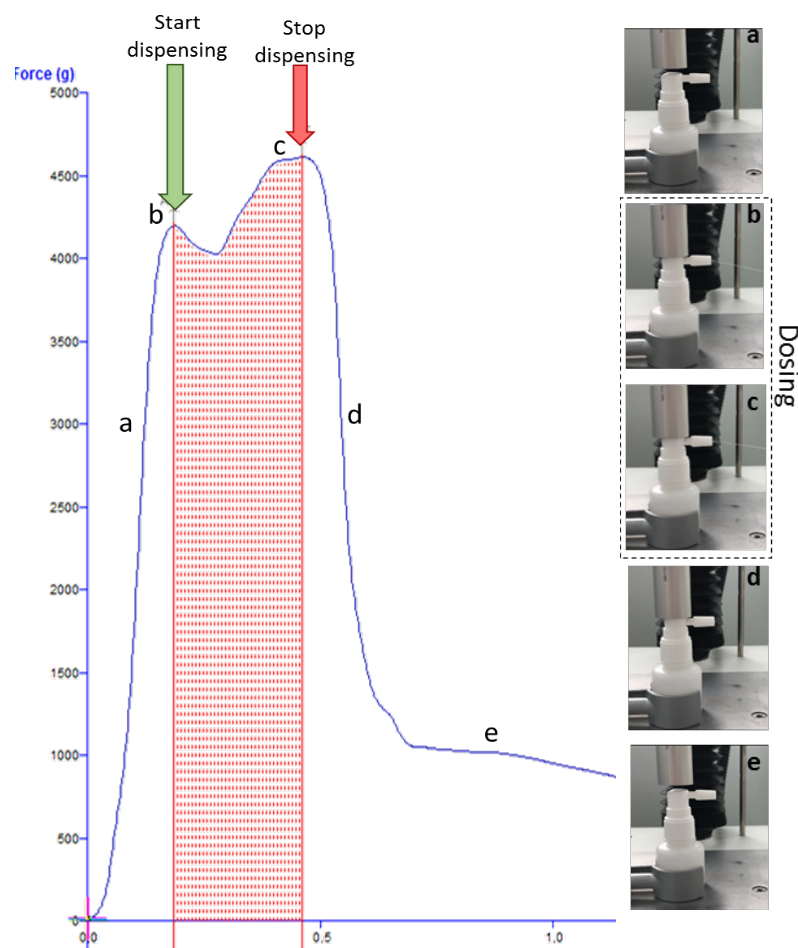
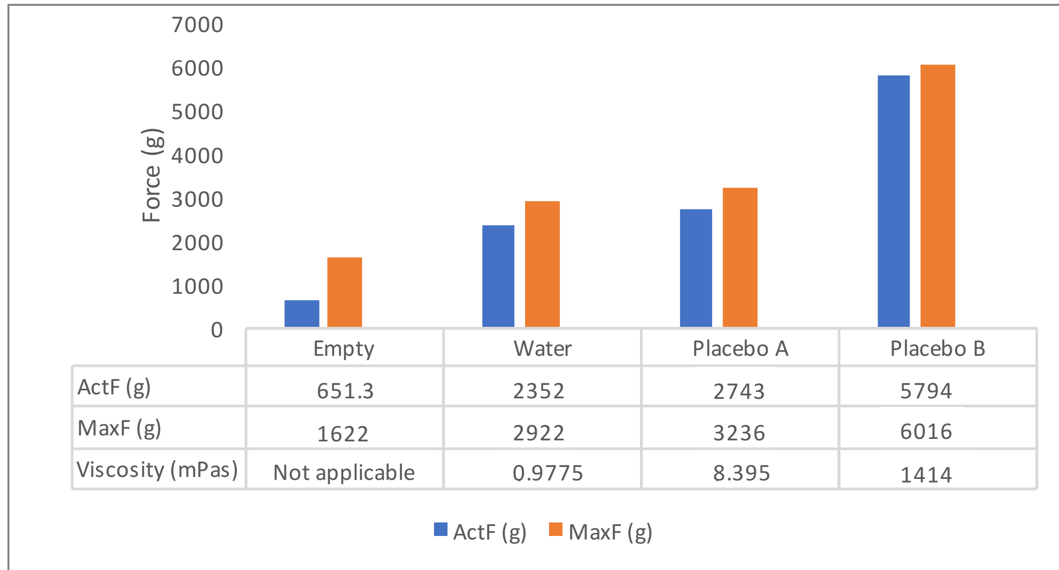


Figure 40 Representation of spray characterization using Texture Analyser. Actuation force corresponds to the "start dispending" and the maximum force corresponds to the "stop dispensing". Spray duration is the red zone between the peaks.

Texture Analyser is a specialized equipment that allows the measurement of actuation and maximum intensity forces, both identified during the spray actuation test. **Figure 40** shows a schematic representation of how Texture Analyser works and gives an example of the force profile obtained. Throughout the test, Texture Analyser applies a continuous force on the top of the spray device, mimicking the human use of the device, but under controlled settings. As can be observed, initially, Texture Analyser detects increasing resistance forces, up to a first peak (**Figure 40a**). This corresponds to the increasing force required to start dispensing the formulation (filling of dip tube and spray housing), although not yet released from the device. After this initial step, the Texture Analyser detects the first peak of force that corresponds to the detection of the actuation force (**Figure40b**). At this point, the formulation starts being released from the device. From this point on, Texture Analyser detects increasing resistance forces that corresponded to the formulation being dispensed through the nozzle of the device. During the actuation, there is a specific point where Texture Analyser detects the second peak, corresponding to the maximum intensity force required to push out the formulation (**Figure 40c**). From this point on, the resistance force detected abruptly decreases (**Figure40d),** indicating that actuation was reaching the end (**Figure40e**).



	Empty	Water	Placebo A	Placebo B
ActF (g)	651.3	2352	2743	5794
MaxF (g)	1622	2922	3236	6016
Viscosity (mPas)	Not applicable	0.9775	8.395	1414

Figure 41 Viscosity influence on actuation and maximum forces.

To evaluate the influence of the formulation composition on actuation and maximum intensity forces, two different formulations (placebo A and placebo B) were studied and compared. **Figure 41** graphically represents the required forces when the spray device was tested empty, with water, with placebo A or filled with Placebo B. Each of these samples had a specific viscosity (as showed in **Figure 41**), and the results demonstrate that as higher the viscosity of the formulation, higher the actuation and maximum forces required. These results were in accordance to the observed when samples were actuated manually, where higher viscosity formulations required more "effort to press" the actuator with the finger. This test was performed on the Texture Analyser using the same spray device and pre-defined settings. The viscosity of both placebos tested is within the range of viscosity that allows the ability to be released as a spray (11 until 1500 mPas).

Different suppliers, with the same formulation were evaluated in order to understand the influence of the type of spray device on spray performance. The spray performance of 4 different suppliers was studied and compared to a commercialized reference spray product (**Figure 42**).

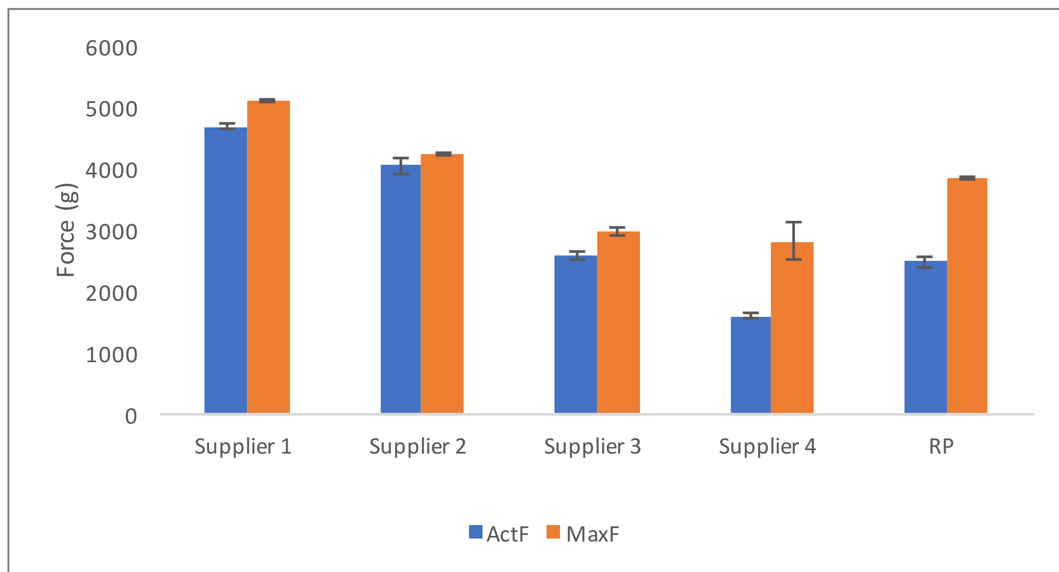


Figure 42 Actuation force and maximum force of different suppliers and reference product (RP).

The results show that depending on the type of spray device supplier, the actuation and maximum forces varies from 1598.90 to 4687.70 g and 2824.90 to 5113.50 g, respectively (**Figure 42**). These extended ranges are due to the intrinsic characteristics of the spray device since the formulation is the same in the 4 different sprays analyzed. The spray device features may be, for example: dispensed volume, closing system of spray device and the material constituting the spray. These different characteristics lead to the definition of settings for each type of spray device, which justifies the observed differences. It is important to note that besides the challenging in comparing the results, the obtained results with commercialized reference spray product are within the ranges specified above obtained from the suppliers analyzed.

Doughty et al. [55] made a study where he determined and compared adults and pediatric settings on nasal spray products and the maximum force obtained by adults are 5.82 ± 1.40 kg. Both results demonstrated in **Figure 41** and **Figure 42** are within this adult maximum force which indicates that these DP under development are suitable to use.

3.1.2.2. Pump Delivery/Shot weight

Pump delivery evaluates the weight delivered after the actuation of the spray device. The acceptable criteria established by FDA authority limit weight delivered after each individual actuation to be within 15% of the target weight claim in the label of the product. Moreover, the mean weight delivered should be within 10% of the target weight [12]. The spray devices suppliers specify the amount of sample (by weight) that is released per actuation, normally using water, at 25 °C. Nevertheless, this value is only indicative and slightly differs from formulations to formulation.

Figure 43 shows an example of a pump delivery study, obtained through sequential controlled actuation (force, velocity and distance) by texture Analyser. The results were obtained using a spray device containing 5 g of a specific formulation with a density of 0.967 g/cm^3.

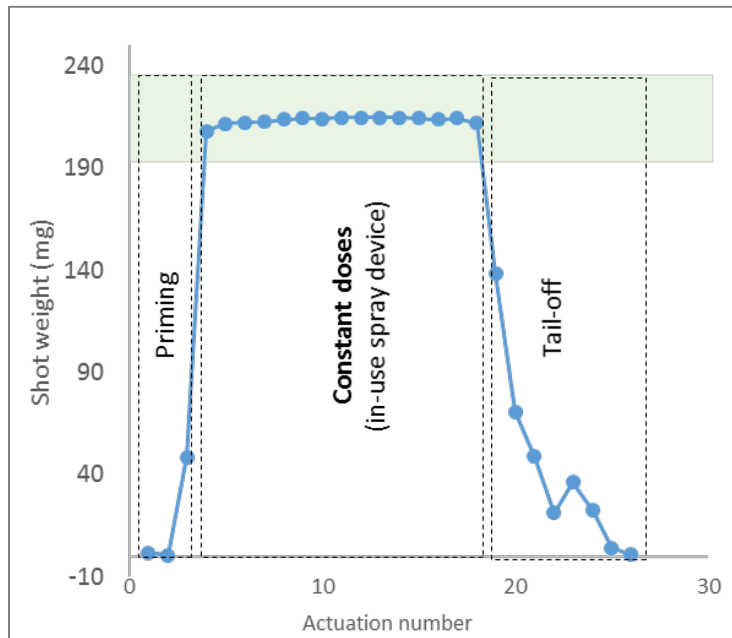
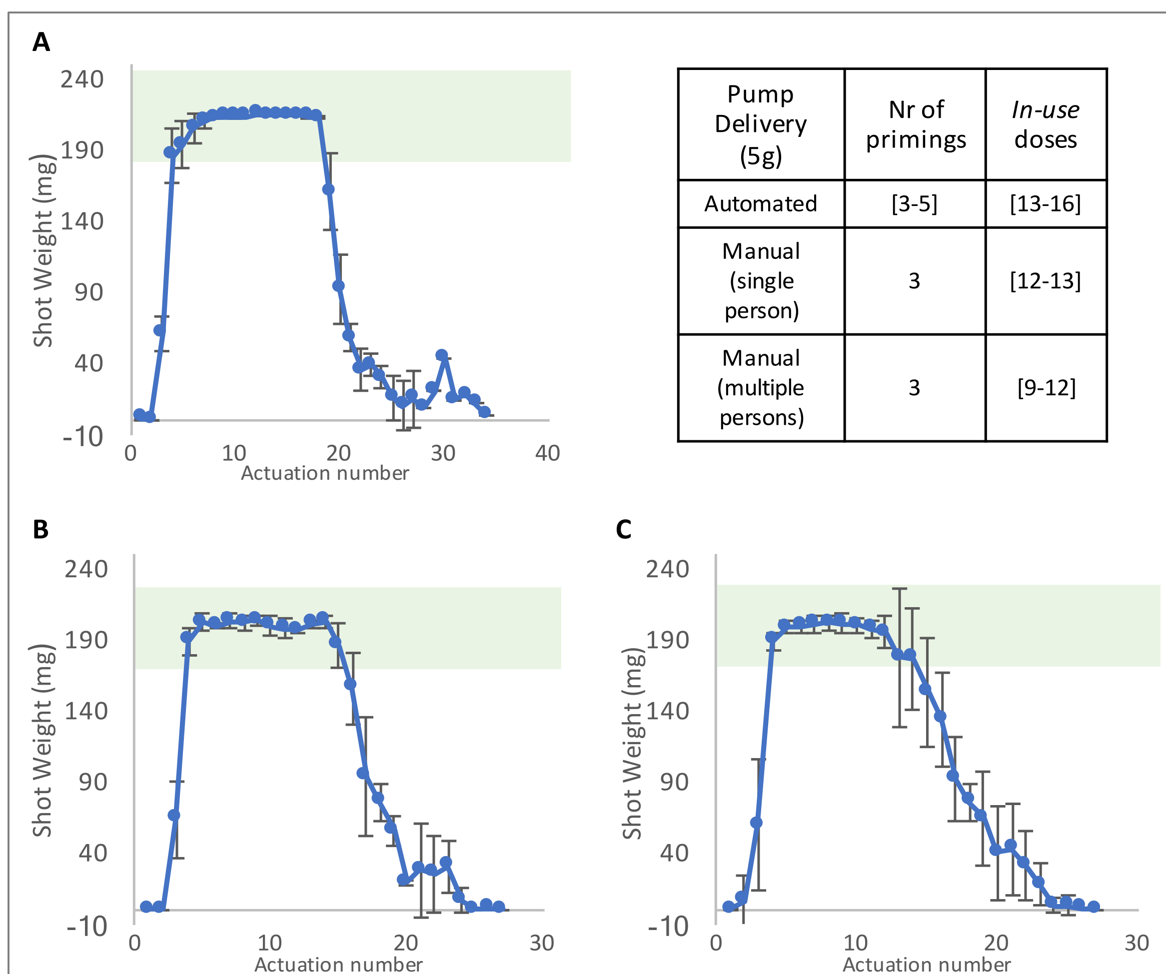


Figure 43 Pump delivery with 5 g of formulation. Priming actuations, constant doses and tail-off characteristics are represented.

The results demonstrate that after the initial actuations ("priming actuations"), the shot weight is constant during 15 actuations and it is within the acceptable limit defined by FDA authority [12] ("in use" actuations). Near exhaustion of the device, the weight delivered decreases abruptly to a point that stops releasing formulation (named "tail-off"). From the initial 5 g of formulation, 3.2 g of the formulation was released and around 1.8 g remained un-usable within the device. The mean of the shot weight obtained was 213.36 mg.

It is important to note that in order to accurately control each actuation, a specialized equipment was used – Texture Analyser – where force, velocity and distance were controlled during actuation of the device. Some studies were also performed to compare automated pump delivery using Texture Analyser and pump delivery performed manually (**Figure 44**).



Pump Delivery (5g)	Nr of primings	*In-use* doses
Automated	[3-5]	[13-16]
Manual (single person)	3	[12-13]
Manual (multiple persons)	3	[9-12]

Figure 44 Impact of automated and manual settings on spray performance. Automated settings using Texture Analyser (**A**); Manual settings but single person (n=3) (**B**) and manual settings but multiple person (**C**).

The results suggested that for the priming actuations, Texture Analyser requires between 3 and 5 actuations (**Figure 44A**) whereas manual activation only requires 3 actuations (**Figure 44B** and **Figure 44C**). This difference could be related to a more intense force applied manually on the actuation of the device. As expected, during the constant doses, the actuations with Texture Analyser (controlled settings) present lower inter variability (small standard deviation). Moreover, with controlled actuations, the number of constant actuations was slightly higher when compared with manual actuation (13-16 vs. 9-13). Overall, we could notice that the pump delivery slightly varies from individual to individual, being clear that with a single person (**Figure 44B**) the obtained results become less variable when compared to a multiple person (**Figure 44C**).

Quantification of drug substances is out of the scope of this thesis. Nevertheless, it is important to note that pump delivery only evaluates the total dose weight released in each actuation and it is also important to guarantee that the amount of drug substance in each of them

is the same. In the cases where the product is a solution, which guarantees the homogeneity of the medium, the amount of drug substance in each actuation can be estimated. However, tests like delivery dose uniformity (for multiple use spray), uniformity of dosage units (for single use spray) and mean delivered dose are required by the regulatory authorities.

3.1.2.3. Priming

The number of actuations that is required to discard to activate the spray device is determined during the priming test. This test corresponds at the stage before the patient uses the DP for the first time in order to guarantee that the dose used by the patient is that described in the drug product label. The influence of different spray devices suppliers on the number of primings was evaluated (**Figure 45**).

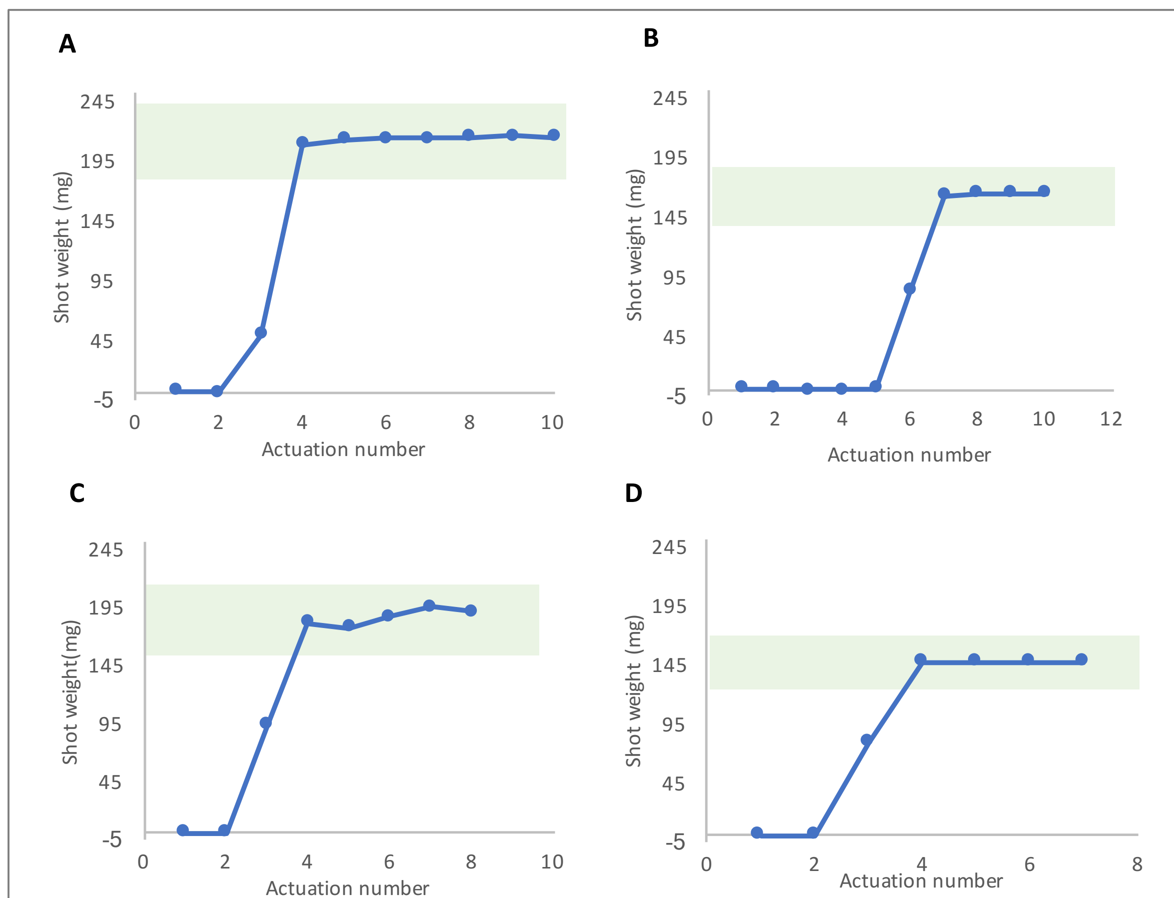


Figure 45 Priming on different suppliers (**A**, **B**, **C** and **D**). It seems to be necessary to discard 5 to 6 actuations before the first administration to the patient.

The priming test was performed with different suppliers but with the same formulation, using automated actuations. The criterion for selecting the number of actuation to discard is made

based on the pump spray weight delivery acceptable criteria mentioned by FDA that should control the weight of the individual sprays to within 15% of the target weight [12].

Generally, all suppliers presented similar profiles. The results show that it seems to be necessary to discard 3 SPR since they are below the range referred above (**Figure 45A**, **C** and **D**). However, in Figure **45B**, it seems to be necessary to discard 6 SPR before the first administration to the patient. Whereas the same formulation was used, these results show some variability that occurred due to different spray devices suppliers tested.

A comparative study was also performed with two commercialized reference spray products (**Figure 46**).

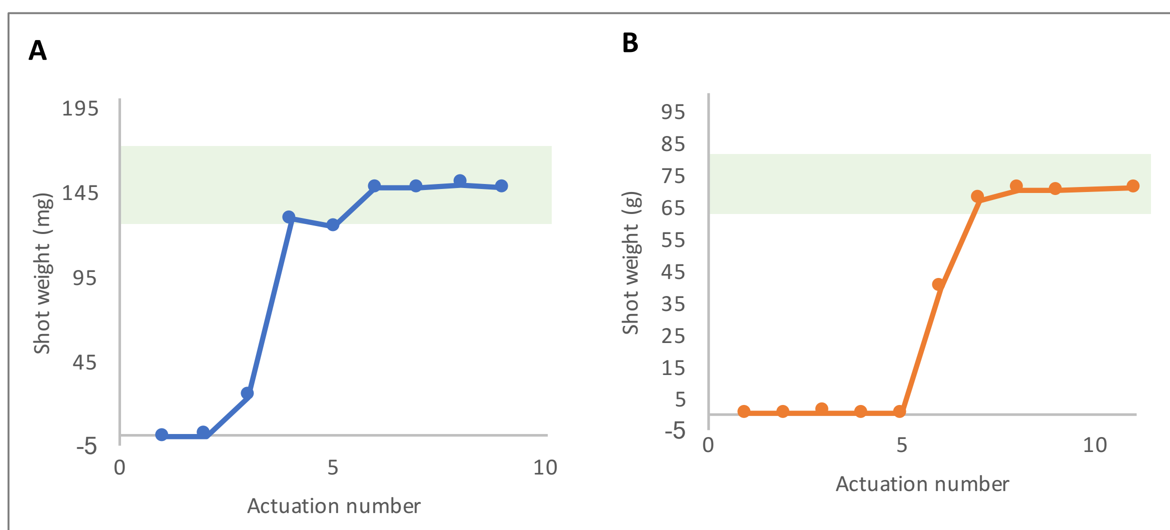


Figure 46 Priming actuations on different commercialized reference products. It seems to be necessary to discard 5 to 6 actuations before the first administration to the patient

The results obtained show that it seems to be necessary to discard 5 to 6 actuations before the first administration to the patient. These results are similar from the analyzed suppliers with the developed formulations (**Figure 46**).

The priming information is crucial to include on the drug product label claim in order to ensure the correct usage of the DP and to achieve therapeutic effect.

3.1.2.4. Number of doses/actuations

Another parameter that was referred by the regulatory authority to accomplish the drug product label is the number of doses, that will depend from drug product to drug product.

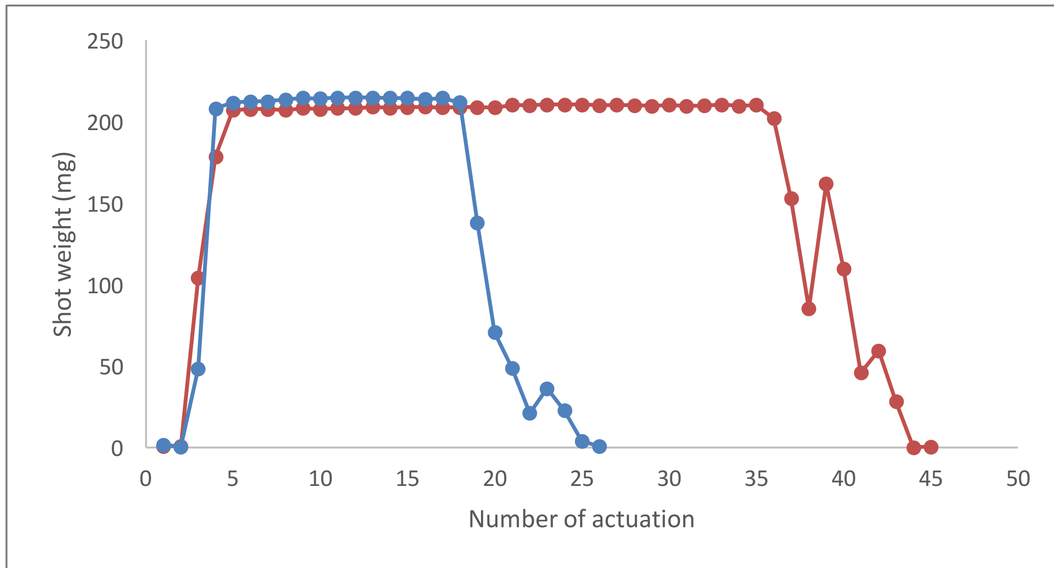


Figure 47 Number of actuation on containers with different formulation amounts (containers filled with 5 g and 10 g).

Figure 47 shows the pump delivery profile of 2 spray devices filled with 2 different amounts of formulation. Both spray devices were actuated using Texture Analyser until the complete exhaustion of the content. The results show that for 5 g of formulations, the constant doses were 15 actuations and for 10 g was 32 actuations (**Figure 47**).

It is important to take into consideration that for counting the number of doses present on the DP, the actuations using for priming and repriming as well as the tail-off doses must be discarded. In order to ensure that the formulation amount used to fill the spray ensures the constant doses mentioned on the DP label, minimum fill test should be performed.

3.2. Pre-Stability studies

3.2.1. Characterization of the formulation

3.2.1.1. Definition/Description/Appearance

Some formulations samples were stored and re-analyzed over time. When the formulations were packaged on a spray device, the evaluation of the spray performance, features and integrity of the packaging system as well as the formulation appearance are required. When it was packaged on a glass bottle, we visually evaluate the appearance of the formulation.

Figure 48 Appearance of spray device that fails during non-formal pre-stability study.

Figure 48 represents an example of a visual examination of a spray device that fails during pre-stability. The damage occurred during the evaluation of the spray performance wherein a portion of the spray nozzle was expelled. Therefore, the spray was discarded from the pre-stability study.

The appearance of the formulation was also investigated. In **Figure 49** the appearance of two different formulations packed in amber bottles was compared. The first formulation (**Figure 49A**) stay stable remaining clear and without foreign particles over 12 months at room temperature and it was classified with "1.00". In opposition, the other formulation (**Figure 49B**) is jellified on the bottom of the flask over 12 months. This may be due to some excipients incompatibility and, consequently, it is classified with "0.00" (zero).

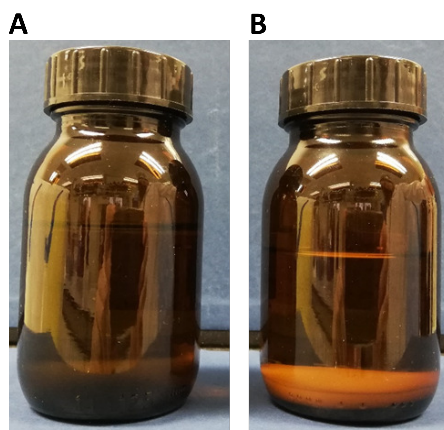


Figure 49 Appearance of different formulations. Normal appearance (**A**) and Sedimentation/solid jellified (**B**).

3.2.1.2. pH

pH monitoring is critical not only because it is a stability indicator of the composition, but also because it is involved in the drug product performance as well as in local tolerability. **Figure 50** presents an example of a pH comparison between two different placebo formulations during a non-formal pre-stability study (at room temperature). The difference between them is the composition (undisclosed).

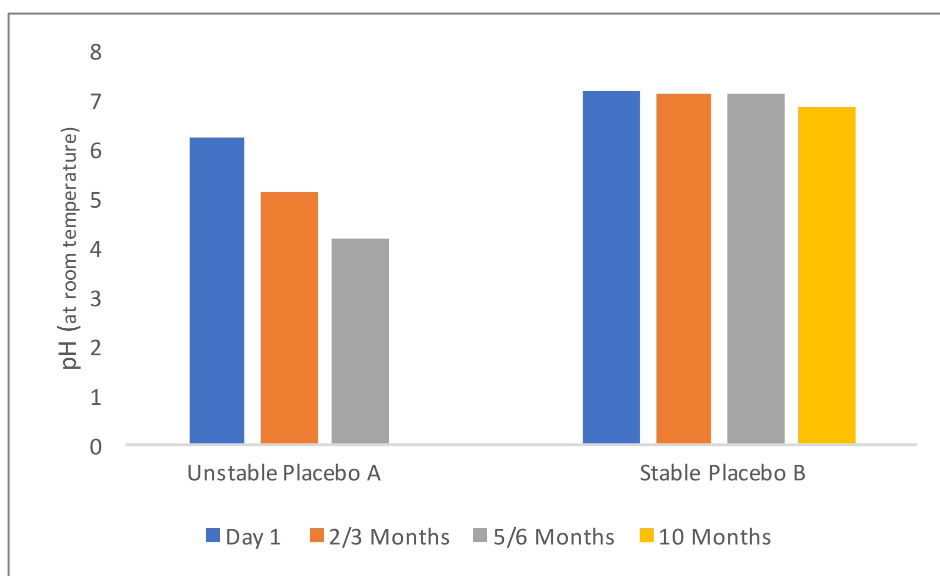


Figure 50 pH measurement of two placebo formulations over time. Placebo A corresponds to an unstable placebo formulation and Placebo B to a stable placebo formulation.

Placebo A depicts a case of an unstable placebo formulation which is, most probably, due to an incompatibility between the excipients used, since it was verified in the initial months. Contrary to placebo A, placebo B report to a stable example (7.1 ± 0.2). In this case, the pH of the formulation remained stable at least for 10 months.

3.2.1.3.Viscosity

As previously referred, the viscosity has high importance for verify formulation stability and to check the capability to being released as a spray over time. **Figure 51** shows an example of a non-formal pre-stability study carried out with different compositions of technologies placebo (undisclosed).

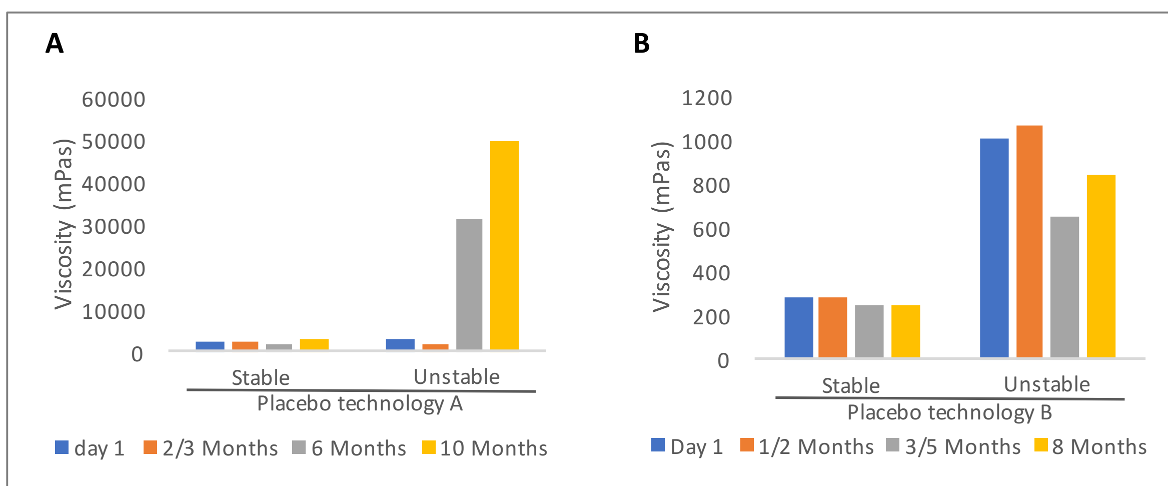


Figure 51 Viscosity measurement on different placebo formulations over time. Placebo technology A (**A**) and placebo technology B (**B**) have stable and unstable formulations.

The results show that both types of placebo technologies have stable and unstable formulations. The formulations are defined as stable or unstable if the viscosity values remain relatively constant over time or if the viscosity increases or decreases sharply, respectively. In the first case (**Figure 51A**) the obtained viscosity values were 2242 ± 580 mPas and 21478 ± 23311 mPas for stable and unstable placebos, respectively. The obtained results for the placebo technology B (**Figure 51B**) was 260 ± 21 mPas for stable placebo and for unstable was 889 ± 189 mPas. The lack of stability can be observed by the high standard deviations. The main cause for the instability of formulations is the jellification caused by loss of volatile solvents or even some interaction formed between the formulation excipients.

3.2.1.4. Adhesion

Adhesion measurement over time is critical to ensure an effective drug delivery through mucosa during drug product shelf life. **Figure 52** shows two representative examples of formulations in which adhesiveness property continue stable and another one where this capability disappears.

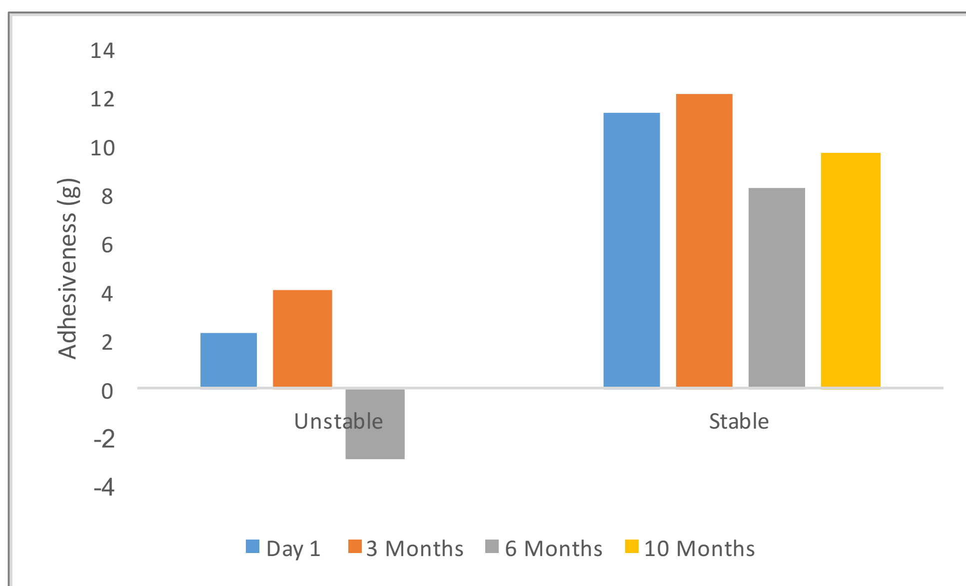


Figure 52 Adhesiveness measurement over time. Unstable and stable formulations are represented.

Specifically for the DP under development, it was desired that the DP maintains the adhesion property over time in order to guarantee the DP performance. The results show an example of an unstable formulation and a stable formulation. The positive range of adhesiveness is associated with adhesive formulations and negative range with non-adhesive formulations. After 6 months of stability, unstable formulation decreases the adhesiveness for negative values and became a non-adhesive formulation. The other formulation was stable over 10 months since the adhesion property remains positive.

The obtained results suggested that it is necessary to develop formulations with higher adhesions (more than 4 g) in order to maintain their adhesive properties over time.

3.2.2. Spray performance

3.2.2.1. Spray actuation test

The evaluation of the forces that are required to promote the actuation of a spray device (actuation and maximum force) was investigated during pre-stability. **Figure 53** demonstrates a study performed on different suppliers (different pre-defined settings) but with exactly the same formulation.

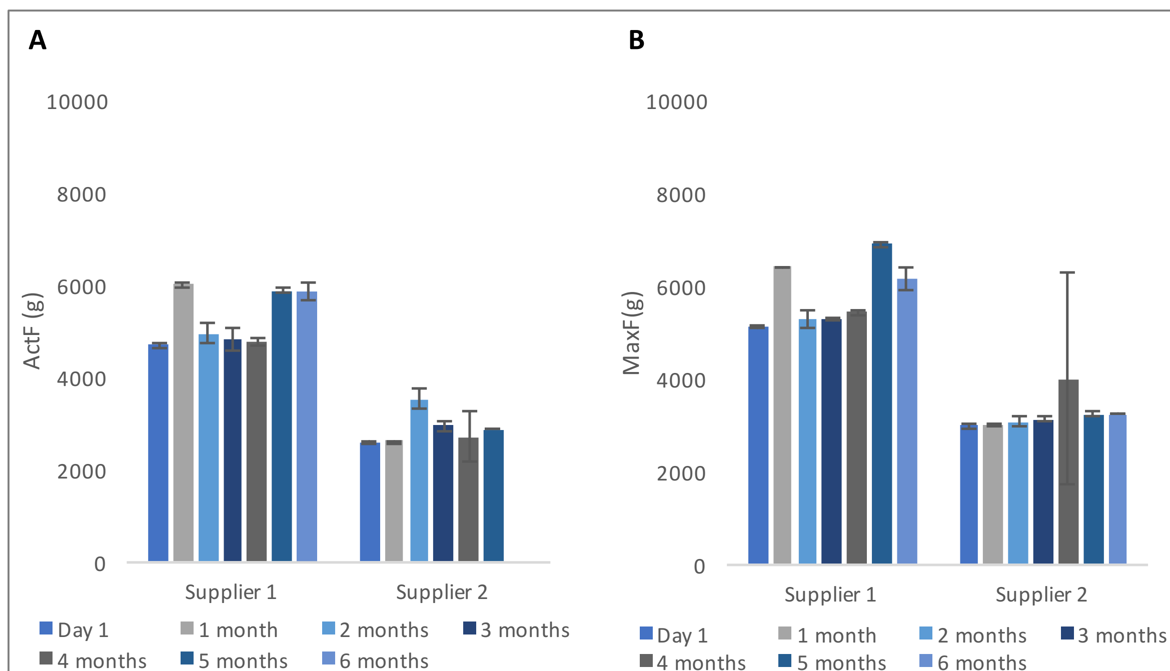


Figure 53 Spray actuation test over time. Actuation (**A**) and maximum (**B**) forces with 2 different suppliers.

Since this test was not performed with the same type of spray device, the results cannot be directly compared. However, the evaluation of the spray device performance over time demonstrate some fluctuations of the actuation force (**Figure 53A**) and maximum force (**Figure 53B**) on both types of sprays (Supplier 1 and Supplier 2). Since spray actuation test is totally dependent on the characteristics of the DP, namely the physicochemical characteristics of the formulations and the type of spray device, the results obtained may be due to some changes in the formulation itself, for example, the viscosity of the formulation. Despite these fluctuations, all values remained within the range reported by Doughty *et al.* [55].

3.2.2.2.Weight loss (stability)

Weight loss test is only performed over time and because of this, it is only referred in this subchapter. This test is related to both non-sterile liquid preparation and container closure system. Depending on the physical integrity of the packaging system, the weight loss of formulations with volatile excipients could be more or less accentuated. On guidance for industry related with stability, it is referred that a 5 percent loss in weight from its initial value is considered a significant change for a product packaged in a semipermeable container after an equivalent of 3 months. For non-aqueous products (solvent-based products) other comparable approaches can be developed and reported [42].

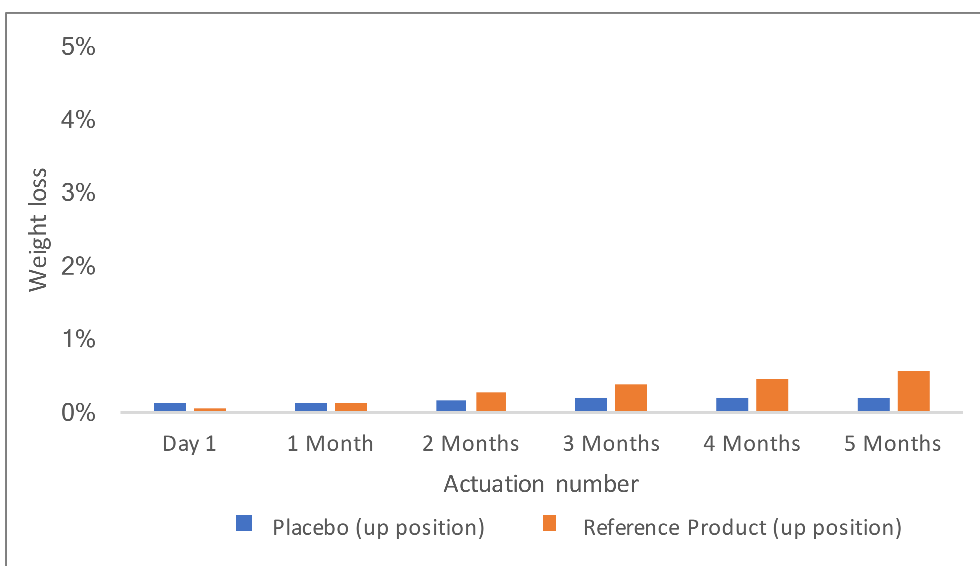


Figure 54 Weight loss of placebo formulation and commercialized reference product up to 5 months.

Figure 54 shows that both spray devices analyzed have weight loss within the acceptable limit of 5% [42]. The reference product weight loss is higher than the obtained with the placebo formulation which may be due to a lower isolation capacity of the container or due to a higher amount of volatile solvents. The results with placebo formulation demonstrate that the selected container closure system has the suitable capability of isolation.

3.2.2.3. Repriming (stability)

Spray performance of different spray devices suppliers were evaluated after specific intervals without use and, because of this, it is only referred in this subchapter. On the drug product label, the number of actuations that is required for re-activate the spray device, should be mentioned. In the graphic below, different time points were selected according to the intended use. A monthly evaluation was performed in 3 different suppliers and in one commercialized reference spray product. The three different suppliers were filled with the same formulation and any differences in the results only depend on the spray device design (**Figure 55**).

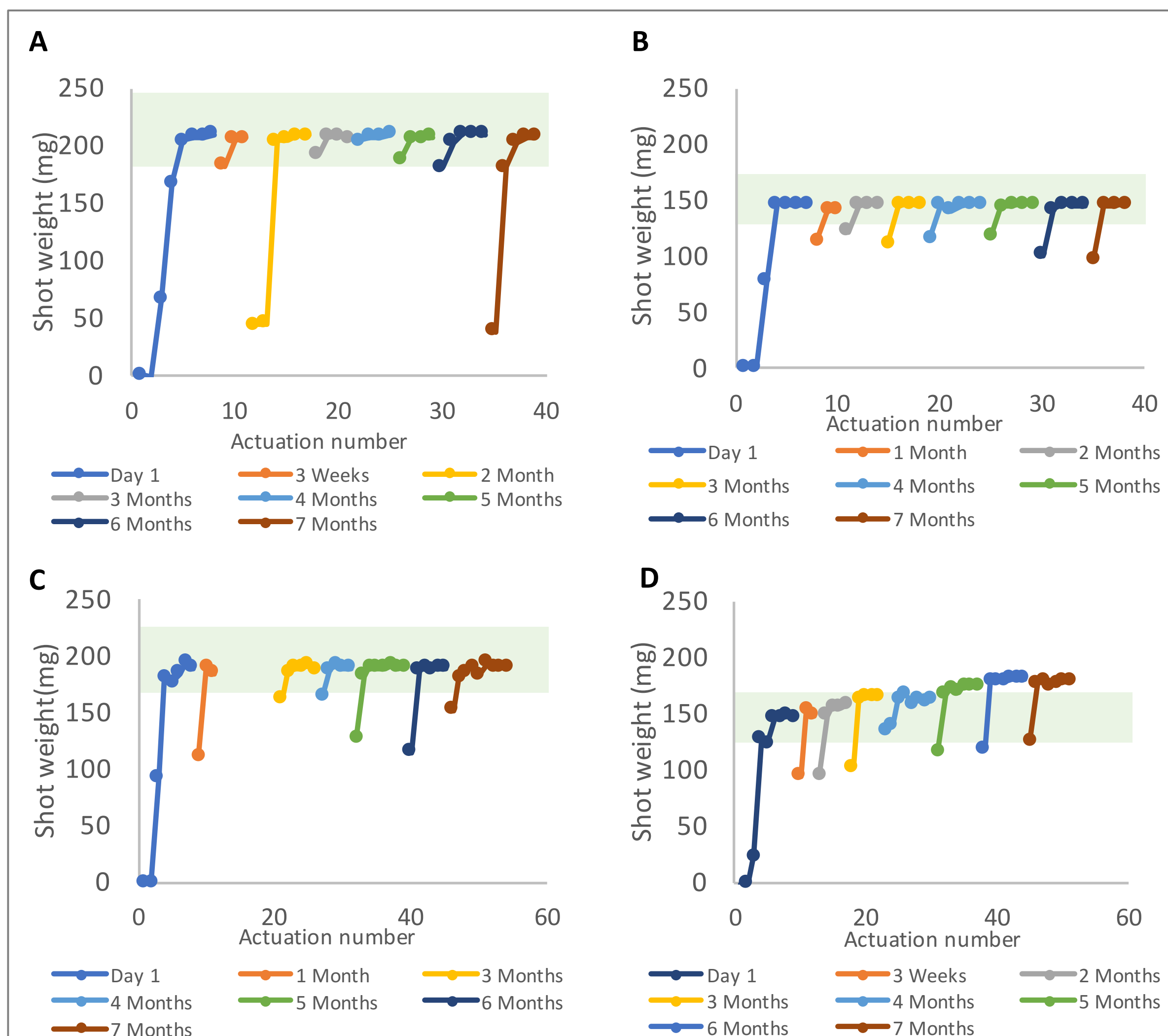


Figure 55 Repriming actuation on different spray devices suppliers (**A**,**B** and **C**) with the same formulation and reference product (**D**).

For the identification of the number of actuations required to be discarded prior re-use of the product, we use the acceptable criteria defined by FDA authority of variations within 15% of the target weight [12]. If the first actuation is outside this interval, we considered that it is required to discard this actuation. Generally, the results reveal that is required to discard at least one actuation prior use (after an unusable interval about 1 month) either for the developed formulations in all suppliers tested (**Figure 55A**, **B** and **C**) and for the commercialized reference product (**Figure 55D**).

Beyond to the pre-stability studies after 1 month, we also evaluate the spray performance of two spray devices, with the same formulation, after an unusable interval of 4 and 6 months (**Figure 56**).

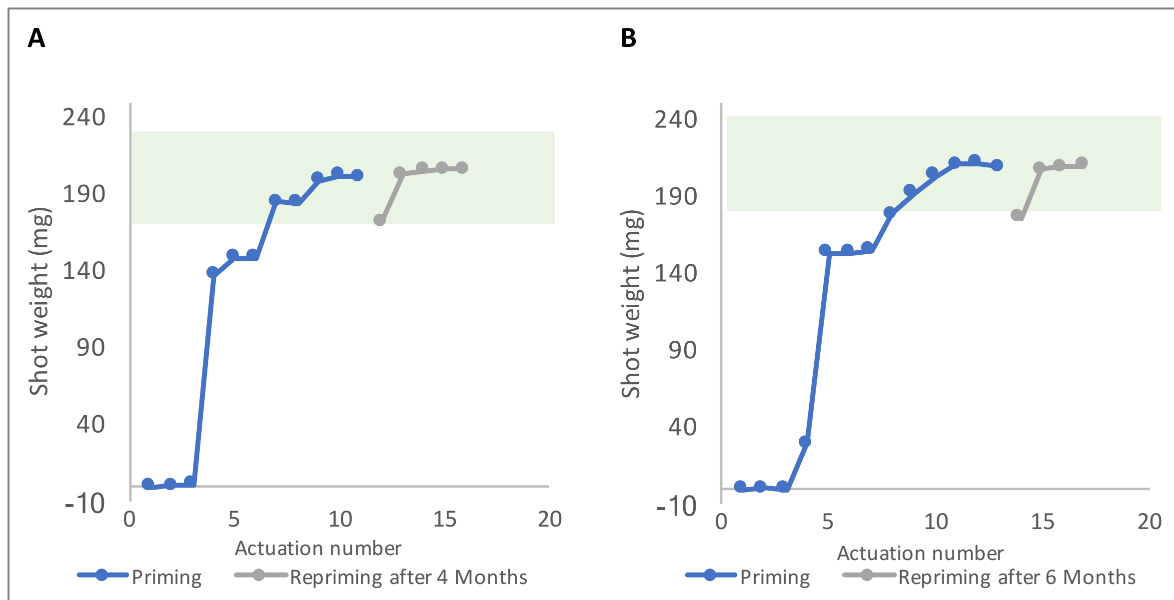


Figure 56 Repriming actuations in two spray devices with the same formulation over 4 (**A**) and 6 (**B**) months.

Figure 56 shows that over 4 and 6 months, using the same spray device type and the same formulation composition, it is also required at least 1 actuation for re-activate the spray device. The repriming actuation is crucial to include on the DP label claim in order to ensure the correct usage.

CHAPTER 4

CONCLUSION

The drug product under development is composed by a non-sterile liquid preparation and a container closure system and it is classified as a liquid dosage form intended to release the DS in the internal mucosa through immediate or extended release.

The interest of the pharmaceutical industries for the development of this pharmaceutical dosage form has emerged in part because it improves the drug delivery safety and efficiency over the direct access to the systemic circulation via capillaries and venous drainage. Mucosal drug delivery has some challenges related to the DS solubility and permeability, however it presents several advantages, in particular:

- Enhanced patient compliance and convenience: easy administration, self-administration and painless;
- Increase the safety of highly metabolized drugs in the liver by reducing the dose that needs to be administered to achieve the therapeutic effect;
- Potential to enhance bioavailability: possible reduction of GI degradation and minimizes hepatic inactivation (first pass metabolism);
- Decrease the variability of GI absorption due to food presence;
- Faster onset of action when compared with solid oral dosage forms;
- Reduce the time needed to achieve the therapeutic effect particularly in emergencies.

The main purpose of this work is to contribute to establish the instructions for the correct usage of the drug product, and guarantee that the use is proper and result in suitable product performance. In that effect, product quality and performance tests were selected according to CQA identified for technology/product under development and were based on pharmaceuticals guidances and pharmacopoeias chapters.

The conclusions of the executed tests for the characterization of the formulation are the following:

- Definition/Description/Appearance: Normal appearance (clear, without foreign particles).
- Specific Gravity/Density: Should be well-defined for each DP.
- pH: Mucosal dependent parameter: should ensure local tolerability and does not compromise the DS solubility and permeability.
- Viscosity: Acceptable range was not higher than 1500 mPas for spray drug products.

- Adhesion: Considering the intended use, adhesion should be positive and apparently not lower than 4g.
- *In vitro* release test: Useful test for DP comparison studies and determination of release profile – immediate DS release.

The spray performance evaluation leads to the following conclusions:

- Spray actuation test: Actuation and maximum forces are below the maximum adult limit referred on the literature (5.82 ± 1.40 kg).
- Pump delivery/Shot weight: Shot weight depends on pump type, pump weight capacity and type of actuations. Individual spray weight delivery should be within ± 15% of the target weight.
- Priming: It seems to be necessary to discard 3 to 6 actuations before the first administration of the DP.
- Number of doses/actuations: Dependent on the amount of formulation filled into packing material.
- Weight loss: The weight loss of the spray device tested is within the acceptable range. Not higher than 5% weight loss.
- Repriming: It seems to be required at least one actuation after an unusable interval about 1, 4 and 6 months.

The evaluation of DP immediately after its preparation determines the intrinsic characteristics of the formulation and supports the formulation development team. On the other hand, the tests that have been evaluated over time – pre-stability studies - are only preliminary tests, since they should be done with well-defined conditions of temperature and humidity, according to the guidances and pharmacopoeias.

To conclude, I consider that my internship in this company has responded to the objectives that have been proposed to me. The characterization of the DP – formulation and spray performance – was studied and discussed as intended. Overall, it is possible to develop a safe and effective DP if the formulation and spray performance exhibit the above characteristics.

CHAPTER 5

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ATTACHMENT

Table A1 Special spindles for viscosity measurement.

	Special Spindles		
	TL5	**TL6**	**TL7**
Speed (rpm)	**Viscosity (mPas)**		
0.1 (V2 only)	3×10^4	3×10^5	6×10^5
0.2 (V2 only)	1.5×10^4	1.5×10^5	3×10^5
0.3	1×10^4	1×10^5	2×10^5
0.5	6×10^3	6×10^4	$1,2 \times 10^5$
0.6	5×10^3	5×10^4	1×10^5
1.0	3×10^3	3×10^4	6×10^4
1.5	2×10^3	2×10^4	4×10^4
2.0	1.5×10^3	1.5×10^4	3×10^4
2.5	1.2×10^3	1.2×10^4	2.4×10^4
3.0	1×10^3	1×10^4	2×10^4
4.0	7.5×10^2	7.5×10^3	1.5×10^4
5.0	6×10^2	6×10^3	1.2×10^4
6.0	5×10^2	5×10^3	1×10^4
10.0	3×10^2	3×10^3	6×10^3
12.0	2.5×10^2	2.5×10^3	5×10^3
20.0	1.5×10^2	1.5×10^3	3×10^3
30.0	1×10^2	1×10^3	2×10^3
50.0	60	6×10^2	1.2×10^3
60.0	50	5×10^2	1×10^3
100.0	30	3×10^2	6×10^2
200.0	15	$1,5 \times 10^2$	3×10^2
Increment	0.1 mPas	1 mPas	10 mPas

Table A2 Standard spindles for viscosity measurement.

	Standard Spindles			
	L1	**L2**	**L3**	**L4**
Speed (rpm)	**Viscosity (mPas)**			
0.1 (V2 only)	6×10^4	3×10^5	$1,2 \times 10^6$	6×10^6
0.2 (V2 only)	3×10^4	$1,5 \times 10^5$	6×10^5	3×10^6
0.3	2×10^4	1×10^5	4×10^5	2×10^6
0.5	1.2×10^4	6×10^4	2.4×10^5	1.2×10^6
0.6	1×10^4	5×10^4	2×10^5	1×10^6
1.0	6×10^3	3×10^4	$1,2 \times 10^5$	6×10^5
1.5	4×10^3	2×10^4	8×10^4	4×10^5
2.0	3×10^3	1.5×10^4	6×10^4	3×10^5
2.5	2.4×10^3	1.2×10^4	4.8×10^4	2.4×10^5
3.0	2×10^3	1×10^4	4×10^4	2×10^5
4.0	1.5×10^3	7.5×10^3	3×10^4	1.5×10^5
5.0	1.2×10^3	6×10^3	2.4×10^4	1.2×10^5
6.0	1×10^3	5×10^3	2×10^4	1×10^5
10.0	6×10^3	3×10^3	1.2×10^4	6×10^4
12.0	5×10^2	2.5×10^3	1×10^4	5×10^4
20.0	3×10^2	1.5×10^3	6×10^3	3×10^4
30.0	2×10^2	1×10^3	4×10^3	2×10^4
50.0	1.2×10^2	6×10^2	2.4×10^3	1.2×10^4
60.0	1×10^2	5×10^2	2×10^3	1×10^4
100.0	60	3×10^2	1.2×10^3	6×10^3
200.0	30	1.5×10^2	6×10^2	3×10^3
Increment	1 mPas	1 mPas	10 mPas	10 mPas