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A Quality by Design approach on pharmaceutical development of magnesium dosage forms: Tablet and Oral Solution

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Preface

The work presented in this thesis was performed at the company Grupo Medinfar (Amadora, Portugal), during the period March-September 2019, under the supervision of Master Mariana Viegas Caeiro Gonzalez Teixeira de Almeida. The thesis was co-supervised at Instituto Superior Técnico by Professor Margarida Maria Portela Correia dos Santos Romão.

Declaration

I declare that this document is an original work of my own authorship and that it fulfills all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

Acknowledgments

I would like to start by thanking my parents for all the support and encouragement they gave me. None of my accomplishments would have been possible without their care, love and friendship. I owe them the person I am today! Luzia and Manel, thank you so much for believing in me and for being two fighters that have done everything to give me the very best. I love you both very much and hope to give you many reasons to be proud. I would also like to thank my sisters, for being my best friends, my support, my role models and for always believing in me. Thank you Catarina, for being a good listener and for all the good advises you gave. You have supported me through thick and thin. You are truly what an older sister should be; kind, concern and protective. Thank you Sara, for being generous, concerned and loving. For being near me, even though you live in a different country. Thank you for celebrating my victories as your own, and for taking my sours away with your genuine unique tenderness. Without you both, I would never had accomplished this goal (and many others...). I can not put in words the importance you have in my life. You are both unique and very special to me. Thank you for everything.

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To each and every one of you – Thank you.

Abstract

Different approaches can be applied to the development of pharmaceutical dosage forms. However, recently, a systematic approach to pharmaceutical development, known as Quality by Design (QbD), has been strongly recommend and analyzed by the regulatory agencies: European Medicines Agency (EMA) and Food and Drug Administration (FDA). QbD promotion began with the recognition by the regulatory agencies that quality must be built into the pharmaceutical product, and that increased testing does not imply the improvement of product quality. The overall aim of this work was to develop magnesium dosage forms, tablets and oral solutions, taking in consideration the QbD approach. Therefore several concepts and tools of QbD approach were applied, such as definition of the Quality Target Product Profile (QTPP), risk assessment and Design of Experiments (DoE). DoE was performed in order to evaluate the effect of formulation factors variation on the products Critical Quality Attributes (CQAs). Regarding tablets development, it was studied the impact of glidant, lubricant and superdisintegrant level variation on the appearance, friability and disintegration time attributes. In addition, response surface analysis was applied in order to predict and establish optimal formulation settings, culminating in a immediate release tablet complying with QTPP criteria. Concerning the oral solution development, a DoE was applied in order to select the combination of excipients that enable an oral solution complying with the appearance, taste and pH quality targets.

Keywords

Quality by design, Design of experiments, Immediate release tablet, Oral solution, Food supplement development.

Resumo

Diferentes abordagens podem ser aplicadas ao desenvolvimento de formas farmacêuticas. No entanto, nos últimos anos, uma abordagem sistemática mais conhecida como *Quality by Design* (QbD), tem vindo a ser fortemente recomendada e analisada pelas entidades reguladoras: *European Medicine Agency* (EMA) e *Food and Drug Administration* (FDA). Especificamente, a promoção desta abordagem começou com o reconhecimento por parte destas entidades de que a qualidade deve ser incorporada no produto farmacêutico e de que o aumento do número testes ao produto acabado não implica, necessariamente, a melhoria da qualidade do produto. O objetivo do presente trabalho é desenvolver formas farmacêuticas contendo magnésio, comprimidos e soluções orais, considerando a abordagem QbD. Desta forma, vários conceitos e ferramentas inerentes a esta abordagem foram aplicados, nomeadamente, definição do *Quality Target Product Profile* (QTPP), avaliação de risco e delineamento experimental. O delineamento experimental foi executado de forma a avaliar o efeito da variação da composição das formas farmacêuticas nos atributos críticos de qualidade de cada produto. Em relação ao desenvolvimento dos comprimidos, estudou-se principalmente o impacto da variação na concentração dos excipientes: deslizante, lubrificante e desagregante, nos atributos: aparência, desagregação e friabilidade. Desta forma, foi possível otimizar a formulação dos comprimidos e cumprir com os atributos estabelecidos no QTPP, obtendo-se assim comprimidos de libertação imediata com a qualidade desejada. Por sua vez, o desenvolvimento da solução oral passou pela realização de um delineamento experimental que permitiu selecionar a combinação de excipientes que melhor se adequa aos critérios pré-estabelecidos de aparência, sabor e pH.

Palavras Chave

Desenvolvimento farmacêutico, Comprimidos, Solução oral, *Quality by Design*.

Contents

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Acronyms

ANOVA	Analysis of Variance
API	Active Principle Ingredient(s)
CMAs	Critical Material Attributes
CPPs	Critical Process Parameters
CQAs	Critical Quality Attributes
DGAV	*Direção-Geral de Alimentação e Veterenária*
DoE	Design of Experiments
EMA	European Medicines Agency
EP	European Pharmacopoeia
EU	European Union
FDA	Food and Drug Administration
GMP	Good Manufacturing Practices
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
MAs	Material Attributes
SD	Standard Deviation
SSF	Sodium Stearyl Fumarate
SSG	Sodium Starch Glycolate
QbD	Quality by Design

QTPP Quality Target Product Profile

USA United States of America

1

Introduction

Contents

The aim of pharmaceutical development is to design quality products and their manufacturing process, in order to consistently achieve the desired performance of the pharmaceutical product [1]. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) defines quality, in the guideline Q6A, as "the suitability of either a drug substance or a drug product for its intended use. This term includes such attributes as the identity, strength, and purity" [2].

Until recently, the approach of pharmaceutical development was based on the quality by test method. This method comprises a process in which the quality of the product is assured, alone, by testing raw materials, drug substances and the manufacturing process [3]. Quality by test relies on the idea that raw materials and drug substances can only be introduced in the manufacturing process when all the specifications and criteria defined by the several regulatory agencies, such as the European Medicines Agency (EMA) and the Food and Drug Administration (FDA), are fulfilled. In a scenario where those specifications and criteria are not complied, a re-processing of the raw materials, drug substances and formulations is needed. The fundamental causes of failure are usually not well understood, due to poor understanding of the process or of the relation between raw material and product quality attributes [4]. Consequently, in order to better understand them, the development procedure has to be restarted [4]. Summarizing, quality by test development may lead to product variation, resulting in low drug safety, and poor cost-efficiency [3, 4].

Recently, a new approach - Quality by Design (QbD) - has been strongly suggested and analyzed by both regulatory agencies, EMA and FDA [3,5]. In September of 2004, the FDA published the final report on its new initiative: "The Pharmaceutical Current Good Manufacturing Practices for the 21st century - A Risk-Based Approach". In this document, the American agency encourages the pharmaceutical industry to adopt risk-based approaches, and to apply QbD principles in pharmaceutical development, manufacturing process and quality assurance [6, 7]. Moreover, the QbD promotion began with the recognition by FDA and EMA that quality must be built into the pharmaceutical product, and that increased testing does not imply the improvement of product quality [7]. The concept of QbD has been gaining importance along the years in the pharmaceutical industry panorama. In particular, due to the publishing of the ICH guidelines Q8 (R2) (Pharmaceutical Development) and Q9 (Quality Risk Management) [7]. In the ICH Q8 guideline, it is stated that the manufactures may choose between two different approaches to pharmaceutical development: an empirical approach or a more systematic approach (also mentioned as QbD). Finally, in the same document, the ICH defines QbD as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management." [1].

Nowadays, it is possible to notice an increasing tendency in the scientific and commercial interest in food supplements, consequently, the research and development in this field has been growing. In the past few years, several pharmaceutical companies have started to develop and manufacture food sup-

plements. Unlike pharmaceutical development, there are no guidelines suggesting or recommending the application of different approaches to the development of food supplements. Moreover, the food supplements regulation is significantly less strict than the pharmaceuticals regulation. Thus, food supplements are easier to marketing and its development is, by far, cheaper than pharmaceutical development [8–10]. Nevertheless, food supplements can interact with drug substances or nutrition substances normally absorbed in the daily diet. In addition, some nutritional substances when consumed in high doses can cause adverse reactions, e.g., magnesium can cause diarrhea [8, 11]. Regardless of the mentioned before, currently, there is no post-market monitoring system for food supplements that, similarly to the pharmacovigilance system, manage adverse reactions after the product is introduced in the market [8].

QbD is not a new concept in the food industry, but there seems to be a limited number of studies concerning the application of QbD approach to food supplements development. These may be to the lack of guidelines suggesting its application but, it can not be discarded that, food supplements are marketed in dosage forms similar to pharmaceutical products and that a certain level of quality and safety is expected from these products. Nevertheless, there are nutritional substances that, depending on the dosage, can be considered as medicines, e.g., magnesium [8, 9, 11, 12]. Concluding, it seems to be relevant to understand the potential of QbD as an approach to the development of food supplements.

In the following sub-chapters a review of the state of the art on the several concepts mention before is presented. First, it is presented a review on the QbD concept, followed by the definition of risk assessment and design space. After, an analysis on food supplementation with focus on magnesium supplementation is presented. Finally, a review on two typical food supplements dosage forms, oral solutions and tablets, is provided. I would like to remark that the present work was developed jointly with the company Grupo Mendifar and that the dosage forms approached were suggested by the company itself.

Hereinafter, it should be taken in consideration that the terms ”pharmaceutical development” and ”product development” are used with the same meaning. It is acknowledged that the definitions presented in literature, including the in ICH guidelines Q8 and Q9, do not consider food supplements. Although, for the purpose of this work, pharmaceutical development includes food supplements development, unless otherwise mentioned. Regarding the term ”product”, this term encompasses pharmaceutical and food supplements products. Additionally, the term ”drug substance” is used to mention Active Principle Ingredient(s) (API), although, in the scope of this work, this term can be extended for nutritional substances used in food supplements. Nevertheless, it is widely recognized that the conventional definition of API do not include nutritional substances. The definition being ”a substance used in a finished pharmaceutical product, intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to have direct effect in restoring, correcting or modifying physiological functions in human beings” [13]. Henceforth, the reader

should consider that whenever the terms drug substance or API are used, the nutritional substances are included (unless otherwise mentioned).

1.1 Quality by design

Development scientists can choose different approaches to the development of a product: an empirical approach or a more systematic approach, or even, a combination of both. Nevertheless, the product should be formulated to meet patients needs and the intended product performance [1, 7, 14]. As mentioned before, the systematic approach to development is also known as QbD, being defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" [1]. The QbD goals can be narrowed to: i. increase the efficiency of product and process development, ii. increase the process/product understanding in order to increment process capability and reduce product variability and defects, iii. promote and enhance the root cause of failure analysis, iv. achieve relevant product quality specifications; and finally, v. enhance post-approval change management [4, 7, 14]. The application of such systematic approach encompasses many improvements onto traditional approach to product development (quality by testing), as illustrated in the Figure 1.1 [4, 7, 15].

According to what it is suggested in the ICH Q8, a pharmaceutical development should, at a minimum, include:

- The definition of a Quality Target Product Profile (QTPP) considering aspects such as, the route of administration, dosage form, bioavailability, strength, stability, etc., and how these attributes relate to quality, safety and efficacy;
- The identification of potential Critical Quality Attributes (CQAs) of the product, with the aim of studding and controlling them;
- Determining the quality attributes of the drug or nutritional substance and excipients (raw materials), also mentioned as Material Attributes (MAs).
- Selecting the type and amount of excipients to deliver a product of the intended quality and performance;
- Defining an appropriate manufacturing process;
- Defining a control strategy [1].

While, a QbD approach additionally includes:

- "A systematic evaluation, understanding and refining" of both the formulation and manufacturing process of the product, which includes:

  - The identification of MAs and process parameters that possible have an effect on product CQAs, considering prior knowledge, experimentation and risk assessment;
  - Determining and understanding the linkage between MAs and process parameters to the product CQAs;

- The establishment of a design space as a part of a defined control strategy based on enhanced product and process understanding combined with quality risk management [1].

As a result, the QbD approach facilitates continual improvement along the product life-cycle and can support more flexible regulatory approaches [1, 7]. In the following sub-chapter, a more in-depth review on those QbD elements (starting with QTPP) is presented.

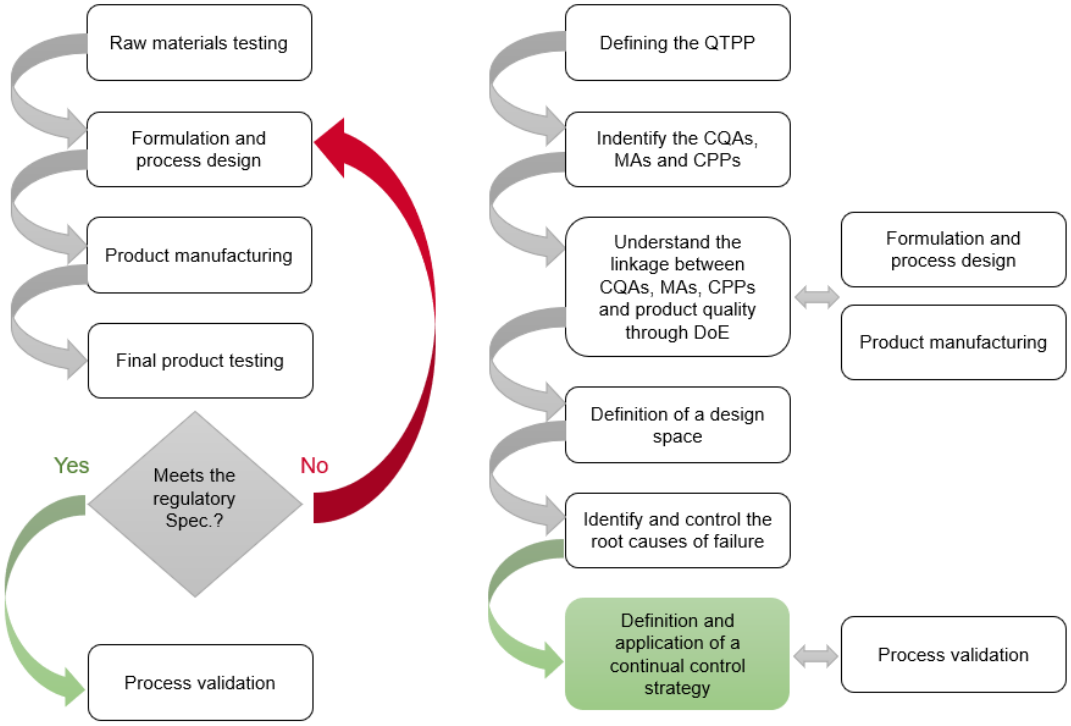

Figure 1.1: Steps of Quality by Test approach (left) *versus* Quality by Design approach (right) to product development. Spec.: Specifications, QTPP: Quality Target Product Profile, CQAs: Critical Quality Attributes, MAs: Material Attributes, CPPs: Critical Process Parameters, DoE: Design of Experiments.

1.1.1 Quality Target Product Profile

Like described in the Figure 1.1, QTPP establishment is the first step in pharmaceutical development following the QbD approach. QTPP can be defined as a summary of the quality attributes of a product that ideally will be achieved, in such a way, that the desired product quality and performance is guar-

anteed. The QTPP defines the groundwork for the pharmaceutical development and, therefore, for the establishment of the CQAs, Critical Process Parameters (CPPs), and control strategy [5, 7, 16, 17].

The QTPP may include:

- Intended use, route of administration and dosage form of the product;
- Dosage strength;
- Therapeutic moiety release or delivery;
- Attributes affecting the product pharmacokinetics (such as dissolution or aerodynamic performance);
- Container closure system;
- Appropriate quality criteria of the product such as, sterility, purity and stability [1, 5, 7].

It seems evident that a new product and its desired characteristics should be defined before any development work begins. Yet, the value of defining the target characteristics of the product - by a well defined QTPP - is often underestimated. Resulting in loss of resources, which makes the process of development slower and expensive [7]. Furthermore, this depreciation of a well defined QTPP seems rather unfounded, since the regulatory agencies EMA and FDA, and the ICH guidelines, all suggest its application and benefits. [1, 17, 18].

Having the QTPP established, it is possible to identify the CQAs [19]. In the guideline ICH Q8, CQAs are defined as "a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. CQAs are generally associated with the drug substance, excipients, intermediates (in-process materials) and drug product" [1]. A given product has a variety of quality attributes, e.g., identity, assay, content uniformity, stability, dissolution, microbial limits, friability, moisture content, color, odor, size, etc [20, 21]. These attributes can be critical or not. To understand if a quality attribute is critical, it is crucial to, primarily, assess the severity of harm that could be caused to the patient if the product fall outside the acceptable range for that attribute [7]. The term harm corresponds to any damage to health, including those that can arise from the loss of product quality or availability [18]. Risk assessment facilitates this process, i.e., facilitates the identification of CQAs [7, 18].

Risk analysis, as a part of risk assessment, is defined in the ICH guideline Q9 as the process of linking the probability of occurrence of a given harm with its severity. The capability of detecting such harm, known as detectability, is also considered in some risk management tools. The risk can be assessed qualitatively or quantitatively [18]. To understand the relation between risk and criticality of a quality attribute, it is necessary to take in consideration the following:

- Risk is commonly defined as ”the combination of the probability of occurrence of harm and the severity of that harm” and, in some cases, it also considers the detectability of that harm. Therefore, the level of risk can change as a result of risk management [18].
- The criticality of a quality attribute is initially established by the severity of harm, thus, it does not change as result of risk management [18].

A more exhaustive review on risk assessment as part of QbD product development, is further presented in the sub-chapter 1.2.

Summarizing, QTPP establishment is a crucial step on the process of product development. It represents the basis for all the further steps included in the QbD pharmaceutical development. Having all the product quality targets defined is possible to design the formulation and its manufacturing process. Following, a review on those product development steps is presented.

1.1.2 Product design and understanding

Product design is an essential step of QbD since, its main objective is to design a robust product that meets the QTPP. Product design, not only, determines the ability of the product to fit the patients needs, but also, determines whether the product is stable through its shelf life or not. Moreover, it is generally accepted that an extended product understanding could have prevented some historical stability problems and should be substantially applied when formulating new products [7].

Product design may follow different pathways, but its key steps can be summarized as the following:

1. Characterization of the drug substance (physical, chemical and biological characteristics);
2. Identification and selection of the excipients and its grade;
3. Identification of the possible interactions between the drug substance and the selected excipients;
4. Identification of the critical MAs (including excipients and drug substance) and consequent optimization of the formulation [7].

The first key step - characterization of the drug substance - should include the characterization of physical, chemical and biological properties of the API. The physical properties include particle size distribution and morphology, polymorphism, solubility, dissolution rate, melting point and hygroscopicity. Chemical properties may include oxidative stability, light stability, pKa, chemical stability in solid state and in solution. Finally, biological properties include membrane permeability, bioavailability and partition coefficient. It should be given much attention to these characteristics, since many of them can impact the manufacturing process and product quality attributes such as dissolution rate, stability and bioavailability [7].

The next step is to select and identify the excipients and its grade. Excipients are the components of a pharmaceutical product that do not correspond to the API. Excipients can be added to the product with different objectives. There are excipients that are added to support, protect or enhance product stability, bioavailability or even patient acceptability, while others, facilitate the product manufacturing or may enhance the effectiveness or safety of the product during its life-cycle. Excipients are classified by the intended function in the product, for example, there are sweeteners, preservatives, dispersing agents, pH modifiers (buffers), binders, disintegrants, and many others. These components have respective defined and recommended concentration limits, that may vary over dosage form. Moreover, excipients have specifications (purity, identification, etc.) they must comply with and can be found in the European Pharmacopoeia (EP) [7, 20–22].

The identification of the possible interactions between the drug substance and the selected excipients is a step of particular relevance to pharmaceutical development. Regardless of the well known impacts that excipients can have in the product performance, there is not a well-defined strategy to effectively select excipients. Excipients are selected to meet its desired function and the compatibility tests between drugs and excipients are often left out. However, methodical compatibility tests enable the early identification of interactions between the drug substance and the excipients, facilitating the identification of sources of failure. Moreover, it minimizes the occurrence of unexpected stability problems, making the development process more cost and time efficiently, and consequently, maximizes the stability of the formulation through its life-cycle [7].

Formulation optimization and identification of critical MAs is the final key step of the product design and understanding. The optimization studies are essential for the development of a robust formulation, since these tests provide information on the existing critical MAs of the drug substance, excipients and intermediate products and on the relation between CQAs and critical MAs. The formulation optimization culminates in the development of a control strategy for the drug substance and also, for the excipients. It is important to be aware that in a QbD approach the relevance of the studies and the knowledge gained determines the design of a quality product, and not, the number of studies performed. Thus, Design of Experiments (DoE) is a useful tool in the formulation optimization process and in the over-all QbD process. Through DoE it is possible to obtain higher quality information, when compared to the traditional trial and error method of experimentation [1, 7].

The critical MAs correspond to the physical, chemical, biological or microbiological proprieties of the raw materials that should comply with an given limit or range, to ensure the desired quality of the final product. Critical MAs should not be mistaken for CQAs, since CQAs corresponds to the critical attributes of the final product, i.e., output materials, while, critical MAs correspond to the input materials also mentioned as raw materials. The critical MAs of the input materials can impact the CQAs of the outputs materials. It seams obvious that there are a wide range of MAs that may have an impact on

the CQAs and, therefore, it is not reasonable to study all the MAs. Thus, risk assessment represents an essential tool to prioritize the MAs worthwhile studying (see sub-chapter 1.2) [1, 7, 18]. A MAs can be considered as critical when variation in that material attribute can significantly affect the final product performance and quality [7].

The steps that are taken during the product design that enhance the product understanding are outlined in Figure 1.2. Following product design and understanding, it is necessary to design and understand the manufacturing process to gain knowledge on its influence on the final product quality. In the next sub-chapter the points to consider in this step are discussed.

1.1.3 Process design and understanding

Pharmaceutical products are manufactured by a process that combines several unit operations. Unit operations are individual activities that include physical or chemical transformations. The extent of unit operations included in a given manufacture process depend on the desired product and its complexity. For example, immediate release tablets manufactured by direct compression may simply include two unit operations: mixing and compression. While a more complex formulation, like coated tablets, may include in its manufacturing process a variety of unit operations. Unit operations may be performed in a

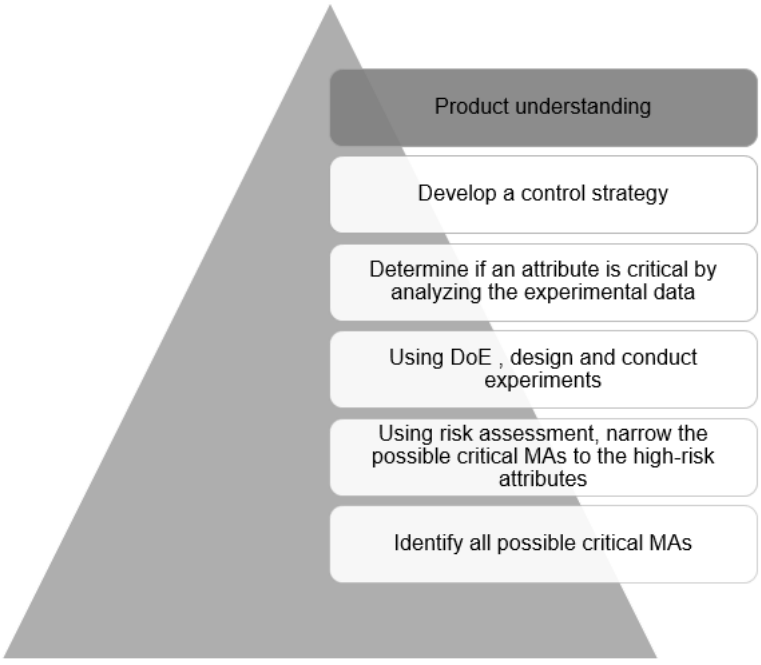

Figure 1.2: Steps in product design following the Quality by Design approach that contribute to an enhanced product understanding. MAs: Material attribute; DoE: Design of Experiments. Adapted from: L. X. Yu, G. Amidon, M. A. Khan, S. W. Hoag, J. Polli, G. K. Raju, and J. Woodcock, "Understanding Pharmaceutical Quality by Design," The AAPS Journal, vol. 16, no. 4, pp. 771–783, 7 2014.

continual or in, a more common, batch mode of manufacturing process [7].

In order to attain a well-understood process it is necessary to identify and explain all the sources of variability, manage the variability within the process, and accurately and reliably predict all the final product quality attributes [7].

The quality of an out-put of a given manufacturing process is dependent on the input materials attributes and on the input process parameters. A CPPs corresponds to a process parameter that has an impact on a CQAs, when it varies [7]. The consequences of variations in material attributes and process parameters are analyzed through process robustness studies. Process robustness is defined as the ability of a process to exhibit acceptable quality and performance, while tolerating variability in the inputs materials attributes and process parameters. The robustness studies facilitate the identification of CPPs and the definition of limits for these CPPs that assure the quality of final product [7]. Figure 1.3 describes the relation and linkage between critical MAs, CPPs and CQAs. Each unit operation of a given pharmaceutical process has an input, i.e, critical MAs and CPPs, which impact the output (CQAs) of that unit operation and of the over-all process [7].

The steps that contribute to process understanding are not very different from those involved in product understanding, being the following:

1. Identification of the process parameters that can have an impact on the performance of the process;
2. Identification of all the CPPs using scientific knowledge and risk assessment tools;
3. Establishment of limits for the identified CPPs;
4. Use appropriate tools to design experiments and conduct them;
5. Understand the linkage between the critical MAs, CPPs and CQAs by analyzing the experimental data;
6. Develop a control strategy [7].

The knowledge gained during the formulation and process design allows the establishment of a control strategy. In the next sub-chapter is possible to find a review on this subject.

1.1.4 Control strategy and design space

The overall knowledge gather during the design processes enables the establishment of a meaningful control strategy that allows regulatory flexibility and time-cost efficiency. Accordingly to what is mentioned in the ICH guideline Q8, control strategy consists in a series of control procedures that are established based on product and process knowledge. The control procedures are designed in order

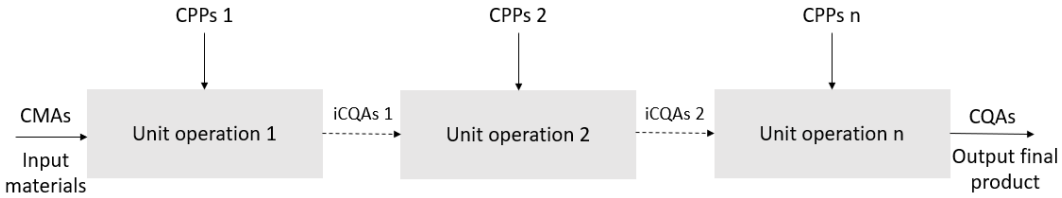

Figure 1.3: Relation between the process inputs, Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs), and the process output: Critical Quality Attributes (CQAs). Each unit operation has an input CMAs and CPPs, and, depending on the stage of the process, a unit operation can have an intermediate CQAs (iCQAs) or a CAQs as an output.

to assure the desired product performance and quality. A control strategy may be composed, among others, by the following actions:

- MAs control, including raw materials, in-process materials and the API,
- Control of the process parameters,
- Understand the linkage of MAs and process parameters with the CQAs,
- In-process measurements and control of CQAs.

In the pharmaceutical industry panorama it is possible to divide control strategy in three levels, as segments of a pyramid. As the Figure 1.4 shows, the bases of the pyramid is the first level, i.e., the traditional pharmaceutical control strategy. This control strategy is mainly based on final product testing, having strict limits for MAs and process parameters. Which culminates in regulatory inflexibility, in the sense that, any changes in the defined MAs and process parameters request regulatory supervision [7]. This control strategy results from poor understanding of the relation between Critical Material Attributes (CMAs), CPPs and the product CQAs [7].

The next level of the pyramid, level two, corresponds to a control strategy that encompasses limited final product testing. In opposite to level 1, level 2 is based on the establishment of a design space, enabling flexible MAs and process parameters. This strategy results from QbD development and relies on extended product and process understanding. The sources of variability that influence the final product are well known and understood, thus, the control procedure is transferred from the final product testing to the begin of the process, being present throughout the product life-cycle. Moreover this control strategy promotes regulatory flexibility, since the impact of any possible variation of MAs and process parameters is described and well-known [7].

The third and final level of the pyramid, corresponds to the most recent control strategy established in the pharmaceutical industry. This strategy relies on real time control of the product CQAs through

advanced technology. The technology is used to control MAs and automatically adjust process parameters, in order to achieve the desired final product quality attributes. This strategy allows real-time product release. A common method applied by this strategy is process analytical technology [7].

The second and third level of the pyramid have in common the promotion of regulatory flexibility, mainly possible due to extended knowledge about the process and product. A good representation of such knowledge is a design space. According to the ICH guideline Q8, design space is a multidimensional representation of the relation between the process inputs, i.e., MAs and process parameters, and the product CQAs. This combination of variables provides assurance of a quality product with the desired performance [1]. In the same document, there are given examples of several possible approaches on presenting a design space. Regardless of the approach taken, it is assumed that a procedure within the design space will deliver a product that complies with the product quality target profile [1]. The selection of variables to be included in a designed space can identified through the application of risk assessment tools. As it is further presented in the sub-chapter 1.2, risk assessment tools contribute for the identification of the product CQAs and, furthermore, for the understanding of the impact that MAs and process parameters have on those. Such MAs and process parameters may be included in the design space [1]. Figure 1.5 shows a design space representation that encompasses the relation between the variation of two CQAs (friability and dissolution) and the variation of two process parameters. The design space is represented by the white region where the friability and dissolution criteria are both met [1].

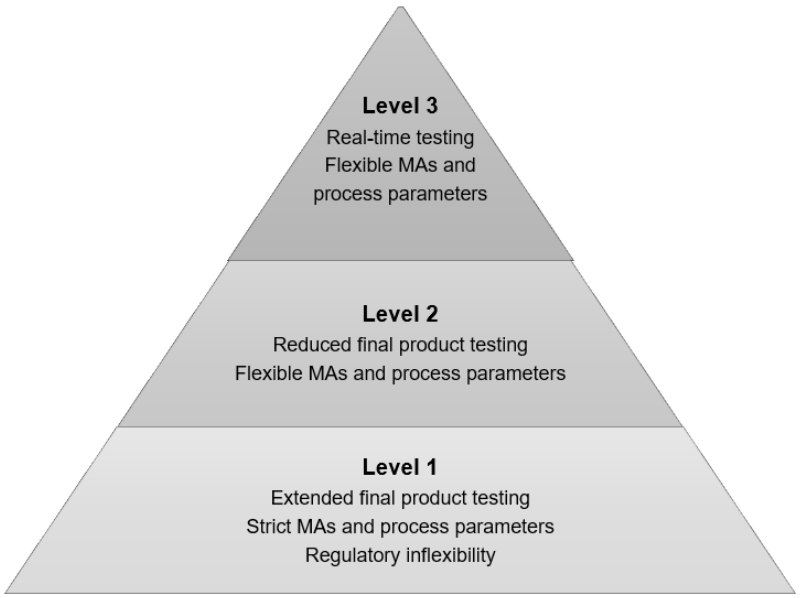

Figure 1.4: Levels of pharmaceutical control strategy.

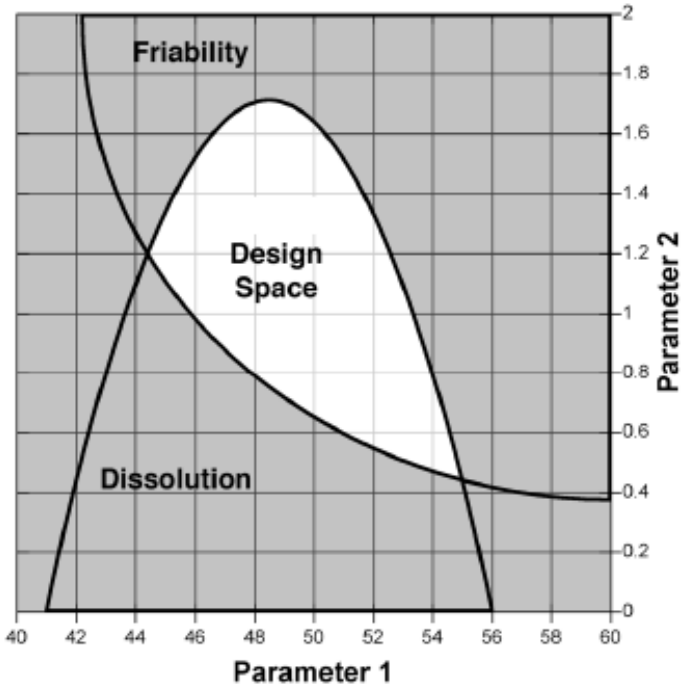

Figure 1.5: Design space representation. Source: International Council for Harmonisation, “Pharmaceutical Development Q8(R2),” vol. 8, no. August, pp. 1–28, 2009.

1.2 Risk assessment

Risk assessment is an integrating step of the quality risk management process. This procedure is defined as ”a systematic process for the assessment, control, communication and review of risks to the quality of the product across the product lifecycle” [18]. A wide number of industries rely on quality risk management principles to manage the risk built-in their products. Regarding the pharmaceutical industry, it is commonly recognized that quality risk management is a valuable component of an effective quality system [18, 23]. Furthermore, it can be largely applied to many aspects of pharmaceutical quality, such as product development, technology transfer, production, pharmacokinetic studies, distribution, validation and life cycle management. Concerning product development, the principles of quality risk management can be used in selection of raw materials, excipients, packaging and labeling materials, formulation development, manufacturing process development and process improvement [23]. When applying quality risk management in the pharmaceutical industry, it should be recognized that the protection of the patient is the main concern. It is evident that the manufacturing and use of a pharmaceutical product as an inherent degree of risk, thus, the risk to its quality is just one component of the over-all risk [18].

To understand the importance of quality risk management and, in particular, of risk assessment it is crucial to understand the concepts of risk, harm and hazard. As mentioned before, risk is defined as the

combination of the severity of a harm and the probability of occurrence of that harm. Harm corresponds to any damage to health resulting from the usage of a product, including those that can occur from loss of product quality or availability. And finally hazard corresponds to the potential source of harm [18].

A typical quality risk management process is composed by three main components: i. Risk assessment, ii. Risk control and iii. Risk review. These components are divided in smaller steps. A summary of the typical quality risk management steps is presented in Figure 1.6. As shown, risk assessment is the first step of quality risk management. Risk assessment consists of three different stages: i. identification of hazards, ii. analysis of those hazards and iii. evaluating the risks associated with exposure to those hazards. These stages will be further discussed. Furthermore, in order to facilitate risk assessment during pharmaceutical development, there are three main questions that should be addressed:

1. What might go wrong during product development and process?
2. What is the probability of that going wrong?
3. What are the consequences and their severity?

As shown in Figure 1.6, risk assessment begins with risk identification, i.e., by answering to the question: ”what might go wrong?”. The aim of risk identification is to identify hazard referring to the proposed risks. Risk identification should consider historical data, theoretical research, informed opinions and the concerns of the company. Ultimately, risk identification is perceived as the basis for the quality risk management process [18].

Risk analysis can be considered as the step in which a estimation of the risk associated with the previously identified hazards is made. Risk analysis is a quantitative or qualitative process of associating the probability of occurrence and the severity of harms. In several risk analysis tools, the detectability of harm is also a component that can be associated with probability and severity in order to estimate the risk [18].

The third and last step of risk assessment process is risk evaluation. In this final step the identified and analyzed risk is compared with a given risk criteria, in which, the strength of evidence is used to accomplish the risk assessment process [18].

The output of a risk assessment can be a quantitative estimate of risk (numerical probability) or a qualitative definition of a range of risk, e.g., ”high”, ”medium” or ”low”. In some cases, risk is expressed by a raking of scores instead of ranges, in which, the highest score represents the higher risk. In these particular cases, a quantitative risk estimation can be used in the intermediates steps of scoring [18, 23]. The quality of these out-puts, quantitative or qualitative, is largely affected by the robustness of the data set. Acknowledging the reasonable sources of uncertainty on the data will increase confidence in the out-up and, additionally, it will help in the identification of its limitation. Uncertainty arise from the combination of lack of knowledge about a process and about its expected (or not) variability. An example

of sources of uncertainty are, gaps in knowledge about pharmaceutical science and process science, sources of harm poorly understood and insufficient data on the probability of detection of harms [18,23].

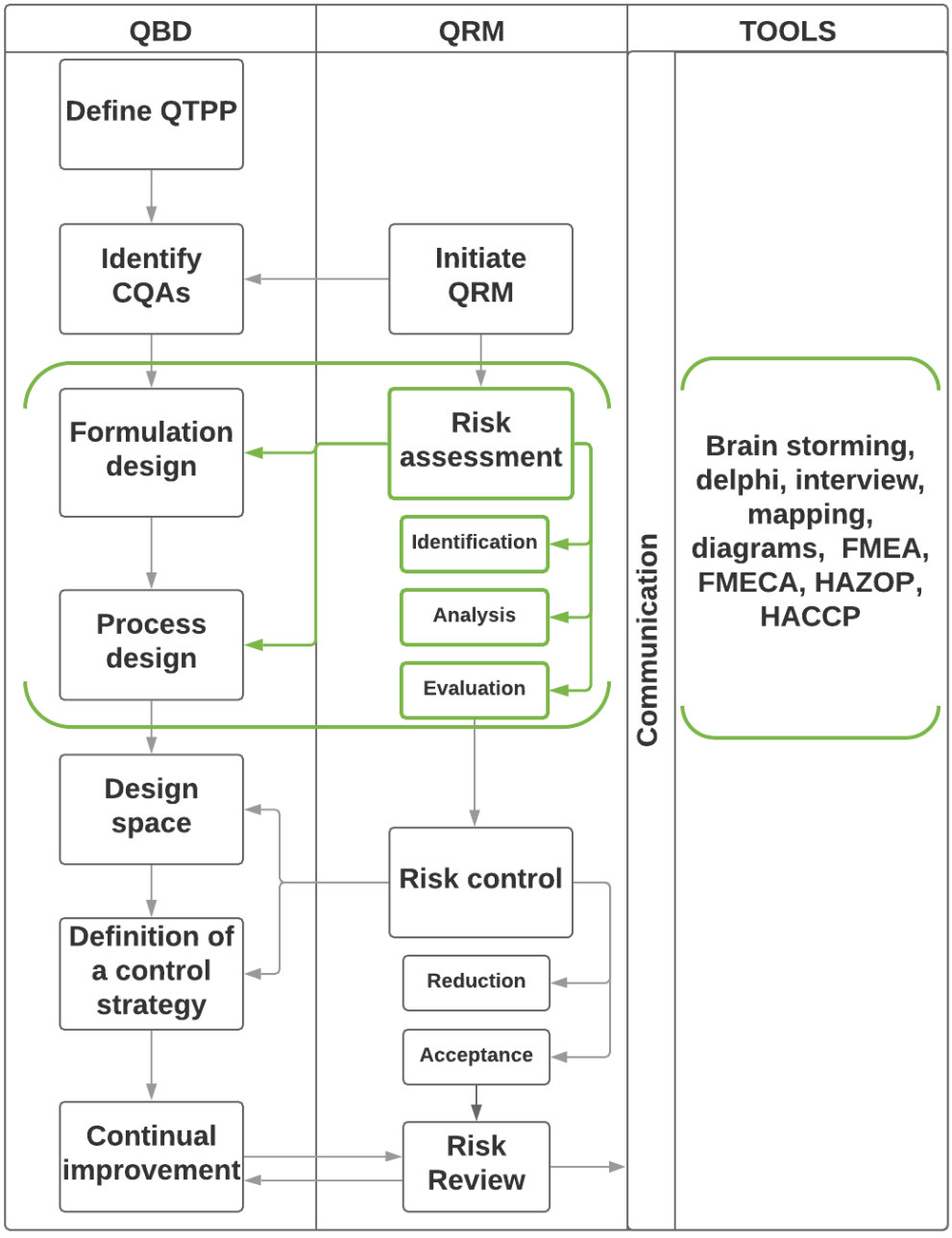

Figure 1.6: Application of quality risk management process in quality by design product development. The green area highlights the risk assessment interference. QBD – Quality By Design, CQAs – Critical Quality Attributes, QTPP – Quality Target Product Profile, QRM – Quality Risk Management, FMEA – Failure Mode and Effects Analysis, FMECA – Failure Mode, Effects and Critically Analysis, HAZOP – Hazard Operability Analysis, HACCP – Hazard Analysis and Critical Control Point. Adapted from: International Council for Harmonisation, "Pharmaceutical Development Q8(R2)," vol. 8, no. August, pp. 1–28, 200.

In conclusion, risk assessment represents an efficient tool to identify risks to the product CQAs. Risk

assessment allows product and process to be further design in order to gain knowledge about these risks and to comply with the target attributes set in the QTPP. Figure 1.6 describes how risk assessment relates to these steps of QbD. Moreover, the knowledge gained in analyzing the risks to CQAs allows the establishment of a meaningful design space, in which, variations to critical MAs or CPPs within the defined limits consistently result in a product with desired quality characteristics [1, 18, 23].

Regardless of the benefits that risk assessment can bring to a product development process, risk assessment is not required for the approval and market introduction of food supplements. While this concept seams rather new in the development of food supplements, there are FDA guidelines suggesting the application of risk assessment tools to assess the risk built-in food. Likewise, the European Food Safety Authority recently published guidance for applying risk assessment in food development. However, it is possible to note a lack of examples to follow concerning food supplements [8].

In the next sub-chapter a review on several food supplements topics is presented, in order to better understand the supplementation panorama and how the already introduced QbD topics may be applied to food supplements development.

1.3 Food supplements

Food supplements can be defined as concentrated sources of substances with a nutritional or physiological reaction, these substances may include, but not limited to, vitamins, minerals, amino-acids, essential fatty acids, fibre and so on. Food supplements are formulated and marketed in dosage forms similar to pharmaceutical products (e.g., tablets, capsules and liquid formulations) [24, 25]. Food supplements are not medicinal products, since their use aims ”to correct nutritional deficiencies, maintain an adequate intake of certain nutrients, or to support specific physiological functions” and not to lead to a pharmacological, metabolic or immunological effect. Summarizing, these products are not suitable to treat or prevent diseases or to modify physiological reactions in humans. Thus, they may be used to complement the ingestion of nutritional substances by a normal diet but not to treat or prevent a given health issue or its symptoms. [24, 25].

It is important to highlight that a normal diet should offer, under normal circumstances, the sufficient intake of nutritional substances. Although, due to recent changes in human lifestyle, reality is not always in line with the ideal scenario or with the normal circumstances, resulting in nutritional intake deficiencies [10, 24, 25]. Consequently, the consume of food supplements is becoming a reliable alternative. The consumption of food supplements has been increasing in the European Union (EU) and in the United States of America (USA), wherein adolescents and elder people represent the main consumers [10, 24]. Statistic data on this topic shows that 20% of the European consumers is using at least one food supplement. In Portugal a study, held by the Lisbon School of Economics and Management, found that

65% of the population is using or has used vitamin food supplements and 52% of the population is using or has used mineral food supplements. The main reason to consume food supplements seems to be tiredness and mental concentration problems [10].

In Europe food supplements are regulated as food and by the European Food Safety Authority. While in Portugal, the competent authority is the *Direção-Geral de Alimentação e Veterenária* (DGAV). In the EU, each state member is responsible to take the decision, in respect with the current legislation, if a given product is considered as a food supplement or a medicine. Therefore, the classification of the same product may vary between different countries. The same dosage form, of the same substance, can be registered as a medicine in one European country and as a food supplement in another. Consequently, when a pharmaceutical company aims to introduce a nutritional substance in several European countries it must considered the classification of that substance in the different countries and therefore adapt the development process to each classification. Following a review on the food supplements manufacturing and marketing regulation is further presented. Likewise, it is presented an analysis focused on magnesium supplementation and on the magnesium products marketed in Portugal.

1.3.1 Food supplements manufacturing and marketing regulation

In June 2015, the Decree-Law n.º 136/2003 was changed and updated to the Decree-law n.º 118/2015, in which it is stated that food supplements can only be introduced in the market as pre-packaged products and are considered as food stuffs. In the same document, it is mentioned the existence of a list of vitamins and minerals that may be added to food supplements dosage forms. This list was established by the European Commission and was published in the food supplements Directive 2002/46/EC and in the European Commission Regulation n.º 1170/2009. Only the vitamins and minerals listed in the mention documents are allowed to be added to food supplements. Moreover, all the food supplements manufactures in the European Union member states must comply with this list. Additionally, the European Commission has established a maximum and minimum level for each vitamin and mineral presented in the mentioned list, these levels should not be exceeded in order to market the product as a food supplement [8, 26–28].

The process of introducing food supplements containing nutritional substances listed in the European Commission Regulation n.º 1170/2009 in the EU market is simple and straightforward. The manufacture that intends to place a food supplement in the market must notify the competent regulatory authority about the market introduction. Along with the notification, the manufacture must send a copy of the product label and information leaflet. Nevertheless, the competent authority may demand to the food supplement manufacture all the scientific data, documents and papers that verify the compliance of the product with the current food supplements law in Portugal. The notification is sent *via* e-mail to the competent authority, i.e., DGAV. Following the notification, a process of appreciation is initiated by the

DGAV, culminating in a final decision of accepting or denying the food supplement marketing. In the Figure 1.7 it is possible to see a description of the notification process and the following processes of appreciation and decision. As presented in the Figure 1.7, if the DGAV does not give an answer with its decision in the deadline of 60 days, the decision is considered as positive. Furthermore, if the composition, manufacture process, label or distribution of the food supplement is changed, the competent authority must be informed within 10 days after the modification [29].

The notification process in Portugal comprises the filling of a specific table with 30 different items that must be filled correctly. The table template is available on the DGAV website. The table is divided in two main fields: information about the manufacture process and marketing, and information regarding the product [29]. In the first field, the notifier should presented the contact information of the manufacture, distributor and importer, for example. And in the second field (information regarding the product),the notifier should, for example, present the list of ingredients and claims used in the food.

Finally, regarding the purity criteria for vitamins and minerals, it is stated in the Decree-law n.º 118/2015 that the same criteria that it is applied to those vitamins and minerals when used in the manufacture of food stuffs, can be applied to the manufacture of food supplements [28].

Concluding, food supplements manufacturing and marketing regulation is by far simpler and less strict when compared to drug products. This may suggest that food supplements development should also be a simple and none strict process. However, as menti oned before, food supplements are presented in dosages forms similar to the pharmaceutical products and may be simultaneously taken, which could lead to interactions between the nutritional substance and the API. Additionally, nutritional substance may degrade or be contaminated with microbial growth. All these aspects represent harm for the patient and must be understood and controlled during product development, QbD development may represent a good approach to do so.

A review focusing on the health benefits of magnesium supplementation is further presented.

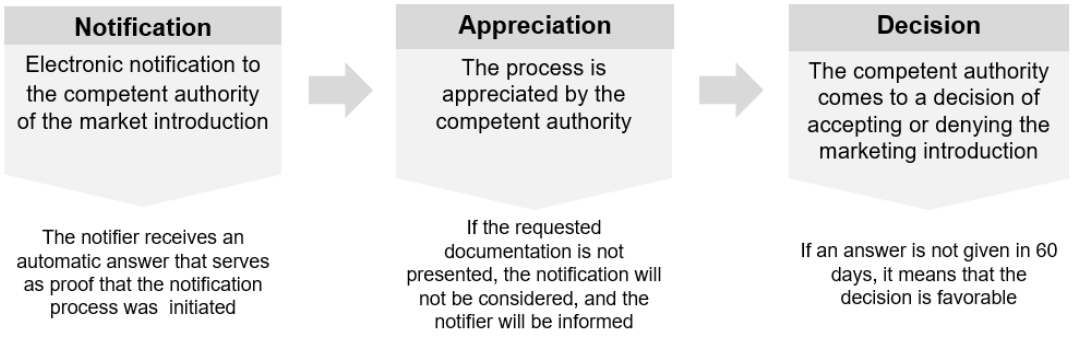

Figure 1.7: Representative scheme of the notification, appreciation and decision process of marketing introduction of food supplements.

1.3.2 Magnesium supplementation

Chemically, magnesium is classified as an alkaline earth metal. It occurs as the mineral part of a large variety of compounds (chlorides, carbonates and hydroxides) and as the free cation Mg^{2+} in aqueous solutions [11, 30].

Magnesium (Mg^{2+}) is an essential nutrient (mineral), i.e, it is required for normal physiological and biochemical body function but can not be produced in the body, meaning that, it has to be provided by the daily diet [31]. This mineral is a co-factor of a wide range of enzymatic reactions, more than 300, especially for reactions involving ATP, being required in several physiological and biochemical functions. For example, magnesium takes part in biochemical pathways such as macronutrients (proteins, fatty acids, etc) degradation, neurological and muscular excitability, DNA and protein synthesis, and regulation of the parathyroide hormone secretion. At a physiological level, magnesium acts as an antagonist for calcium channels, affecting the mechanisms that are regulated by the intercellular concentrations of calcium. So, magnesium is essential for the normal neurological and muscular function. Additionally, by its interaction with phospholipids, it is involved in the regulation of the cellular membrane permeability and affects blood pressure and vessel tone [30–32].

The total body content of magnesium varies between 22 and 26 g in adults. From this amount, 99% is located in the intracellular space, while, less than 1% is located in the extracellular space. Magnesium is mainly stored in the bone tissue (60-65%), whereas only 34-39% of this mineral is stored in the muscle and soft tissues [30, 31].

Magnesium absorption takes place in the intestine, mainly as an ion form. The highest absorption occurs in the ileum and jejunum fractions of the small intestine and, considering physiologic doses, the magnesium absorption can be described as biphasic curve. The intestinal magnesium absorption can be affected by several factors. For example, the dose, the food matrix, the meal composition, and the enhancing and inhibiting factors can influence magnesium absorption. Some known enhancers are vitamin B6 and vitamin D. Calcium, on the other hand, is pointed in the literature as an inhibitor of magnesium absorption [32]. Furthermore, there is evidence that the type of magnesium salt (organic or inorganic) can also affect the magnesium intestinal uptake [31, 32]. The bioavailability of the different magnesium salts seems to be strongly influenced by the solubility in water of the salt. The relation between these two characteristics seems to be: the highest the solubility in water, the highest the bioavailability. Organic magnesium salts tend to have a greater water solubility when compared with inorganic salts, such as magnesium oxide or magnesium sulfate, and therefore tend to have a higher bioavailability [32].

Concerning magnesium elimination, this process occurs mainly by renal excretion. Approximately 100 mg of magnesium are excreted into urine *per* day, while the losses through sweat are relatively low. Nevertheless, during intense exercise, the magnesium losses *via* sweat can increase [30–32].

Magnesium excretion plays a very important role in the regulation of the total amount of magnesium stored in the body, i.e., in the magnesium homeostasis. Since, intestinal absorption interplays with magnesium renal excretion in such a way that, if the level of magnesium stored in the body is superior to its normal value, the extra amount is immediately excreted via renal excretion [30, 31]. When the magnesium intake is low, magnesium may be detached from the bone tissue, in order to assure the the normal serum magnesium level. Hence, a normal magnesium serum level may not be a reliable indicator of hypomagnesia absence. Moreover, this process may induce health issues such as osteopenia or osteoporosis [33].

The recommended daily intake of magnesium varies between different countries and regions. In the EU, the European Food Safety Authority recently defined the daily "adequate intake" of magnesium as 300 and 350 mg for adult women and men, respectively. Moreover, the same Authority defined the dietary reference value of magnesium as 375 mg [11, 30, 31].

Magnesium deficiency, or hypomagnesemia, has many causes. Specifically the most common are insufficient intake of magnesium, alcoholism, aging, antacid therapeutics, diurectics therapeutics, laxatives consumption, chronic stress, diarrhea, menorrhagia, gastrointestinal disorders, extreme practice of exercise, low vitamin B6 intake, low sodium intake and low selenium intake. The clinical signs of magnesium deficiency are mainly muscular weakness, painful cramps, tremor, ataxia, anxiety, irritability and even depression.

In the literature, it is possible to find several studies suggesting beneficial health effects of oral magnesium supplementation. Among others, the magnesium supplementation is suggested to have an effect on: blood pressure, cardiovascular events (stroke and coronary heart disease), arrhythmia, metabolic syndrome, Diabetes Mellitus type 2, cancer, immune system, bone health and fatigue [11, 34, 35].

Studies focusing on the relation between magnesium intake and blood pressure reduction seem to have inconclusive results. A study from 1998 [34] concluded that there may be an inverse association between magnesium intake and systolic and diastolic blood pressure, whereas a more recent study (2014) [35], compromising 14 healthy men, conclude that magnesium supplementation with a 368 mg daily dose did not affect the systolic and diastolic blood pressure of the group study [11, 34, 35]. Regarding cardiovascular events, a meta-analysis carried out in the year of 2013, showed that magnesium intake and risk of cardiovascular disease events (stroke, coronary heart disease and cardiovascular disease) have an inverse relation. Moreover, the same analysis, showed that a magnesium intake between 150 and 400 mg *per* day enables the biggest risk reduction of the cardiovascular events already mentioned [36]. The metabolic syndrome also seems to be affected by the daily intake of magnesium. There is evidence that the intake of 150 mg of magnesium per day has an inverse relation with the risk of metabolic syndrome [11, 37, 38]. It is well known that magnesium plays an important role on bone health, since it has a very important function in the structure of hydroxypatite crystals in the bones.

Along the years, several studies have been made in order to understand if the increment in the daily intake of magnesium improves bone mineral density or bone mineral content, with a big number of them concluding that there is a positive relation [39–41].

1.3.3 Comparative analysis of magnesium salts

Magnesium may be added to food supplements in the form of organic or inorganic salts. The different magnesium salts have different physical-chemical characteristics, that may have a different impact on the product performance. Thus, selecting a magnesium salt is a very important aspect while formulating a magnesium dosage form. The European Food Safety Authority has elaborated a list of all the authorized magnesium sources that can be used in food supplements, such list is presented in the Appendix A.

Initially, to select the magnesium salts to be formulated in the pretended dosage forms, there were analyzed three salt characteristics: solubility in water, molecular weight (g/mol) and the percentage of magnesium weight in each salt molecule. Afterwards, the salt mass needed to accomplish a magnesium dosage of 0,25 g and 0,375 g was calculated (dosages desired for the oral solutions and tablets, respectively). Such characteristics are presented in the Table 1.1 for all the magnesium salts authorized by the European Food Safety Authority (authorized magnesium salts).

A study of magnesium bioavailability from ten organic and inorganic magnesium salts in magnesium-depleted rats showed that the organic magnesium salts have a higher bioavailability then the inorganic salts [42]. Although, like it is possible to observe in Table 1.1, the organic salts have less magnesium in its composition, making it difficult to incorporate in formulations that are meant to deliver a high dosage of magnesium. Taking in consideration the same Table (1.1), it is possible to verify that the magnesium oxide salt is the one with the highest percentage of magnesium (60,30%), meaning that 0,62 g of magnesium oxide provides 0,375 g of magnesium. For this reason, magnesium oxide is largely used in tablets as a magnesium source (see the sub-chapter 1.3.4). However, in the literature magnesium oxide is described as a very low bioavailable compound and, additionally, it seems to cause diarrhea more often then any other authorized magnesium salt [42, 43].

On the other hand, magnesium gluconate only has 5,66% of magnesium weight, being the authorized magnesium salt with fewer magnesium in its composition. However, magnesium gluconate is described in the literature as the authorized salt with the highest bioavailability [42]. Concluding, this compound presents two main disadvantages:

1. Very low percentage of magnesium, making it impossible to compress into *per os* tablets with the desired magnesium dosage;

2. Very low microbial stability in water, making it difficult to formulate into oral solutions.

Regarding the salts bioavailability, the study mentioned above have shown that from the magnesium salts studied, magnesium oxide and magnesium carbonate were the salts with the lowest intestinal absorption. While the salts with the highest excretion were magnesium sulfate and carbonate, and therefore, the ones with lowest body retention. Finally, the study have shown that magnesium gluconate and lactate were the salts with the highest body retention [42].

Considering magnesium citrate, a study held by Lindberg et al. have shown that this salt has significantly higher bioavailability than magnesium oxide, and moreover, is more soluble in water. The same was found by Coudray et al. [42,43]. Magnesium citrate has 11.34% of magnesium and a high solubility in water, representing a good source of magnesium to be used in aqueous solutions. However, its low magnesium percentage make it difficult to compress into a swallowable tablet. Yet, there is a modified molecule of magnesium citrate, trimagnesium citrate, that has a higher percentage of magnesium (16.16%). This molecule is composed by three magnesium ions. It would be interesting to understand if this salt can be compressed in swallowable tablets of 187.5 mg or 375 mg of magnesium. Another salt that may represent a good source of magnesium in oral solutions, is magnesium L-pidolate. As shown in the Table 1.1, this salt is considerably soluble in water. Regarding the bioavailability, a study carried in 2005, showed that magnesium L-pidolate has a similar bioavailability to magnesium citrate [42].

In conclusion, organic magnesium salts have higher bioavailability and are better sources of magnesium when compared to inorganic salts. Nonetheless, some magnesium inorganic salts also show satisfactory bioavailability and therefore can be considered as good magnesium sources, such as magnesium chloride [42]. So, considering what was mentioned before, three magnesium salts were selected to be formulated into tablets:

- Magnesium oxide,
- Magnesium chloride,
- Magnesium citrate.

And, in the same line of thought, three magnesium salts were selected to be formulated into oral solutions:

- Magnesium citrate;
- Magnesium chloride;
- Magnesium L-pidolate.

It is important to highlight, that in order to choose a suitable magnesium salt, it is crucial to consider, besides the salt characteristics, the magnesium dosage desired, the dosage form and the manufacturing process. Thus, to choose the appropriated one it is necessary to study the behavior of the selected salts during the manufacture process and in the formulation.

In order to understand which salts are commonly used as magnesium sources in food supplements a marketing analysis was performed.

Table 1.1: Physical and chemical characteristics of the magnesium salts authorized by the European Food Safety Authority.

Magnesium Salts	Molecular formula	Solubility in water	Molecular weight (g/mol)	Magnesium weight (%)	Salt mass needed to 0.25 g of Mg (g)	Salt mass needed to 0.375 g of Mg (g)
Magnesium oxide	MgO	0.0086 g/100 mL	40.30	60.30	0.41	0.62
Magnesium hydroxide	H_2MgO_2	0.0009 g/100 mL	58.319	41.68	0.60	0.90
Magnesium carbonate	$MgCO_3$	<0.2 g/100 mL	84.31	28.83	0.87	1.30
Magnesium sulfate	$MgSO_4.7H_2O$	51.8 g/100 mL	120.361	20.193	1.24	1.86
Magnesium fumarate	$C_4H_2MgO_4$	4.9 g/100 mL	138.361	17.57	1.42	2.13
Magnesium succinate	$C_4H_4MgO_4$	10 g/100 mL	140.377	17.31	1.44	2.17
TriMagnesium citrate	$C_{12}H_{10}Mg_3O_{14}$	<0.2 g/100 mL	451.113	16.16	1.55	2.32
Magnesium aspartate	$C_8H_{12}MgN_2O_8$	<0.2 g/100 mL	155.392	15.64	1.60	2.40
Magnesium malate	$C_4H_4MgO_5$	<0.2 g/100 mL	156.376	15.54	1.61	2.41
Magnesium glycinate	$C_4H_8MgN_2O_4$	<0.2 g/100 mL	172.423	14.10	1.77	2.66
Magnesium glycerophosphate	$C_3H_7MgO_6P$	Insoluble	194.362	12.505	2.00	3.00
Magnesium pyruvate	$C_6H_6MgO_6$	10 g/100 mL	198.413	12.25	2.04	3.06
Magnesium lactate	$C_6H_{10}MgO_6$	<0.2 g/100 mL	202.445	12.01	2.08	3.12
Magnesium chloride hexahydrate	$Cl_2H_{12}MgO_6$	167 g/100 mL	203.31	12.00	2.08	3.13
Magnesium citrate	$C_6H_6MgO_7$	52.6 g/100 mL	214.41	11.34	2.21	3.31
Magnesium taurate	$C_4H_{12}MgN_2O_6S_2$	Very soluble	272.573	8.917	2.80	4.21
Magnesium L-pidolate	$C_{10}H_{12}MgN_2O_6$	32.2 g/100 mL	280.519	8.664	2.89	4.33
Magnesium salicylate	$C_{14}H_{10}MgO_6$	0.000686 g/100 mL	298.533	8.14	3.07	4.61
Magnesium gluconate	$C_{12}H_{22}MgO_{14}$	43.2 g/100 mL	414.60	5.86	4.26	6.40

1.3.4 Magnesium dosage forms in the Portuguese market

A review on the products sold in Portugal containing magnesium was made in order to understand the market composition and its tendencies. Initially, it was made a research concerning the magnesium supplements and medicines present in the Portuguese market. The aim of this research was to understand which dosage forms are used, and which excipients and magnesium salts are the most used in the formulation of magnesium products. Two different sources of information were used: the Infomed website and the *Farmácias Portuguesas* website. The Infomed website (an Infarmed data base of all the medicines present in the Portuguese market) was used to search for magnesium medicines and the *Farmácias Portuguesas* website was used to find the magnesium supplements sold in pharmacies.

Table 1.2 lists the magnesium oral solutions resulting from this search. Additionally it is presented a list of the excipients which compose such products. The most used magnesium salts in oral solutions are magnesium chloride and magnesium L-pidolate. The magnesium salt dosage varies between 1500 mg and 83.3 mg. Regarding the excipients, it should be noted that all the magnesium oral solutions have sodium saccharin, being unappropriated for diabetics. Another important feature of these products is the common combination of magnesium with vitamin B6 (pyridoxine hydrochloride). Apart from its well known nutritional benefits, vitamin B6 appears to enhance the magnesium intestinal absorption, hence the combination [44–46].

Table 1.3 shows an exhaustive list of the magnesium products sold in the form of tablets in Portugal. Magnesium oxide is the most used salt in tablets. As mentioned before, this salt has very low bioavailability and, moreover, is the magnesium salt with more adverse reactions. Within this, it would be expected that this component was not used in magnesium supplements. However, the magnesium oxide salt has a high percentage of magnesium and a low molecular weight, meaning that, in order to achieve a considerable amount of magnesium in one tablet it is necessary a smaller amount of magnesium oxide than of any other magnesium salt. Concluding, magnesium oxide is largely used as a magnesium source in tablets since it facilitates the tablet manufacturing, resulting in small tablets that deliver a considerable amount of magnesium. Taking in consideration the existing products in both dosage forms it is possible to conclude the following:

- There is a lack of magnesium oral solutions appropriated for diabetics in the food supplementation market,
- Most of the magnesium tablets in the market are composed by a poorly absorbed salt, jeopardizing the efficiency of the product. Thus, it is necessary to develop a magnesium product that is satisfactorily absorbed and therefore effective.

In the following sub-chapters it is possible to find a review on the formulation aspects of the magnesium dosage forms to be developed: oral solution and tablet.

1.4 Oral solutions

A solution can be defined as two or more substances homogeneously mixed. While solubility corresponds to a chemical property that expresses the ability of a given substance, the solute, to dissolve in a solvent. Solubility is measured by the maximum amount of a solute dissolved in a solvent at equilibrium, resulting in a saturated solution. Regardless of that, in pharmacy technology, solutions dosage forms are not saturated in order to prevent precipitation of crystals due to seeding of particles or changes of pH, and temperature [47]. The EP classifies oral solutions as liquid preparations for oral use that contain

one or more API in a proper vehicle, and are supplied in single or multidose containers [21].

Liquid formulations, including oral solutions, offer many benefits, e.g., are easy to dose and administer. Additionally, oral solutions offer the advantage of eliminating many rate-limiting steps in the gastrointestinal absorption of the API, since the API is already in the dispersed phase. Nevertheless, those benefits of oral solutions are proportional to the many problems that can occur in their formulation, such as, chemical and physical instabilities or taste-masking needs. These problems may represent great challenges for the development scientist and may require highly specialized formulation techniques to overcame them. In the next sub-chapter some of the most important challenges in formulating oral solutions are discussed [48].

Table 1.2: List of magnesium oral solutions present in the Portuguese market.

Brand name	API or nutritional substance	Dosage	Excipients	Marketing classification
Magnoral	Magnesium chloride hexahydrated	1028,4 mg/10 ml	Sodium saccharin, Sodium methyl benzoate, Sodium propyl benzoate, Ponceau red, Citric acid, Strawberry flavor, Ethanol 96%, Neohesperidine, Sorbitol (70%), Purified water.	Over the counter medicine
Magnesona	Magnesium L-pidolate	1500 mg/10 ml	Sucrose, liquid sorbitol, methyl p-hydroxy benzoate, propyl p-hydroxybenzoate, sodium saccharin, mandarin essential oil, E110 dye, pyroglutamic acid, and purified water	Over the counter medicine
Absorvit Magnésio Resist	Magnesium L-pidolate, dried extract of Siberian ginseng, vitamin B6	200 mg + 50 mg + 1,4 mg/10ml	Purified water, sorbitol, citric acid, aroma, potassium sorbate, sodium riboflavin, sodium benzoate, sucralose, saccharin sodium	Food supplement
Movitum Magnésio Fos	Magnesium chloride, fructooligosaccharides, vitamin B6	400 mg + 1425 mg + 0,82mg/10ml	Purified water, citric acid, aroma, potassium sorbate, sodium benzoate, aspartame, sodium cyclamate, sucralose, sodium saccharin, sodium riboflavin	Food supplement
Bio-Ritmo	Arginine Aspartate, Magnesium Citrate, Vitamine B6, D-Biotin	950 mg + 83,3 mg + 4 mg + 150 μg	Water, citric acid, potassium sorbate, sodium benzoate, aspartame, sodium cyclamate, dye (E 102 and E 124), sodium saccharin	Food supplement

Table 1.3: Magnesium tablets present in the Portuguese market.

Brand name	API or nutritional substance	Dosage	Marketing classification
Magnespasmil	Lactato de magnésio	60 mg	Over the counter medicine
Miostenil	Magnesium Aspartate, potassium Aspartate	250 mg + 250 mg	Medicine
Solgar: Magnesium with vitamine B6	Magnesium oxide, vitamin B6	400 mg + 25 mg	Food supplement
ABSORVIT® MAGNÉSIO B6	Magnesium oxide, vitamin B6	495 mg + 2 mg	Food supplement
Magnésio rapid	Magnesium oxide, vitamin C, niacin, zinc, potassium, pantothenic Acid, manganese, vitamin B6	375 mg + 80 mg + 16 mg + 10 mg + 8 mg + 6 mg + 2 mg + 1,4 mg	Food supplement
Formag magnésio marinho	Magnesium oxide, vitamin B6	150 mg + 1 mg	Food supplement
Magnésio xamane	Magnesium carbonate, magnesium oxide, vitamin C, vitamin B6	850 mg + 175 mg + 40 mg + 1,4 mg	Food supplement
Varimine Magnésio AP	Magnesium citrate, magnesium glycerophosphate	187,5 mg (magnesium)	Food supplement
MyThera magnésio	Magnesium carbonate	144 mg (magnesium)	Food supplement
Forté Magnésio Marinho 300	Magnesium oxide, Vitamin B6	300 mg + 2 mg	Food supplement
Valdispert Noite magnésio	Magnesium oxide, passiflora extract, Vitamin B6	187,5 + 300mg + 0,7 mg	Food supplement

1.4.1 Challenges in formulating oral solutions

While formulating oral solutions there are several characteristics and attributes of the raw materials, of the dosage form itself and of the manufacturing process that must be considered and studied. Such aspects and attributes may represent a challenge for the pharmaceutical development scientists.

An important characteristic to consider is the solubility of the API. The amount of API dissolved *per* unit of a solvent is a critical parameter that is impacted by many factors such as temperature, presence of electrolytes, hydration, crystalline form and the nature of the drug crystals [47–49]. The solubility of a drug can be enhanced or reduced depending on its interaction with other components of the dosage form or even depending on the pH of the solution. Complexation is an example of a process that results form interactions between the API and other components of the dosage form and modify the solubility of the drug substance. Organic substances in solution commonly tend to associate with each other by weak bonds, forming a complex that readily disassociates. However, there are processes of complexation that form stronger complexes, in these cases the solubility of the drug can be extremely impacted and modified. Complexation frequently results in the loss of the API. Furthermore, variability in the solution pH may modify the API solubility and the microbial preservatives activity, which may cause serious stability problems. [48].

The taste of oral solutions is another aspect of main importance and can represent a challenge in the formulation process. A lot of API have a salty, bitter or sour flavor and provoke an unpleasant sensation when administered *per os*. Thus, a combination of efforts is required to mask these tastes. There is a large number of sweeteners and flavor agents available to mask the unpleasant flavor of dosage forms compounds. The choice of a sweetener and flavor may seam simple and less important when compared with other aspects of the formulation process, but the role of flavors and sweeteners should not be minimized since it may determine if a patient comply with the therapeutics or not [47–49]. Concluding, the selection of a flavoring or sweetener may not only represent a challenge, but also an opportunity. Since, there is not a defined method to deal with elegance issues but the development scientist have the flexibility to develop a product that is well received by the patient [47].

The most commonly used solvent in oral solutions is water, which serve as a good medium for microbial growth. Microbial growth in oral solutions represents a potential source of harm for the patient and must be prevented and controlled. Therefore, microbial preservatives are always a component of oral solutions. The preservative efficacy must be proved, i.e, it should be proved that the preservative encompasses adequate protection against microbial growth during the shelf-life and the use of the product. There is a large number of approved preservatives that can be used in the manufacture of dosage forms. The allowed concentrations of each preservative are presented in the Pharmacopoeia. Nevertheless, the preservative concentration in the final product must be the minimum concentration possible, i.e., the minimum concentration that delivers the appropriate level of efficacy. Thus, European Pharmacopeial

tests must be performed in order to justify the preservatives concentrations concerning its efficacy and safety. It should also be taken in consideration that the preservative efficacy may be impacted by several factors, such as the physical and chemical characteristics of the final product, especially the solution pH, and the type and level of microbial contamination [50].

It is important to underline that, even though, preservatives represent an essential component in almost every oral solution, they commonly give an unpleasant taste to the dosage form and may cause allergic reactions in some individuals. Thus, the selection of a preservative may represent a challenge in pharmaceutical development since it is essential to protect the dosage form against microbial growth, but it is fundamental to make the oral solution safe and pleasant for the patient. Moreover, it is crucial to ensure that the preservative efficiency is not impacted by the formulation physical and chemical characteristics. Concluding, the choice of a given preservative should be supported by appropriate literature and experimentation, and not by trial and error [47–49].

Another aspect of main importance, when designing a formulation, is the selection of excipients. An oral solution may have several excipients, with different proposes, in its composition. Sweeteners, flavorings, coloring agents, antimicrobial preservatives, antioxidants, electrolytes and buffers are just an example of the wide range of excipients that may compose oral solutions. The excipients attributes may impact the performance, safety and quality of the final product and, additionally, affect the manufacturing process. A clear example, is the relation between microbial contamination of the excipients and further microbial contamination of the final product. Thus, it is essential to evaluate the microbial quality of the excipients in order to prevent and control the microbial growth in the final product. Regarding impurities, the same line of thought can be followed. An example of an oral solution excipient that must follow a tight evaluation of its microbial quality is water. The water used in oral solution should, at least, comply with the specifications for purified water. Even though, there are no regulations requiring the use of purified water in oral solutions, it is widely recommended in Good Manufacturing Practices (GMP) guidelines and pharmacopoeias [48]. Another characteristic of excipients that should be evaluated regarding its possible impact on the manufacturing process and final product, is the particle size. The particle size may impact the rate of dissolution of the excipients during the manufacturing process. Excipients with thinner particles may dissolve faster as a result of a bigger surface area in contact with the solvent. While, particles with a larger size have a smaller surface area and therefore may have a slower dissolution rate. The particle size of a given substance can be modified by its crystal form, salt form or by the breaking process. Regarding the manufacturing process, mixing at higher speed leads to a breakage of the particle and a to faster dissolution rate. Finally, it is always necessary to evaluate the chemical and physical stability of the excipients and, obviously, the possible incompatibilities that the excipients may have between them or with the API [47].

Regarding the stability of oral solutions, there are several reactions that can generate chemical in-

stability. Such instabilities are mainly caused by interactions between the components of the dosage form or by interactions with the container materials. The main chemical instability reactions occurring in oral solutions are hydrolysis, isomerization and oxidation. Moreover, nearly all the physical instabilities occurring in oral solutions are a result of a prior chemical instability. Such reactions may be precipitation, crystal formation, swelling of the container, droplets of fog inside the container or cloudiness [47]. Therefore the final product stability is a very important aspect to consider and study while formulating a new product. The perception of stability problems enables the definition of a design space and, consequently, enables the establishment of a product control strategy [47].

Summarizing, through a literature review, it was possible to identify five main aspects of oral solutions that can represent a challenge for the development scientist: solubility of the API, taste, selection of the microbial preservative, selection of the excipients and the stability of the final product. To overcame these challenges it is important to give special attention to these aspects. Nonetheless, there are other aspects that should be considered during the formulation process of oral solutions. Such aspects may be less critical for the quality of the final product or for the manufacturing process, but have to be considered and study as well. Following a review on these aspects is presented.

1.4.2 Formulation aspects of oral solutions

Along with the aspects that represent an important challenge in oral solution formulation, there are other aspects, although not so impacting, that must be considered during the development process in order to produce an oral solution with quality [47, 48, 51].

The appearance of the oral solution is an example of those formulation aspects. The appearance itself it is not a determinant quality factor but, if the appearance attributes do not meet the patients expectations, it may lead to therapeutic interruption. Therefore, it is important to consider the appearance of the solution, concerning the color and the particles in solution. The formulation should look homogeneous, without any detectable particles or precipitates in solution and, with a uniform color. Moreover, these characteristics should be maintained during the shelf-life and the usage time of the product. There are many coloring agents available in the market that maybe used to give an attractive color to the dosage form. Normally, the color of the oral solution is coordinated with the flavour, e.g., an oral solution tasting like strawberry normally is pink or light red [47, 48, 51]. The presence of colouring agents in pharmaceuticals and nutritional products is highly regulated. Thus, the introduction of such compounds in dosage forms can represent an issue and the choice of the colouring agent must always be in accordance with the current regulation. Specifically, colouring agents must satisfy the requirements of Directives 78/25/EEC, as amended and/or 94/36/EC [47, 48, 50, 51].

Another aspect to consider is the order of addition of the solutes to the solvent. The order of addition should be adapted to the quantity of each excipient, their solubility, physical stability and the solution

temperature. In the sense that, less soluble raw materials will take longer to dissolve, and the same may happen for large amounts of a given raw material. Thus, it is a common procedure to start by adding to the solvent the less soluble, or with a higher concentration, raw materials. The solution temperature should be appropriate to the raw material being added, in order to prevent its precipitation or evaporation. This aspect is specially important concerning the order of addition of flavors and other volatile substances (e.g. vitamins). For example, if the addition of a certain raw material to the solvent generates an exothermic reaction, it is important to add this raw material as far as possible from the addition of volatile substances, in order to avoid losses by evaporation [47, 48, 51].

Finally, the oral solution filling process should be considered. Since this unit operation is impacted by the solution viscosity, surface tension and by the compatibility with the filling machine, it is important to study such aspects and to design the filling process accordingly. The filling process of liquids usually occurs at high temperatures and positive pressure to facilitate the liquid flow. In these cases, special attention should be taken with volatile substances in order to avoid losses [48].

Summarizing, while formulating oral solutions there are several aspects to consider, some represent bigger challenges then others but, by a prioritize manner, all should be considered and studied in order to produce an oral solution with quality, safety and that complies with the regulatory specifications.

Since it was suggested the development of two dosage forms (oral solution and tablets), it is presented a review on the state of the art of tablets formulation.

1.5 Tablets

Solid dosage forms are the most frequently vehicles for the administration of API and nutritional substances by the oral route. Tablets are unit dosage forms and are the most frequently used solid forms. these solid dosage forms are designed as a single rigid unit, comprising a mixture of ingredients, that contain a well defined and accurate dose of a drug or, concerning food supplements, a nutritional substance [47]. In the EP, tablets are defined as ”solid preparations each containing a single dose of one or more active substances. They are obtained by compressing uniform volumes of particles or by another suitable manufacturing technique, such as extrusion, moulding or freeze-drying. Tablets are intended for oral administration” [20, 21].

Tablets can be presented in different degrees of complexity such as, immediate release systems that are relatively simple, or modified release tablets which represent more complex systems. Normally, tablets are classified according to its release behaviour, e.g., immediate or prolonged release tablets. But can also be classified according to its manufacturing process, for example, the term direct compressed tablets is common in literature, referring to tablets that result from a direct compression tableting process [47].

Most tablets are designed to be swallowed and then rapidly disintegrated in the gastrointestinal tract, where, in some point, the absorption of the drug or nutritional substances occurs. Nevertheless, there are tablets intended to be placed in the oral cavity and to to be absorbed there or to be chewed before swallowing. Additionally, sustained and controlled release tablets are widely produced formulations [20, 21, 47].

The present work will be focused on the formulation of immediate release tablets. These formulations are designed to immediately release the drug (or nutritional substance) in the gastrointestinal system, after *per os* administration [47]. Specifically, the EP states that immediate release tablets must disintegrate in water in a maximum time of 15 minutes [20, 21].

The manufacturing process of immediate release tablets, in general terms, can fallow two different processes: direct compression and granules compression. Direct compression is the most simple process. This process encompasses two unit operations: mixing and tableting. Regarding its simplicity and consequent time/economic efficiency, direct compression is the preferable process for the manufacture of tablets. However, in order to successfully execute direct compression it is crucial that the raw material particles have good flowability characteristics, high compactibility and a low segregation tendency. Not all particles present such characteristics and, therefore, an alternative manufacture process must be applied, i.e., granules compression. In the granules compression process, a pre-compression unit operation is performed, in order to increase the compactibility and flow properties of the materials. Such unit operation is called granulation. There are different methods of granulation, the most common being wet and dry granulation. Regardless of the method, granulation causes particles to adhere to which other and form agglomerates, making the size of the particles bigger. For the purpose of this work, granules compression (or indirect compression) will not be further discussed, focusing only on the process of direct compression. Since, this was the selected manufacture process, by Medinfar, for the manufacturing of magnesium tablets [51].

Following, it is presented a review on the challenges of formulation immediate release tablets, manufactured by direct compression.

1.5.1 Challenges in formulating immediate release tablets

Immediate release tablets may seem less complex systems to formulate than those of prolonged or retarded release, however, the formulation process of these systems can encounter some challenges that need special attention to be exceeded. As mentioned before, there are two main processes of immediate release tablets manufacture: direct compression and granules compression. This work will be focused on the formulation of immediate release tablets by direct compression.

Not all materials have the characteristics necessary to successfully be direct compressed. A powder must have good flowability characteristics, high compressibility and a low segregation tendency in order

to successfully be compressed into a tablet. The assessment of those characteristics, in order to select the excipients, may represent a challenge while formulating immediate release tablets. Therefore, increased efforts should be taken to understand the influence of those characteristics in the product performance [51–53].

To better understand how the manufacturing process impacts the excipient selection and the product quality attributes, it is necessary to understand the stages in tablet manufacture. Those stages are represented in the Figure 1.8. The first stage is the die filling with powder, following, the compaction process is initiated as the upper punch descends and a given pressure starts to be applied to the powder (see Figure 1.8: B). As the pressure increases, the compression and consolidation process of the powder happens (Figure 1.8: C). When a maximum defined value of pressure is reached, the upper punch rises and the compaction process is completed, resulting in a tablet. Finally, the formed tablet is ejected as the the lower punch rises and a pushing device removes the tablet from the equipment (see Figure 1.8: E) [51].

The compaction step is the most critical stage of tablet manufacture, in which compression and further consolidation of particles occur, as a consequence of an applied force. During an initial state of compaction, the material particles are brought together, reducing the volume of the material, i.e., compression occurs. As the compaction force increases, the particles start to deform and interact. As a consequence, the mechanical strength of the material increases and the consolidation process is started. Following, the particles create bonds with each other and the consolidation process is complete. Compaction is a very important process regarding the tablet manufacture, since the physical critical attributes of tablets are strongly impacted by this process. An example of those characteristics are density, hardness or friability. Moreover, the integrity of the tablet and the bioavailability of the API maybe influenced by the parameters of the compression process [51].

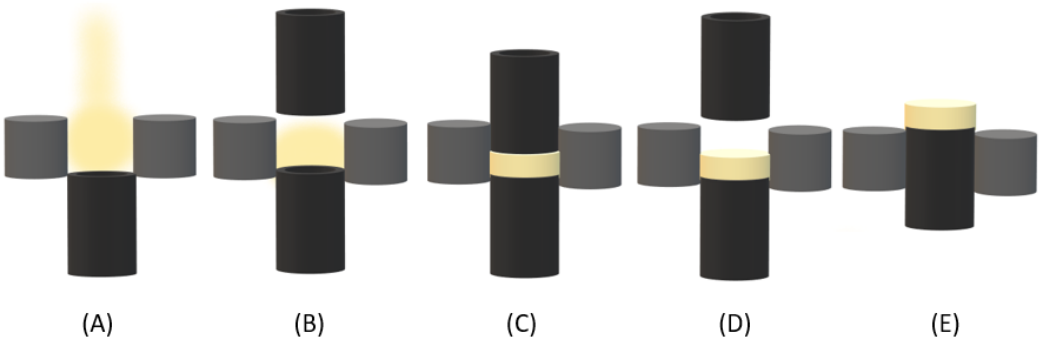

Figure 1.8: Representation of the stages of tablet manufacturing. A – Die filling with powder; B – Compaction: the upper punch descends and enters the die; C – Compaction: The powder is compressed and consolidated; D – Decompression: The powder compaction is completed and the upper punch rises; E – Ejection: the lower punch rises and ejects the formed tablet.

In order to describe the compaction characteristics of powders, two concepts are used, compressibility and compactibility. Compactability is the ability of a given powder to form a concise and strong compact. Whereas, compressibility can be defined as the ability of a material to reduce its volume while a given pressure is applied to it. The volume of the material is reduced as the pressure applied forces the gaseous-particles to leave the system, bringing the solid-particles closer. Compressibility is commonly expressed in the literature by a relationship between the tablet porosity and the compaction pressure applied. Powder compressibility and compactability become a real challenge when tablets have a high dosage of API, in these cases, powder behaviour is highly dependent on the API characteristics. Therefore, understating and controlling the material characteristics is fundamental to control the product quality attributes. Nevertheless, the definition of an appropriate set of material characteristics may be a challenge, since this process requires time and know-how. In order to overcame this challenge, development scientist may performed DoE, varying the factors that critically impact compactibility and studying the impact on the product critical quality attributes [51–54].

The factors that critically impact the powder compactibility can be narrow to the following three:

1. Material and formulation aspects;
2. Processing attributes, such as the operation conditions of the tablet machine;
3. Environmental factors such as the relative humidity [51].

Considering the material aspects, it is possible to find in the literature several papers discussing the influence of the particle characteristics on the compactibility of the powder. Such characteristics can be described as:

- **Particle size and particle size distribution:** Particle size reduction seems to decrease the tablet tendency to fragment. It seems that the tablet tensile strength and the particle size have an inverse relation, i.e, the smaller the particles the bigger the tensile strength of the tablet. Moreover, a well-designed particle size distribution generates favorable blending conditions.
- **Particle shape:** Particle irregularity appears to improve the compressibility of the particles that fragment to a limit extent during compression. On the other hand, for particles that considerably fragment during compression, the initial shape of the particles does not influence the tablets strength. [51, 52, 55, 56]

Alongside with the particles characteristics of raw materials, the amount and type of excipients also have a great impact on the tablet characteristics and in the compression process. The selection of the appropriate excipients and of their concentration in dosage form is critical in the development of a high quality pharmaceutical product. The typical excipients used to formulate solid dosage forms are: diluents, binders, lubricants, glidants and disintegrants. Excipients are added to the formulation

with different intentions and may have different impacts on compression. Binders are an example of a group of excipients that have a great impact on compression. This class of excipients is added to the formulation to promote the cohesive attraction between the powder particles. Consequently, binders facilitate the powder flowability by creating small granules in the mixture, which culminates in a powder with better compression characteristics. Lubricants are another group of excipients that have a positive impact on compression, by reducing the friction between the power and dye wall [51–53].

The direct compression of a powder is also impacted by the process parameters. The the manufacture process has a great influence on the dosage form quality attributes. For example, the mixing time and compression speed may impact content uniformity and, consequently, disintegration time and dissolution. Additionally, the blending process has a well described effect on blend homogeneity. Which has an impact on several product characteristics such as appearance, hardness, friability, dissolution, content uniformity and disintegration time. Blend heterogeneity can also difficult the compression process, originating tablets with appearance defects.

Not well controlled and understood process parameters and formulation characteristics culminate in defective tablets. Preventing such defects may represent a major challenge in formulation development. Table 1.4 and Figure 1.9 describe the most common tablets defects. From those, the most common are capping, lamination and sticking. Even though these defects are considerably affected by the process parameters, the formulation itself has an impact on the occurrence of such defects [51–53]. Inadequate compression force and insufficient binder amounts may lead to capping, lamination or cracking. Insufficient lubricant amount may cause sticking and chipping problems. Summarizing, it is of main importance to understand and control formulation characteristics and process parameters, in order to avoid appearance defects that may lead to batch rejection [54].

Concluding, immediate release tablets are very attractive dosage forms to the pharmaceutical industry, due to its manufacture simplicity and time-resources efficiency. Nevertheless, what may seem a simple dosage form, is in fact a formulation that may lead to several development challenges. The API and excipient selection, the raw materials characteristics control, and the definition of process parameters, represent the bigger challenges when formulating immediate release tablets by direct compression. The QbD approach to pharmaceutical development encompasses several steps and tools that may facilitate the overcoming of such challenges.

Besides the challenges that may arises during immediate release tablets there other aspects that must be considered in order to produce quality tablets.

1.5.2 Formulation aspects of immediate release tablets

During formulation development of immediate release tablets there are some aspects that may represent a bigger challenge and others that are easier to overcome. Nevertheless, the simpler aspects of tablets

formulation should not be despised. these aspects may not be critical for product quality or patient safety, but have to be considered in order to produce a product that meets the desired performance.

Appearance is an example of such aspects. Tablets must comply with minimal elegance criteria, such as color, texture, shape and size. The color and texture of the selected excipients impact the tablet appearance. Depending on the excipients used, the tablets may have a white, gray or yellow color. The texture of some excipients, also have an impact on the surface appearance of tablet (rugous or smooth). An unpleasant tablet appearance (rugous surfaces, heterogeneous color) may discourage patient compliance. Moreover, changes in tablet appearance, specifically in color, commonly represent a sine of excipient-drug substance interaction. In conclusion, the appearance of tablets has to be monitored in order to avoid appearance defects and produce quality tablets.

Table 1.4: Common tablet defects

Tablet defect	Description
Capping	Partial or total separation of the top surface of the tablet, due to air entrapment and particles characteristics.
Lamination	Tablet separation in several layers, due to air entrapment and particles characteristics.
Cracking	Presence of cracks on the upper and lower surfaces of the tablet, rarely on the side.
Chipping	Partial or total breakage of the tablet edges.
Sticking	The compressed material adheres to the surface of the punch.
Picking	The compressed material sticks to the letters or designs on the punch surfaces producing a defected tablet.

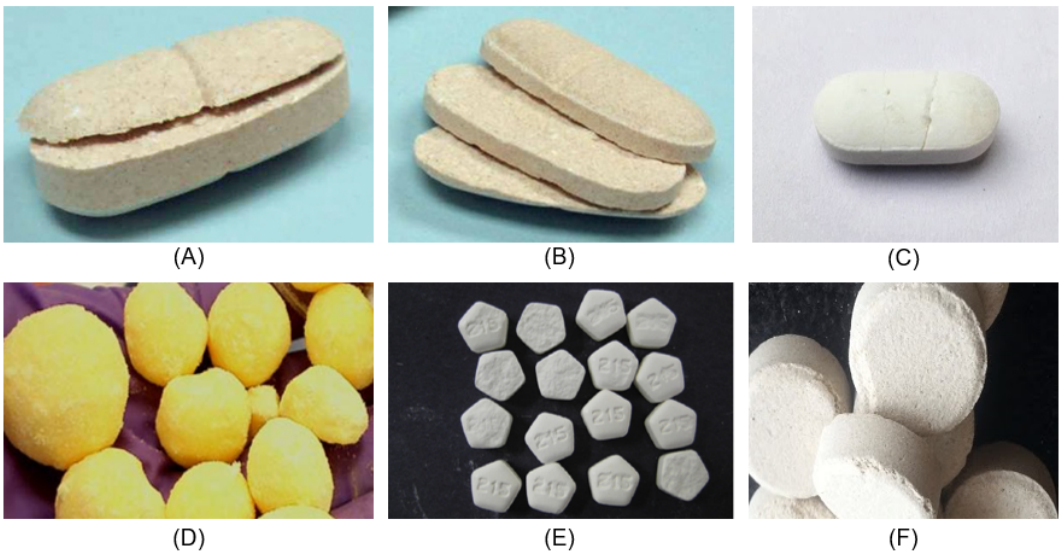

Figure 1.9: The most common tablets defects. A - Capping; B - Lamination; C - Cracking; D - Sticking; E - Picking; F - Chipping.

Another aspect that must be considered is economics. As already mentioned, immediate release tablets may be produced by direct compression or indirect compression. Direct compression is rather a cheaper process when compared with indirect compression. Indirect compression encompasses a granulation unit operation that requires many resources, such as energy, water and time. On the other hand, direct compression performance is highly dependent on the material attributes, consequently it is very common to acquire excipients and drug substances especially treated for direct compression. Such especially raw materials are normally expensive. Concluding, an economic evaluation should be done in order to choose the process that betters meets the company expectations. Additionally, the price of excipients should be considered in order to assure that a cost-efficient formulation is being developed.

Ultimately it should be taken in consideration that tablets must comply with certain specifications, these specifications are presented in the several Pharmacopoeia. Although food supplements do not fall in the scope of the Pharmacopeial criteria, it is common to follow such specifications. Given the purpose of the present work, these specifications will not be discussed.

2

Aim

The overall aim of this work is to develop safe, stable and effective magnesium oral solutions and tablets, that are acceptable and indicated for a large number of patients and fall under the classification of food supplements. Moreover, since the market shows a lack of bioavailable magnesium food supplements, both formulations should encompass considerably bioavailable magnesium and vitamin B6 sources. Thereby, it is suggested the application of QbD approach to the development of both dosage forms. The application of this approach to the formulations development relays on the accomplishment of the following common objectives:

- Establishment of QTPP that enables the identification of the product CQAs;
- Application of risk assessment in order to identify the process variables that may encompass high risk for the accomplishment of the product CQAs;
- Study and understand how the high risk process variables affect the product CQAs in order to control them;
- Selection of a combination of process settings that enable a formulation that complies with the QTPP criteria, using DoE tools;
- Establishment of meaningful specifications.

Additionally, the following specific goals for each magnesium formulation are proposed:

- The oral solution development must culminate in a pleasant solution with a composition free of sugar and low in sodium, therefore, indicated for diabetic and hypertensive patients.
- The tablet development process must culminate in an immediate release tablet produced through direct compression.

3

Materials and methods

Contents

3.1 Materials

In the present sub-chapter the list of raw materials used for the development of the purposed dosage forms is presented.

3.1.1 Tablets

In order to develop magnesium tablets, a large number of raw materials was used. Specifically, there were tested three different magnesium salts: magnesium oxide, magnesium chloride and magnesium citrate. Magnesium oxide was supplied by MAGNESIA (Luneburg, Germany). Magnesium chloride, also mentioned as magnesium chloride hexahidrate, was supplied by Laborspirit (Loures, Portugal). Magnesium citrate was supplied by Dr. Paul Lohmann (Emmerthal, Germany). Pyridoxine Chloride (vitamin B6) was supplied by Farmalabor - Produtos Farmacêuticos, S.A. (Condeixa-a-Nova, Portugal). Regarding excipients, the product Prosolv EASYtab Nutra CM, also mentioned as Prosolv Nutra, was supplied by JRS PHARMA (Rosenberg, Germany). Additionally, the excipients colloidal silicon dioxide (Aerosil 200), Sodium Stearyl Fumarate (SSF), croscarmellose sodium (croscarmellose) and Sodium Starch Glycolate (SSG) were kindly supplied by Farmalabor - Produtos Farmacêuticos, S.A. (Condeixa-a-Nova, Portugal).

3.1.2 Oral solution

Several raw materials were used in the preparation of oral solutions. In particular, magnesium citrate, magnesium chloride (magnesium chloride hexahidrate), pyridoxine chloride (vitamin B6), sodium cyclamate, ponceau Dye, acesulfame, sodium benzoate, potassium sorbate, sodium saccharin, citric acid, neohesperidina were kindly supplied by Farmalabor - Produtos Farmacêuticos, S.A. (Condeixa-a-Nova, Portugal). Additionally, magnesium L-pidolate and sodium gluconate were supplied by MAGNESIA (Luneburg, Germany). Mango flavour was supplied by Mane (Bar-sur-Loup, France). Sodium glutamate was supplied by Ajinomoto (Tokyo, Japan). Stevia (Rebaudiose A $97\,\%$) was supplied by Herboveda (Noida; India).

3.2 Methods

In this sub-chapter are presented the methods used for both development studies.

3.2.1 Risk assessment

Risk assessment was performed during the development process of both dosage forms. The risk assessment procedure applied (risk matrix) was chosen based on the product knowledge detained at the time. The relative risk of each variable was classified as high, medium or low. In Table 3.1 the risk classification matrix is described. The variables that were identified as potential high risk factors required further investigation and understanding. Those that have a medium effect on the products CQAs may demand further investigation and understanding, in order to reduce the risk. Finally, those variables identified as low risk factors do not required further investigation. This relative risk assessment was based on prior knowledge and on literature review (specially based on ref. [57]).

Table 3.1: Relative risk assessment matrix.

	Probability of harm		
Severity	Low	Medium	High
High potential of impact on product quality	Medium risk	High risk	High risk
Medium potential of impact on product quality	Medium risk	Medium risk	High risk
Low potential of impact on product quality	Low risk	Low risk	Medium risk

3.2.2 Magnesium salt mass

In order to find the amount of magnesium salt necessary to accomplish the target magnesium dosages, one needs to know the mass percentage of magnesium in the magnesium salt molecule X_{Mg}. This mass percentage is given by,

$$X_{Mg} = \frac{n \times M(Mg)}{M(S)} \times 100\%, \tag{3.1}$$

where $M(Mg) = 24.31\, g/mol$ and $M(S)(\, g/mol)$ are the molar masses of the magnesium element and magnesium salt molecule, respectively. The term n is the number of magnesium atoms in the salt molecule. Consequently, the mass of magnesium salt needed to accomplish the magnesium dosage Y_S is,

$$Y_S = \frac{D_{Mg}}{X_{Mg}}, \tag{3.2}$$

where D_{Mg} is the target magnesium dosage (mg).

3.2.3 Batch manufacturing for magnesium salt selection

The manufacturing process consisted of direct compression of $100\, g$ batches. First, all the batches components were separately weighted. A precision Mettler Toledo scale (d= $0.1\, g$; maximum weight = $3100\, g$) was used for amounts $\geq 10\, g$, while a Mettler Toledo AG204 analytical scale (d = $0.0001\, g$; maximum weight = $210\, g$) was used for amounts $\leq 10\, g$. Following, the components were blended

using a cube mixer, model AR 402 from ERWEKA at 30 rpm of speed during 15 minutes. For batches consisting of lubricant (SSF) an extra mixing cycle was performed at the same speed for 5 minutes. Finally the powder blend transferred to the single station press machine, model EK 0, brand Korsch and the compression process initiated. The compression parameters were adjusted accordingly to the hardness and weight of the produced tablets.

3.2.4 Effect of lubricant and glidant level on disintegration time

3.2.4.A Design of experiments

To study the effect of lubricant and glidant level on disintegration time a DoE was applied, considering 3 factors varying at 2 levels (2^3 factorial design). The factors considered were the level of Aerosil 200 (varying between $1\,\%$ and $5\,\%$ w/w), the level of SSF (varying between $0.5\,\%$ and $2\,\%$ w/w) and tablet hardness (varying between $10\,kp$ and $18\,kp$). The design was unreplicated due to raw material constrictions. Thereby, 3 center point replicates were added, allowing the investigation of curvature and the estimation of pure error. As illustrated in Table 3.2 the center point corresponds to Aerosil 200 $= 1.00\,\%\,w/w$, SSF = $1.25\,\%\,w/w$ and Hardness = $14\,kp$. All the other formulation and manufacturing parameters were kept invariant, except Prosolv Nutra level. The level of this excipient varied with the amount of glidant and lubricant added to each experiment. From the DoE resulted 11 experiments (experiments number = $2^3 + 3 = 11$), each experiment corresponding to a different formulation, i.e., to a different $100\,g$ batch. The batch production is described further in the sub-chapter 3.2.4.B. The uncoded levels of each factor are demonstrated in Table 3.2 (columns Aerosil 200, SSF and tablets hardness). Disintegration time was considered as the response variable (DT), and tested accordingly to what is described in the sub-chapter 3.2.7. The experimental design, mathematical model, Pareto plots of main effects, interaction plots and the response contour plots were obtained through Minitab 19 software.

3.2.4.B Batch manufacturing

The 11 batches of tablets containing different amounts of Aerosil 200 and SSF were prepared independently and following the order presented in Table 3.2. Every batch was produced with a total weight of $100g$, thus, the level values presented in Table 3.2 also corresponded to the amount weighted in g. For each batch, $77\,g$ of magnesium citrate, the Prosolv Nutra amounts shown in Table 3.2, $0.05\,g$ of vitamin B6 and the amounts of Aerosil 200 and SSF (shown in the same table) were separately weighted. All the components were blended and further compressed as described in sub-chapter 3.2.3. The compression parameters were adjusted accordingly to the out-put tablet hardness and weight. From each batch, tablets were randomly taken for weight and hardness testing (see sub-chapter 3.2.6), disintegration testing (see sub-chapter 3.2.7) and friability testing (see sub-chapter 3.2.8). The resulting tablets

Table 3.2: Batches composition accordingly to the DoE varying Aerosil 200 and sodium stearyl fumarate level. Note: *center points.

Batch	Tablets Hardness (kp)	Aerosil 200 level (% w/w)	Sodium stearyl fumarate level (% w/w)	Prosolv Nutra level (% w/w)
CL1*	14	3.00	1.25	18.70
CL2	18	1.00	2.00	19.95
CL3	10	5.00	0.50	17.45
CL4	18	5.00	2.00	15.95
CL5	10	1.00	2.00	19.95
CL6	18	5.00	0.50	17.45
CL7	10	1.00	0.50	21.45
CL8	18	1.00	0.50	21.45
CL9*	14	3.00	1.25	18.70
CL10*	14	3.00	1.25	18.70
CL11	10	5.00	2.00	15.95

were tested for: i. weight and breaking force and ii. disintegration time; as described in the sub-chapters 3.2.6 and 3.2.7, respectively.

3.2.5 Effect of superdisintegrant level on disintegration time and friability

3.2.5.A Design of experiments

To select a type and level of superdisintegrant, based on the effect on disintegration time and friability, two DoE were separately performed. For the first design, SSG level, and tablet hardness were selected as factors and studied at 2 levels each: minimum and maximum (2^2 factorial design). The experiments were replicated and 3 center points were added to allow the investigation of curvature and the estimation of pure error. Summarizing, 11 experiments were performed, each corresponding to a different formulation and batch (experiments number = $2^2 * 2 + 3 = 11$). For the second design, croscarmellose level and tablet hardness were selected as factors and studied at 2 levels each. As before, the experiments were replicated and 3 center points were added. In conclusion, 11 experiments were performed, each corresponding to a different formulation and batch. For both DoE, all the other formulation and manufacturing variables were kept invariant, except Prosolv Nutra level. The level of this excipient varied with the amount of superdisintegrant added to each experiment. The batches preparation followed the procedures presented in sub-chapter 3.2.5.B. Tables 3.3 and 3.4 list the batches composition. The uncoded levels are demonstrated in the columns SSG level, croscarmellose level and hardness. For both designs, disintegration time (DT) and friability (FRI) were considered as responses and tested accordingly to what is described in sub-chapters 3.2.7 and 3.2.8, respectively. The surface response analysis of the results enabled the establishment of 4 models in uncoded units.

The experimental designs, mathematical models, Pareto plots of main effects, interaction plots and the response contour plots were obtained through Minitab 19 software.

Table 3.3: Batches resulting from the DoE varying the level of sodium starch glycolate and hardness. Note: *center points.

Batch	Tablets Hardness (kp)	Sodium starch glycolate level (% w/w)	Prosolv Nutra level (% w/w)
CS1*	14	3	15.25
CS2	18	4	14.25
CS3	10	4	14.25
CS4	18	2	16.25
CS5	18	4	14.25
CS6*	14	3	15.25
CS7	10	2	16.25
CS8	10	2	16.25
CS9	18	2	16.25
CS10*	14	3	15.25
CS11	10	4	14.25

Table 3.4: Batches resulting from the DoE varying the level of croscarmellose sodium and hardness. Note: *Center points

Batch	Tablets Hardness (kp)	Croscarmellose sodium level (% w/w)	Prosolv Nutra level (% w/w)
CC1	10	0.50	17.75
CC2	10	0.50	17.75
CC3*	14	2.75	15.50
CC4	10	5.00	13.25
CC5	18	5.00	13.25
CC6	18	0.50	17.75
CC7	18	5.00	13.25
CC8	18	0.50	17.75
CC9*	14	2.75	15.50
CC10	10	5.00	13.25
CC11*	14	2.75	15.50

3.2.5.B Batch manufacturing

The batches of tablets containing SSG and of tablets containing croscarmellose sodium were prepared independently and following the order presented in the Tables 3.3 and 3.4, respectively. The batches were produced with a total weight of $100\,g$, thus the level values shown in the mentioned tables also correspond to the amount in (g) weighted. For each batch, $77\,g$ ($\pm 0.1\,g$) of magnesium citrate, the amounts of Prosolv Nutra presented in Table 3.3 and 3.4, $0.05\,g$ of vitamin B6, $4\,g$ of Aerosil 200, $0.7\,g$ of SSF and the amounts (g) of superdisintegrant demonstrated in Table 3.3 and 3.4, were separately

weighted. All the raw materials were blended and compressed as described in the sub-chapter 3.2.3. The compression parameters were adjusted accordingly to the out-put tablets hardness and weight. From each batch, tablets were randomly taken for weight and hardness testing (see sub-chapter 3.2.6), disintegration testing (see sub-chapter 3.2.7) and friability testing (see sub-chapter 3.2.8).

3.2.6 Weight and breaking force testing

In order to perform weight and hardness tests, a sample of 6 tablets was collected for each manufactured formulation. A Mettler Toledo AG204 analytical scale (maximum weight = $210\,g$; d = $0.0001\,g$) was used to individually weight each tablet and a Vanderkamp VK 200 tablet hardness tester was used to individually test each tablet breaking force. Finally, the average and standard deviation of the mentioned values were calculated using Microsoft Excel software. The tablets breaking force is commonly mentioned as hardness, therefore, the term tablet hardness should be interpreted as tablet breaking force.

3.2.7 Disintegration testing

The disintegration tests performed followed what is described in the EP (8^{th} edition) for tablets and capsules of normal size. Therefore, the disintegration time was determined by placing a sample of 6 tablets in a disintegration apparatus (Venkel VK 100). Each tablet was placed in a different basket with one disc, the set of baskets was then submerged in 900 mL of water at $37^o\,C\,\pm 0.5^o\,C$. The test was consider complete when all the tablets were completely disintegrated and the time was registered in minutes. It is important to highlight that all the formulations were separately tested and that all the samples were randomly collected after the compression parameters adjustment.

3.2.8 Friability testing

Friability testing followed the EP (8^{th} edition) standards for uncoated tablets. Consequently, for each formulation, it was collected a sample of 10 whole tablets and each tablet was gently swept, using a bristled brush, in order to remove surface dust particles. The sample was then accurately weighted, using a Mettler Toledo AG204 analytical scale (d = $0.0001\,g$), and the initial weight - W_i - registered considering six significant figures. Following, the tablets were placed in a Vankel friability drum and rotate 100 times. Then, the sample was cleaned and weighted as before; the final weight - W_f - was registered considering six significant figures and, ultimately, the percentage of mass lost by friability - M_L - was calculated, applying the following expression,

$$M_L = \frac{W_i - W_f}{W_i} \times 100\,\%. \tag{3.3}$$

It is important to clarify that the expression ”whole tablets” stands for tablets with a round and consistent appearance, without any signs of fissures or cracks. Moreover, it should be kept in mind that each formulation (batch) was separately tested and that each test was performed immediately after compression.

3.2.9 Magnesium salts palatability characterization

In order to characterize the palatability and stability in water of trimagnesium citrate, magnesium chloride and magnesium L-pidolate, the salts were dissolved in $10\,mL$ of purified water using a magnetic stirrer plate (10 minutes at 30 rpm). After 20 minutes of rest, each solution was qualitatively tested for taste. In addition, the appearance of the solution was recorded: i. immediately after solubilization, ii. after one day, and iii. after seven days. For each salt the amount required to achieve a magnesium dosage of $250\,mg$ was weighted using a Mettler Toledo AG204 analytical scale (maximum weight = $210\,g$; d = $0.0001\,g$) and then dissolve. This amount was calculated through the Equation 3.1 and 3.2.

3.2.10 Oral solution batch preparation

The 12 oral solutions prepared for the excipient selection had a total volume of $50\,mL$ and a magnesium concentration of $25\,mg/mL$. The manufacture process of each solution was as follows:

1. Separately weight every solid solute.
2. Weight $40\,g$ of purified water (EP standards) in a proper vessel. Star agitation (in a magnetic stirring plate) and add the defined amount of citric acid.
3. Add 14.45 of magnesium L-pidolate and homogenize until complete dissolution.
4. Add the designated amounts of sweeteners one by one, in descending order. After complete dissolution, add the corresponding amounts of antimicrobial preservatives, following the same order. Following, add the defined amounts of the flavoring agent designated.
5. Measure the temperature solution (T), if $T \leq 25^{o}\,C$ add $7\,mg$ of vitamin B6 and let homogenize. Add the designated amount of dye and the Mango flavor. When dissolution is complete, stop agitation and make up the solution volume for $50\,mL$ using purified water. Start agitation again and keep it for 10 minutes.

It should be taken in consideration that the agitation was kept constant during the whole process of solutes solubilization (400 rpm). Moreover, each solute was only added to the system when the previous solute was completely dissolved. The procedure was established based on the Company procedures and literature review (in particular, ref. [48, 51]).

3.2.11 Taste score

The oral solutions manufactured during the excipients selection study were tested for palatability by a group of 10 volunteers. Each volunteer was asked to give a sweet and sour score at each solution, accordingly to the descriptions presented in Table 3.5. The final scores were calculated as means and illustrated at bar charts, using Microsoft Excel software.

Table 3.5: Oral solutions taste score.

Score	Sour taste description	Sweet taste description
1	Extremely sour	Not sweet and unacceptable
2	Sour	Very sweet and unacceptable
3	Strong sour aftertaste	Tasteless
4	Moderately sour	Not sweet but acceptable
5	Weak sour aftertaste	Moderately sweet
6	Weak sour	Sweet
7	Not sour	Very sweet but acceptable

3.2.12 Accelerated and real time stability studies

With the propose of performing stability studies over time two batches, one comprising formulation 2.A and another formulation 5.A, were separately prepared with a total volume of $500\,mL$. For each formulation, 16 appropriated containers were filled with $30\,mL$ of oral solution. After properly sealed, 8 of those containers were placed placed on real time storage conditions, i.e., $25^{o}\,C$ and $60\,\%$ of relative humidity. While the remaining 8, were placed in accelerated conditions, i.e., $40^{o}\,C$ and $75\,\%$ of relative humidity. After 30 days one sample (one container) from each storage conditions was taken and analyzed for pH, palatability and appearance. The pH measurements were performed using Metrohm pH meter, model 736 GP Titrino.

The batches preparation followed the order of addition described the sub-chapter 3.2.10. The incorporation percentage of each excipient was based on whats is described in Table 4.17. The mass of magnesium L-pidolate necessary to accomplish the target dosage was calculated based on what is described in sub-chapter 3.2.2.

4

Results and discussion

Contents

In order to develop the two proposed magnesium dosage forms several studies were performed in order to select the their formulations and manufacturing processes. The results and discussion of these studies are presented in this chapter. Initially, there are presented the results from the tablet development and further the results from the oral solution development.

4.1 Tablet formulation and process design

The formulation and manufacturing process selection carried during this research work aimed to develop tablets that meet the QTPP criteria and to select an efficient and reproductive manufacturing process. The QTPP, presented below in the sub-chapter 4.1.1, was established in order to define all the desired characteristics of the product and to support the identification of possible CQAs. Further, there were carried several formulation studies in order to select the magnesium salt and the excipients to be used in the tablet formulation. Equally, different excipients and different combination of these excipients were tested. It was proposed to use a mixture of co-processed excipients specific for direct compression, Prosolv Nutra. This mixture consists of a binder (microcrystalline cellulose), a glidant (colloidal silicon dioxide), a superdisintegrant (croscarmellose sodium) and a lubricant (magnesium stearate). Given the high dosage of magnesium salt, this mixture may not be sufficient for the production of a stable tablet, therefore, other excipients may have to be considered. In Table 4.1 a generic composition of the proposed formulation is shown (including alternative excipients to Prosolv Nutra).

Table 4.1: Generic formulation of immediate release tablets. NA – Not applicable.

Component	Possible substances	Function
Magnesium salt	Magnesium oxide, magnesium citrate and magnesium chloride	Nutritional substance
Vitamin B6	Pyridoxine hydrochloride	Nutritional substance
Prosolv Nutra	NA	Direct compression mixture
Disintegrant	Starch, Sodium starch glycolate, Croscarmellose sodium, Crospovidone	Improve the disintegration time, in the case that Nutra Easy Tab is not sufficient
Lubricant	Magnesium stearate, Magnesium lauryl sulfate, sodium stearyl fumarate, polyethylene glycol 400 or 600	Reduce die-well friction and avoid sticking problems, in the case that Nutra Easy Tab is not sufficient
Glidant	Aerosil 200, magnesium silicate, powdered cellulose	Improve powder flowability and compactability, and promote weight uniformity, in the case that Nutra Easy Tab is not sufficient

4.1.1 Quality target product profile

The development of magnesium tablets starts with the establishment of the QTPP. The QTPP allows the definition of the quality attributes desired for the product and, moreover, represent a support for

the identification of the CQAs of the product. Table 4.2 lists the quality attributes of the tablet and the desired targets for each one. The development of this product should be performed in order to formulate a final product that fulfills all the criteria presented in Table 4.2. Additionally, the initial product CQAs were identified, using the QTPP as a support (see Table 4.2). The initial identification of the CQAs was performed taking in consideration what is described in the literature (in particular ref. [14, 54, 57–59]) and prior knowledge. Having defined the QTPP it is possible to perform the product manufacturing and formulation design studies, further presented.

Table 4.2: Tablet quality target product profile and critical quality attributes identification. CQA – Critical Quality Attribute, NMT – No More Than, NLT – No Less Than, NA – Not Applicable.

Quality attribute	Target	CQA?	Justification
Dosage form	Uncoated immediate release tablet	No	NA
Route of administration	Oral	No	NA
Dosage strength	375 mg	No	NA
Bioavailability	NLT 20% of magnesium is absorbed	Yes	Bioavailability of the nutritional substance impacts the product efficacy and safety. Process parameters and material attributes have an impact on bioavailability.
Appearance	Smooth surface, homogeneous white color and no signs of appearance defects	Yes	Tablet appearance may affect the patient compliance and may be a sign of manufacturing problems. Both the material attributes and the process parameters may have an impact on the tablets appearance.
Weight	1.5 g	No	NA
Identity	Positive for magnesium	No	NA
Uniformity of content	95 % - 105%	Yes	Content variability between tablets may impact safety and efficacy. Process parameters have a strong impact on uniformity of content.
Uniformity of dosage units	Meets the EP specifications	No	NA
Uniformity of mass	Meets the EP specifications	No	NA
Hardness	NLT 10 Kp (98.1 N)	Yes	Hardness may impact bioavailability, since it influences friability, disintegration and dissolution. Process parameters and material attributes have an impact on hardness.
Friability	NMT 0.75%	Yes	Friability should comply with values that ensure the formulation integrity during packaging, transportation and patient handling.
Water content	NMT 1%	No	NA
Disintegration	NMT 15 minutes	Yes	The disintegration time affects dissolution and, therefore, affects bioavailability. Both process parameters and material attributes impact disintegration.
Dissolution	Meets the EP specifications	Yes	Dissolution impacts bioavailability. Both process parameters and material attributes impact dissolution.
Microbiology	Meets the EP specifications	No	NA

4.1.2 Manufacturing process design

The manufacturing process chosen for the production of magnesium tablets was direct compression. This choice was based on a cost-efficiency analysis made by the company and, additionally, on prior knowledge of similar products (with similar physic-chemical characteristics) satisfactorily produced through this process. The manufacturing process is very simple, including only three unit operations: weighting, blending and compression. The excipients and the nutritional substances are separately weight. Then, the magnesium salt and the excipients are mixed at a fixed time and rotation. In some cases a lubrication blending cycle may be needed. Finally, the powder is compressed into 1 g or 1.5 g tablets, depending on the magnesium salt used.

In order to understand which process variables may have an impact on the product CQAs, an initial risk assessment was performed based on prior knowledge and literature review (in particular ref. [57, 58]). Considering the Table 4.3, it is possible to conclude that variation in the formulation seems to encompass high risk for all the product CQAs. Additionally, variation on blending encompasses high risk for the tablets content uniformity. While variation in compression parameters, is a factor of high risk for the tablets hardness, friability, disintegration and dissolution. Considering the blending and compression parameters, it is reasonable to assume that not all parameters impact the product CQAs in the same way. Hence, it is crucial to assess the risk that each process parameter variation can bring to the product CQAs accomplishment. Thereby, a risk assessment tool, known as relationship matrix, was applied. It is acknowledged that there are other risk assessment tools that enable a more complete comprehension of the risk involved but, given that the present stage of product development still comprises a reasonable gap on product and process knowledge, this tool seemed the best option. Table 4.4 describes the results from this risk assessment. Within this, the variables that were identified as high risk factors (e.g. type of compression and ejection force) require further investigation, in particular it should be investigated the effect of variation of such variables on the product CQAs. Regarding the formulation, it is equally assumed that each component has a different impact on the product CQAs. Moreover, the attributes of the excipients and drug substance also impact the product performance. In the following sub-chapter, it is possible to find a more in-depth assessment of such impact.

Table 4.3: Initial risk assessment of the variables that may impact the tablets Critical Quality Attributes (CQAs).

	Variables		
Product CQAs	Formulation	Blending	Compression
Appearance	High	Medium	Medium
Uniformity of content	High	High	Low
Hardness	High	Low	High
Friability	High	Low	High
Disintegration	High	Medium	High
Dissolution	High	Medium	High

Table 4.4: Initial risk assessment of tablets manufacturing process.

		Product CQAs					
Unit operation	Process parameters	Appearance	Uniformity of content	Hardness	Friability	Disintegration	Dissolution
Blending	Type and geometry of mixer	Low	Medium	Low	Low	Low	Low
	Mixer load level	Low	High	Low	Low	Medium	Medium
	Order of addition	Low	High	Low	Low	Medium	Medium
	Number of cycles (speed and time)	Medium	High	Medium	Medium	Medium	Medium
	Holding time	Medium	High	Low	Low	Medium	Medium
Compression	Type of press	High	Low	Medium	Medium	Medium	Medium
	Feed frame type and speed	Low	Medium	Low	Low	Low	Low
	Feeder fill depth	Low	Low	Low	Low	Low	Low
	Press speed	Medium	Medium	Medium	Medium	Medium	Medium
	Compression force	High	Low	High	High	High	High
	Punch penetration depth	High	Low	High	Medium	Medium	Medium
	Ejection force	High	Low	Low	Low	Low	Low

4.1.3 Formulation design

The formulation design studies carried during this research work aimed to identify the combination of excipients and the magnesium salt that result in a formulation that meets all the QTPP criteria. Consequently, the formulation design will encompass tow main steps:

1. Salt selection;
2. Excipients selection;

Regarding the salt selection, it should be taken in consideration that the tablets will be developed with a high dosage of magnesium salt, thus, the formulation performance is highly dependent on the magnesium salt behaviour. Concluding, the procedure of selecting a magnesium salt is fundamental for the development of a quality formulation. In the sub-chapter 4.1.4 it is possible to find the results from the formulation studies performed to select the magnesium salt.

An initial risk assessment was performed in order to understand which formulation variables represent a risk factor for the product CQAs accomplishment. As shown in Table 4.5, the selected magnesium salt represents a high risk factor for the tablet appearance, hardness and friability. Therefore, during the salt selection process it is fundamental to study the impact that the different salts have on those CQAs. Equally, the excipients variation represents a high risk factor for some of the product CQAs, thus, the impact of the different excipients on those quality attributes should studied with special attention. In the sub-chapter 4.1.5 it is possible to find the results from such studies.

4.1.4 Salt selection

Three different magnesium salts were selected based on its physical-chemical characteristics: magnesium oxide, magnesium citrate and magnesium chloride. Magnesium oxide was selected due to its high percentage in magnesium, allowing the production of a small tablet that provides the intended dosage of magnesium (375 mg). Nevertheless, magnesium oxide is referred in the literature as having a very low bioavailability. Summarizing, the magnesium oxide salt presents several manufacturing advantages, as

Table 4.5: Initial risk assessment of tablet formulation variables.

	Formulation variables				
CQAs	Magnesium salt	Lubricant	Disintegrant	Glidant	Binder
Appearance	High	High	Low	Medium	Medium
Uniformity of content	Low	Low	Low	High	Medium
Hardness	High	Low	Medium	High	High
Friability	High	Low	Medium	Medium	High
Disintegration	Medium	High	High	Medium	Low
Dissolution	Medium	Medium	High	Low	Low

it will be further presented, but it does not present a suitable source of magnesium. On the other hand, magnesium citrate is a considerable bioavailable salt, but has a very low percentage of magnesium when compared with magnesium oxide. Consequently, the compressibility characteristics of this salt are of interest, in the sense that, if the magnesium citrate powder presents good compressibility characteristics then low percentages of excipients may be used in order to allow a bigger salt percentage in the formulation. Concluding, the magnesium citrate salt may be a suitable alternative to magnesium oxide, provided that it has good manufacturing characteristics. Finally, the magnesium chloride salt was also considered, since it has a bigger percentage of magnesium than magnesium citrate and a higher bioavailability than magnesium oxide.

4.1.4.A Magnesium oxide

In order to evaluate the performance of the magnesium oxide salt, a formulation containing this salt was produced. Table 4.6 describes the composition of such formulation. More information about the manufacture and calculations of magnesium oxide tablets can be found in the Chapter 3. This formulation comprises the target magnesium dosage (375 mg).

The tablets manufactured had an average weight of 1 g and hardness of 18.6 kp. As shown in the Figure 4.1 ,the magnesium oxide tablets have an acceptable appearance, without any signs of fractures or cracks or any other appearance defects. Moreover, the surface is plain, white and homogeneous. The tablets have a diameter of 1.5 cm and 0.3 mm of thickness. Finally, there were performed disintegration and friability tests, according to what is described in the sub-chapters 3.2.7 and 3.2.8. The tablets presented a disintegration time of 13.00 minutes and a loss of 0.01% of its mass through the friability

Table 4.6: Magnesium oxide formulation composition

Composition	Percentage (% w/w)	Amount per batch (g)	Amount per tablet (g)
Magnesium oxide	62	62	0.62
Prosolv Nutra	38	38	0.38
Total	100	100	1

test. Complying with the QTPP and EP specifications.

Concluding, this formulation have a performance that complies with a certain features of the QTPP. However, as it was mentioned before, the magnesium oxide salt has a very low bioavailability, not complying with these criteria. Thus, and taking in consideration Medinfar expectations, this formulation was rejected and no further tests were executed. In spite of that, the results from the performance tests were used as reference values for further studies, since the formulation showed good manufacturing characteristics.

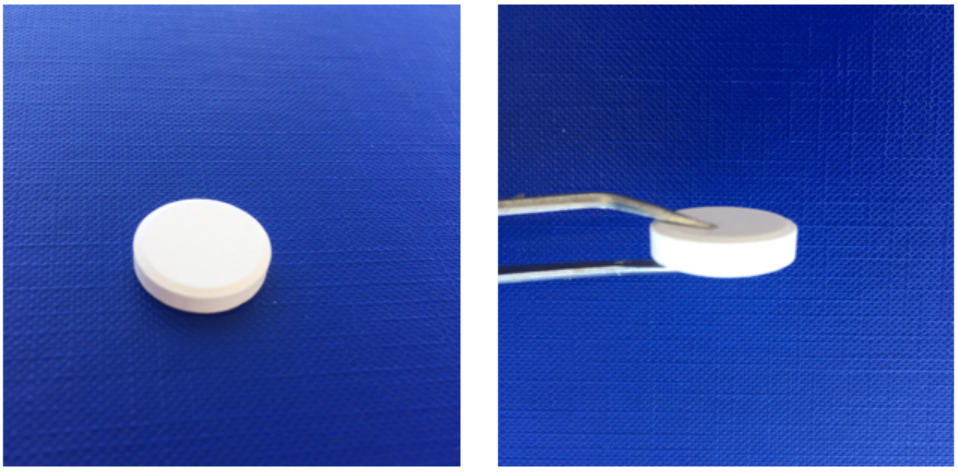

Figure 4.1: Appearance of magnesium oxide tablets

4.1.4.B Magnesium chloride

Magnesium chloride was one of the authorized magnesium salts selected to be included in the pre-formulation studies. Therefore, one formulation was manufactured in order to evaluate the compressibility characteristics of this salt. As mentioned before in the sub-chapter 1.3.3, this salt contains 12.00% of magnesium, which means that it is needed 3.13 g of magnesium chloride to achieve 375 mg of magnesium. Since the maximum tablet weight established was 1.5 g, the development of magnesium chloride tablets with a magnesium dosage of 375 mg is impracticable. Therefore, it was decided to produce tablets with 25 % of the target dosage, i.e., 94 mg of magnesium (see sub-chapter 3.2.2).

The formulation tested was designed with a total weight of 1.5 g, whit 52 % of magnesium chloride and 48% of Prosolv Nutra. The manufacturing process followed what is described in sub-chapter 3.2.3. This formulation presented several manufacturing and performance issues, in which two main limitations were observed: the powder had poor flowability characteristics and very poor compressibility characteristics. The first limitation was observed during the unit operations of mixing and compressing. The powder mixture sticked not only to the mixing machine but also to the compressing machine, making these processes very slow and ineffective. It is speculated that this behaviour is related with

the hygroscopic characteristics of magnesium chloride. The salt is highly hygroscopic and, given that the production area does not possess a humidity control system, it was impossible to control the water absorption by the salt. Furthermore, it was observed that the produced tablets never achieved the target hardness and presented several appearance defects, such as chipping and mottling. As the Figure 4.2 demonstrates, the appearance of the tablets is unacceptable, presenting heterogeneous color, with distinguishable light brown spots and a ridgy surface. Within the previously described, this formulation was considered impracticable and no further tests were performed.

Figure 4.2: Appearance of magnesium chloride tablets.

4.1.4.C Magnesium citrate

Several studies were carried in order to understand magnesium citrate behaviour. The performed studies aimed to evaluate the salt compressibility characteristics and to find the right combination of excipients that enable the production of magnesium tablets through direct compression.

The magnesium citrate salt has a percentage of 11.34 % of magnesium. Taking in consideration the equations presented in the sub-chapter 3.2.2, it is possible to conclude that to achieve a dosage of 375 mg of magnesium per tablet there are needed 3.31 g of magnesium citrate. Therefore the dosage of magnesium per tablet was reduced for 131 mg, i.e, 35 % of the dietary reference value of magnesium (375 mg). In this scenario, there are needed 1.16 g of magnesium citrate per tablet to achieve the target dosage. This salt amount represents 77 % (w/w) of the formulation mass, given that the maximum tablet weight is 1.5 g. Consequently, the formulation can only hold 23 % (w/w) of excipients, which means that the performance of the formulation is mainly impacted by the behaviour of the salt.

In order to evaluate the performance of magnesium citrate when combined with different excipients, three prototype formulations were designed. The composition of such formulations and their identification name can be found in the Table 4.7. Each formulation was tested for weight, hardness, disintegration

and friability.

Table 4.7: Magnesium citrate formulations designed for salt selection studies. DT – Disintegration Time. Note: *Mean value of 6 tablets.

Formulation	Components	Amount (% w/w)	Amount (g)	Hardness* (kp)	DT (min)	Friability (%)
CO1	Magnesium citrate	77	1.16	16.80	20.00	0.35
	Prosolv Nutra	23	0.34			
CO2	Magnesium citrate	77	1.16	16.50	18.00	0.76
	Prosolv Nutra	21	0.32			
	Aerosil 200	2	0.03			
CO3	Magnesium citrate	77	1.16	13.80	19.00	0.56
	Prosolv Nutra	21	0.32			
	Aerosil 200	1	0.015			
	Sodium stearyl fumarate	1	0.015			

Formulation C1 does not comply with what is described in the EP and in the QTPP, given that, the disintegration time is five minutes above the limit (15 minutes). Moreover, as shown in the Figure 4.3, the C1 tablets do not fulfill the desired appearance, presenting sticking defects. Considering the formulation C2, the excipient Aerosil 200 was introduced as a glidant to improve the flowability characteristics of the powder. In the formulation C3, SSF was introduced as a lubricant. As shown in the Figure 4.3, the formulation C2 presents signs of sticking (although less when compared to formulation C1) showing that Aerosil 200 *per se* does not solve the appearance problems. On the other hand, as described in the Figure 4.4, the C3 tablets present a homogeneous surface, with no signs of sticking or other appearance issues, indicating that the introduction of SSF positively impacts the sticking problem observed in formulation C1. Concerning the disintegration time, the formulation C2 has a better time value (as in, it is closer to 15 minutes), suggesting that the introduction of SSF has an adverse impact on this performance test. However, since the presented data is very limited, it is not possible to conclude such relation between the level of SSF and the disintegration time. Therefore, more studies shall be performed.

Considering the results obtained, it was decided to select magnesium citrate as the tablet nutritional

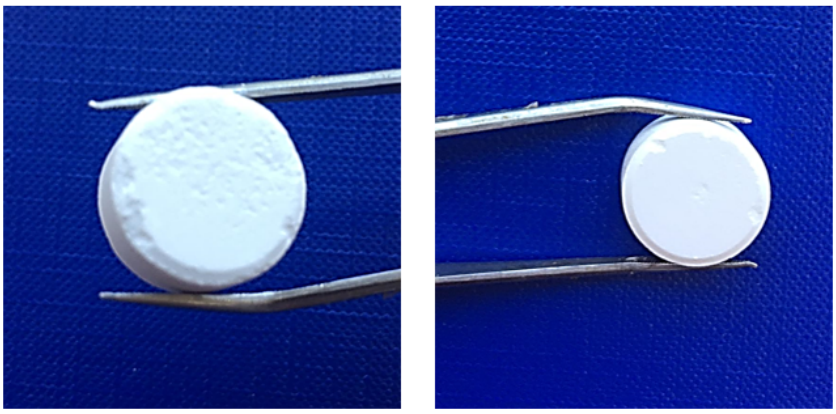

Figure 4.3: Appearance of the tablets produced according to the formulation C1 (left) and C2 (right).

Figure 4.4: Appearance of the tablets produced according to the formulation C3.

substance. The C3 formulation appears to have the best performance. Nevertheless, more studies have to be carried in order to develop a final formulation. Specifically, different combinations of excipients should be studied and their impact on the product CQAs assessed. Moreover, the process parameters should be defined and their impact on product CQAs understood. In the following sub-chapters the mentioned studies and results are presented.

4.1.5 Excipient selection

The magnesium salt selection studies culminated in the selection of magnesium citrate as the tablet nutritional substance. Additionally, a prototype formulation (C3) was selected to be further studied. However, the C3 formulation does not comply with all the QTPP criteria, specifically, in what concerns disintegration time. Disintegration was identified as a product CQAs, therefore special efforts shall be made in order to achieve the desired value. Accordingly to the risk assessment performed previously (summarized in Table 4.5), lubricant variation is a high risk factor for disintegration. Therefore, it is of interest to study the impact of this excipient on the product disintegration. Moreover, through the behaviour of formulation C3, it is possible to conclude that SSF (lubricant) seems to have a positive impact on the tablets appearance but a negative impact on disintegration time. Thus, it is of interest to perform optimization experiments that may enable the definition of an optimum level of lubricant. Equally, disintegrant excipients have an impact on disintegration and dissolution. So, it may be reasonable to add a suitable disintegrant to the formulation in order to achieve the target values for these quality attributes.

In the next sub-chapters such studies and their results are presented.

4.1.5.A Effect of lubricant and glidant level on disintegration time

Taking in consideration the results presented in the previous sub-chapter, a DoE was established with two main aims: i. study the impact of lubricant and glidant level on disintegration time and ii. identify the settings of these factors that produce an optimal response (disintegration time). Consequently, the DoE

was performed with 3 factors varying on 2 levels. The factors considered were the percentage of Aerosil 200 (X_1), the percentage of SSF (X_2) and tablet hardness (X_3). Additionally, the DoE considered 3 center points. Summarizing, 11 experiments were performed as shown in Table 4.8. The tablets produced for the present formulation study were satisfactorily direct compressed, without any appearance defects registered. The tablets were additionally tested for weight, in order to verify the compliance with the target values (results not shown). In the Table 4.8, the experiments and the resulting output, disintegration time, are summarized.

Table 4.8: Formulations resulting from the design of experiments varying the percentage of Aerosil 200 and SSF, and hardness.

ID	Aerosil 200 (% w/w) (X_1)	SSF (% w/w) (X_2)	Hardness (Kp) (X_3)	Disintegration (min)
CL1	3.00	1.25	14	16.80
CL2	1.00	2.00	18	22.80
CL3	5.00	0.50	10	13.75
CL4	5.00	2.00	18	12.82
CL5	1.00	2.00	10	20.00
CL6	5.00	0.50	18	9.80
CL7	1.00	0.50	10	11.88
CL8	1.00	0.50	18	12.83
CL9	3.00	1.25	14	16.50
CL10	3.00	1.25	14	16.20
CL11	5.00	2.00	10	10.63

Accordingly to what is described in sub-chapter 3.2.4, a response surface analysis was applied to fit the experimental data shown in Table 4.8, enabling the following model in uncoded units:

$$\text{DT} = 7.33 + 5.10X_1 + 2.88X_2 - 0.096X_3 - 0.547X_1^2 - 1.516X_1X_2 - 0.0861X_1X_3 + 0.333X_2X_3 + \varepsilon, \quad (4.1)$$

where DT represents the measured response related to each factor-level combination. The terms X_1, X_2 and X_3 represent the main effects of each factor on the response. The terms X_1X_2, X_1X_3 and X_2X_3 represent interaction effects, while the polynomial term X_1^2 represents curvature and ε is the error. The model describes the influence of the factors, Aerosil 200 level, SSF level and tablet hardness, on the response. The model satisfactorily fitted the experimental data, having a high R^2 value (R^2 = 98.45%). However, a large difference was found between the predicted R^2 and R^2 values (predicted R^2 = 7.48%), indicating that the model has low predictive ability and may be over-fitted. This fact implies precaution when analyzing predicted values of disintegration time. The terms significance was evaluated through the Pareto chart of the standardized effects shown in Figure 4.5, considering a significance level of $\alpha = 0.05$. As shown, there are four significant terms that impact disintegration time: Aerosil 200 level (A), the interaction between Aerosil 200 level and SSF percentage (AB), SSF level (B), and finally, the Aerosil 200 squared term (AA). Wherein, the term with the largest effect on disintegration time is Aerosil

200 level and the term with lowest effect is hardness. Moreover, given the significance of the Aerosil 200 squared term (AA), it is possible to conclude that the relation between Aerosil 200 level and disintegration time follows a curved line. It is worth of mention that removing the non-significant terms would improve the precision of predictions from the model, nevertheless, all the non-significant terms include the factor hardness and this factor was considered important for the purpose of this analysis. The non-significance of hardness was found surprising, given that previous studies (ref. [54, 60]) have found that this factor has an important effect on disintegration time. Notwithstanding the above, it was considered crucial to analyze the disintegration time as function of hardness and as function of the interactions between hardness and Aerosil 200, and hardness and SSF.

In order to better understand the relation between disintegration time and the factors a contour plot is presented in the Figure 4.6. As shown, in order to formulate tablets with hardness levels equal to $14\,kp$ and acceptable disintegration time (not more than 15 minutes), the level of Aerosil 200 has to be around or higher $4\,\%$ (w/w). On the other hand, the level of SSF relates in a different way to disintegration time. The same plot shows that it is only possible to achieve acceptable values of disintegration time, if the level of SSF is below or around $1\,\%$. Concluding, the increasing levels of Aerosil 200 have a positive impact on disintegration time, in the sense that, the higher the amount of Aerosil in the formulation the shorter the disintegration time is. Contrary, the percentage of SSF has a negative impact on disintegration time, i.e., the higher the amount of SSF the longer the disintegration time is. These results are in line with what is described in the literature, given that there are several studies indicating a negative impact of high percentages of lubricant on tablets disintegration [61–64].

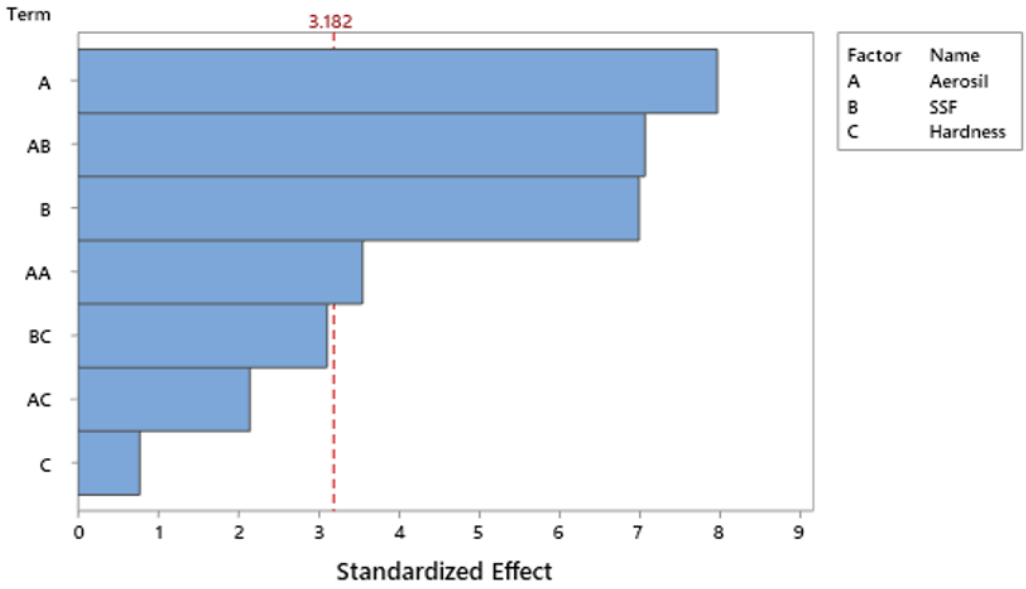

Figure 4.5: Pareto chart of standardized effects of Aerosil 200, SSF and hardness on disintegration time. $\alpha = 0.05$.

Summarizing, the results indicate that formulations containing high levels of Aerosil 200 and low levels of SSF, not only have acceptable disintegration time values but also comply with the target hardness, thus, it is of interest to study the behaviour of such formulations. Therefore, a response optimization was performed for a target disintegration value of 10 minutes and an optimal formulation was identified. Additionally, an alternative formulation was identified through the contour plot presented in the Figure 4.6. The composition of such formulations (CO1 and CO2, respectively) is presented in the Table 4.9. The predicted disintegration time for the formulation CO1 was 10.30 minutes. However, as shown in the Table 4.9, this value was not experimental achieved, the real disintegration time being 14.40 minutes. On the other hand, the formulation CO2 presented a disintegration time of 13.00 minutes, which is in accordance with the predicted range of disintegration values illustrated in the Figure 4.6. Concluding, both formulations have a disintegration time lower than 15 minutes, however, the variable settings suggested by the model response optimizer (formulation CO1) did not generated the predicted disintegration time. This may be explained by the model low predictive ability or by experimental errors. Nevertheless, the disintegration time of both formulations is very close to the acceptance limit, which suggests that the addition of a disintegrant to the formulation would be a way to overcome this issue and achieve the disintegration optimal value (10 minutes).

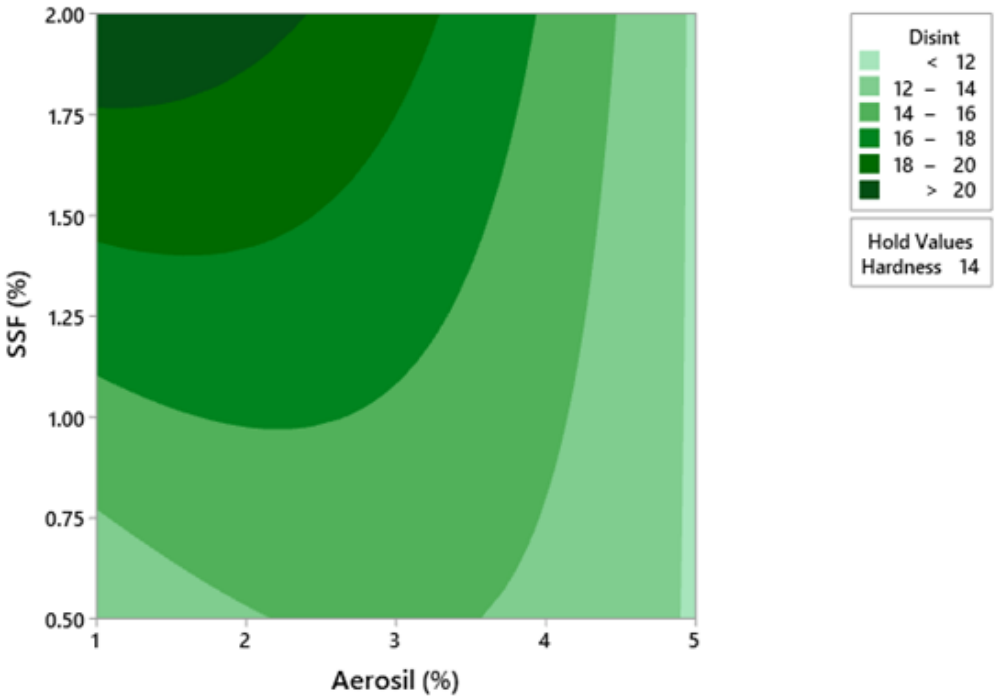

Figure 4.6: Contour plot regarding disintegration time as function of Aerosil 200 and sodium stearyl fumarate level variation. Disint: Disintegration time. Note: consider disintegration time in minutes.

Table 4.9: Optimal magnesium citrate formulations containing Aerosil 200 and SSF. H_{exp} – Hardness expected, H_{obs} – Hardness observed, DT_{pred} – Disintegration time predicted, DT_{obS} – Disintegration time observed.

ID	Aerosil$_{level}$ (% w/w)	SSF$_{level}$ (% w/w)	H_{exp} (kp)	H_{obs} (kp)	DT_{pred} (min)	DT_{obS} (min)
CO1	5	0.5	18	18.70	10.30	14.40
CO2	4	0.7	14	14.76	[12, 14]	13.00

4.1.5.B Effect of superdisintegrant level on disintegration time and friability

Taking in consideration the disintegration results obtain for the optimal formulations (sub-chapter 4.1.5.A), CO1 and CO2, it was decided to proceed the formulation studies by studying the impact of disintegrants on the performance of the formulation CO2. Moreover, the initial risk assessment performed for formulation variables (see Table 4.5) demonstrated that variation in disintegrant excipients represents a medium risk factor for the accomplishment of friability, and a high risk factor for disintegration and dissolution targets. Therefore, it seems reasonable to study the impact of different disintegrants (and different disintegrants levels) on these formulation CQAs. With that purpose, two superdisintegrants were selected based on a literature review (ref. [57, 65]) and on prior knowledge: croscarmellose sodium and SSG. It is worth of mention that the Prosolv Nutra mixture, included in the previous tablet formulations, contains croscarmellose sodium (also mentioned as croscarmellose). However, the amount of croscarmellose used seems to be insufficient to achieve the optimum disintegration time (10 minutes). The study of the impact of a different superdisintegrant, i.e. SSG, in the formulation performance is based on the recent suggestion that there is synergism between superdisintegrants [65]. This possibility may be a consequence of the different disintegration mechanisms presented by superdisintegrants (topic discussed elsewhere) [65]. Actually, a recent study [66] found evidences of disintegrants synergism at particular compression pressures and disintegrant amounts.

The aim of this study is to select one of the mentioned excipients based on the outup disintegration time and friability values. Thus, two DoE, one considering SSG and another considering croscarmellose as factors, were separately performed and the resulting models compared. In both the DoE, disintegration time and friability were considered as responses. The performance of disintegration and friability tests followed the procedures describe in the sub-chapters 3.2.7 and 3.2.8, respectively. Below, there are presented the results and interpretation of the mentioned studies. First, there are presented the results regarding SSG and secondly the results concerning croscarmellose.

Regarding SSG, a DoE was performed varying the percentage of this superdisintegrant (K_1) between $2\,\%$ and $4\,\%$, as demonstrated in the Table 4.10. Simultaneously, hardness (K_2) was varied between 10 kp and 18 kp. There were considered 3 center points (3% of SSG and 14 kp of hardness) and 2 replicates

of each experiment, making a total of 11 experiments. As shown in Table 4.10, the lower disintegration time registered, 12.00 minutes, corresponds to the CS11 formulation (4% of SSG and 10 kp). On the other hand, the formulation with the highest disintegration time corresponds to the formulation CS4, with a disintegration time of 18.90 minutes. The same formulation presents the lowest percentage of mass lost through friability testing (0.41 % w/w). Contrary, the highest friability value (1.98 % w/w) corresponds to the formulation CS7.

A response surface analysis was applied in order to fit the experimental data shown in Table 4.10. Accordingly, two separate regression models were applied to fit the experimental disintegration time data and the experimental friability data. The SSG model for disintegration time (DT) in uncoded units is given by,

$$DT = 24.37 - 2.001K_2 + 1.263K_1 + 0.1075K_2^2 - 0.2013K_2K_1 + \varepsilon, \tag{4.2}$$

while the SSG model for friability (FRI) corresponds to,

$$FRI = -0.08 + 0.501K_2 - 0.572K_1 - 0.02701K_2^2 + 0.0397K_2K_1 + \varepsilon, \tag{4.3}$$

where DT and FRI represent the measured responses related to each factor-level combination. The terms K_1 and K_2 represent the main effects of each factor on the response (SSG level and hardness, respectively). The term K_2K_1 represents the interaction effect, the polynomial term K_2^2 represents curvature and ε is the error. The terms significance was evaluated through the Analysis of Variance (ANOVA) test (results not shown) and the Pareto chart of the standardized effects, considering a significance level of $\alpha = 0.05$.

Table 4.10: Disintegration time and friability values of the formulations resulting from the design of experiments varying sodium starch glycolate and hardness.

ID	SSG (% w/w) (K_1)	Hardness (kp) (K_2)	Disintegration (min)	Friability (% w/w)
CS1	3	14	12.60	1.68
CS2	4	18	13.80	0.87
CS3	4	10	12.30	1.51
CS4	2	18	18.90	0.41
CS5	4	18	13.70	0.65
CS6	3	14	12.90	1.35
CS7	2	10	13.20	1.98
CS8	2	10	14.00	1.78
CS9	2	18	18.00	0.54
CS10	3	14	12.70	1.75
CS11	4	10	12.00	1.55

The R^2 value for the disintegration model indicates that the model explains $98.44\,\%$ of disintegration time variance, which means that the model satisfactorily fitted the data. The predictive ability of the

model was also considered adequate, given that the predicted R^2 corresponds to $R^2_{(pred)} = 93.95\%$. Considering the Pareto chart demonstrated in the Figure 4.7, it is possible to conclude that all the terms included in the model significantly impact disintegration time, wherein hardness variation represents the highest impact. Additionally, it is possible to notice that the relationship between hardness and disintegration time follows a curved line, since the squared term for this factor is significant (AA). Finally, as illustrated in the mentioned figure, the interaction between hardness and SSG (AB) has a significant impact on disintegration time. Meaning that the relationship between SSG level and disintegration time depends on hardness values and *vice-versa*. To better understand such relation the interaction plot of these factors for disintegration time mean is presented in the Figure 4.11. Through this plot it is possible to conclude that at a SSG level of $4\,\%\,(w/w)$, regardless the tablet hardness values, the disintegration time will always be less than 14.00 minutes. Moreover, an optimal combination of factor settings for a minimal disintegration time seems to be: Hardness $= [12\,kp, 14\,kp]$ and SSG $= 4\,\%\,(w/w)$. Nevertheless, the optimal factors settings are also dependent on the friability data distribution, thus a response optimization will be further presented. Through the contour plot illustrated at Figure 4.9, it is possible to have a more in-depth comprehension of how the variation in tablet hardness and SSG level affects disintegration time. As demonstrated, at SSG levels superior to $3.5\,\%$ and tablet hardness values between $12\,kp$ and $14\,kp$, the disintegration time is always less than 12.00 minutes.

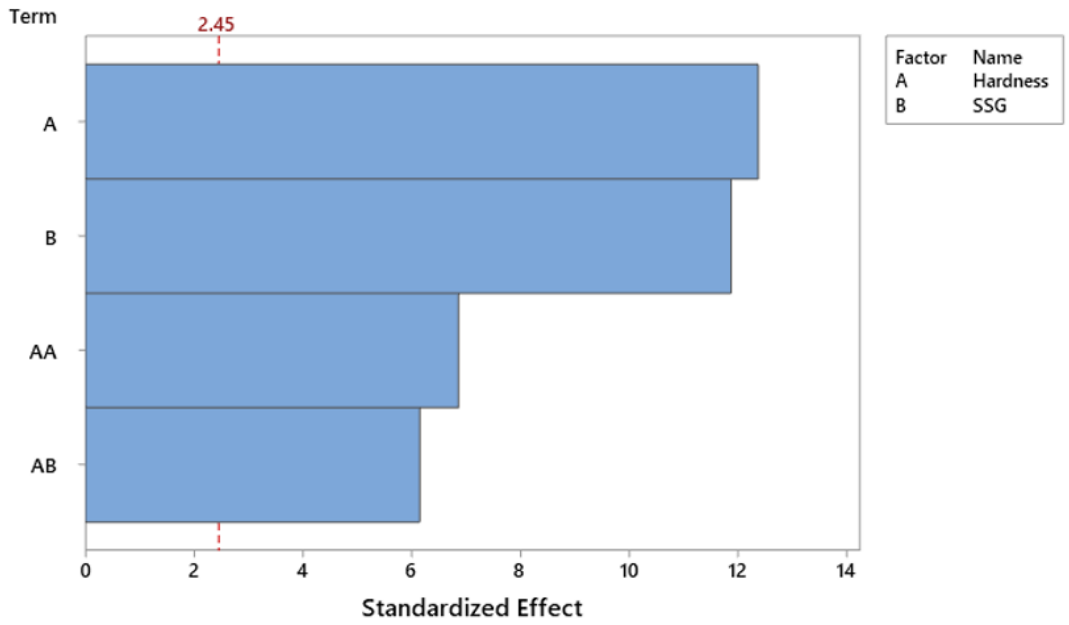

Figure 4.7: Pareto chart of standardized effects of sodium starch glycolate and hardness on disintegration time. $\alpha = 0.05$.

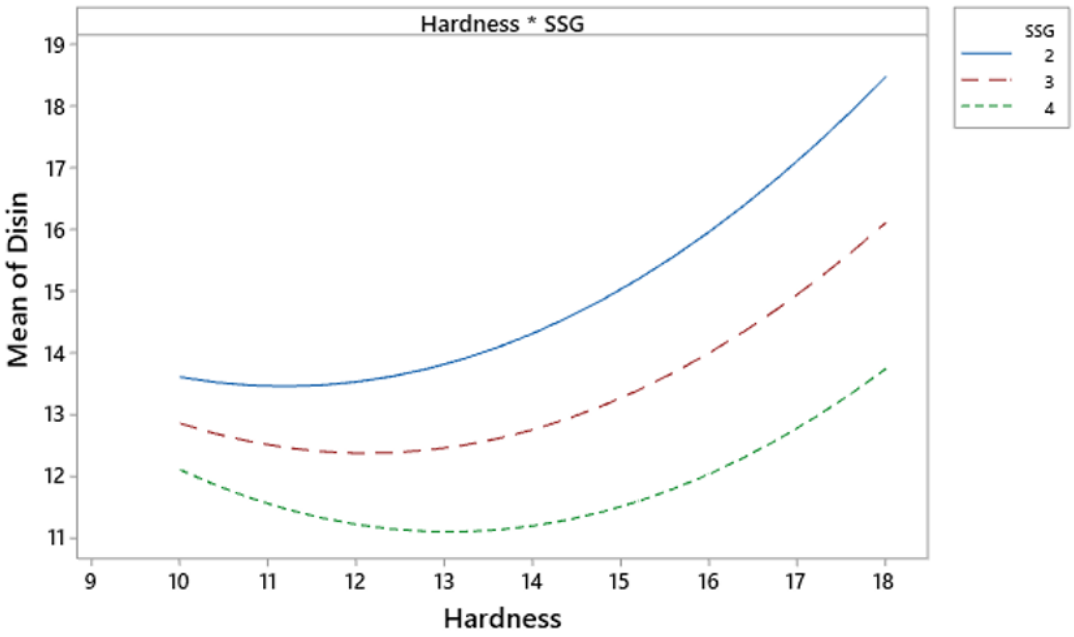

Figure 4.8: Interaction plot hardness-sodium starch glycolate for disintegration time. Disint: Disintegration time, SSG: sodium starch glycolate level. Note: consider hardness in kp, sodium starch glycolate level in % (w/w) and disintegration time in minutes.

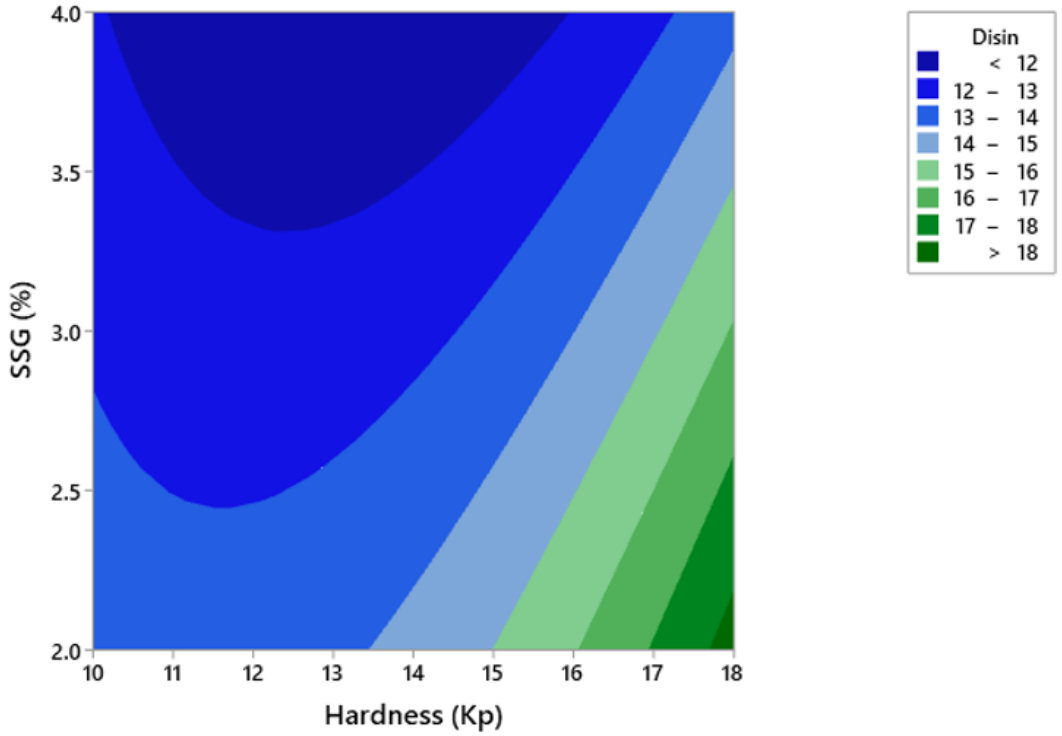

Figure 4.9: Contour plot regarding disintegration time as function of sodium starch glycolate level and hardness variation. Disin: Disintegration time. Note: consider disintegration time in minutes.

Regarding the friability data model (see equation (4.3)), it was obtain a R^2 value equals to $95.36\,\%$ and a predicted R^2 corresponding to $R^2_{(pred)} = 93.95\%$. Thus, the model adequately fitted the friability data and its predictive ability is satisfactory. Considering the Pareto chart presented in the Figure 4.10, it is possible to conclude that all the terms included in the model have a significant effect on friability variability, except for SSG level (B). Additionally, it is possible to note that the interaction term (AB) has a significant effect on friability, meaning that the relation between friability and hardness is dependent of SSG level. To better understand such relation, the interaction plot for such factors affecting friability is demonstrated in Figure 4.11. As illustrated, around a low friability point (friability $\leq 0.75\,\%$) the increase in SSG level has a negative impact in the friability values, in the sense that, friability values increase with the increment of SSG level. In this plot area it is also possible to notice that the friability values significantly vary with hardness and suffer a moderate variation with SSG variation. This means that a tight control of these parameters, especially hardness, should be performed in order to achieve the low friability values. To better understand how the relation between SSG level and tablet hardness impact friability mass losses, a contour plot is presented in the Figure 4.12. As illustrated, it is only possible to achieve low values of friability (friability $\leq 0.75\,\%$) if the tablets hardness is around $18\,kp$ and the SSG values are lower that $4\,\%$. Actually, $4\,\%\,(w/w)$ of SSG seems to be a critical value, in which, no matter the harness values, values of friability $\leq 0.75\,\%$ will never be achieved.

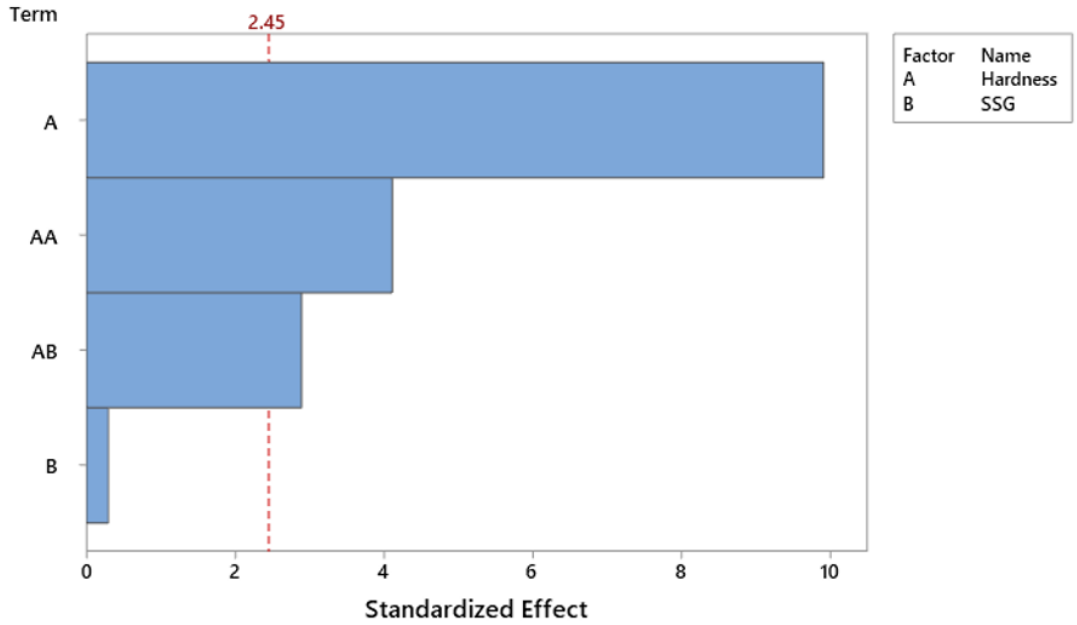

Figure 4.10: Pareto chart of standardized effects of sodium starch glycolate and hardness on friability mass losses. $\alpha = 0.05$.

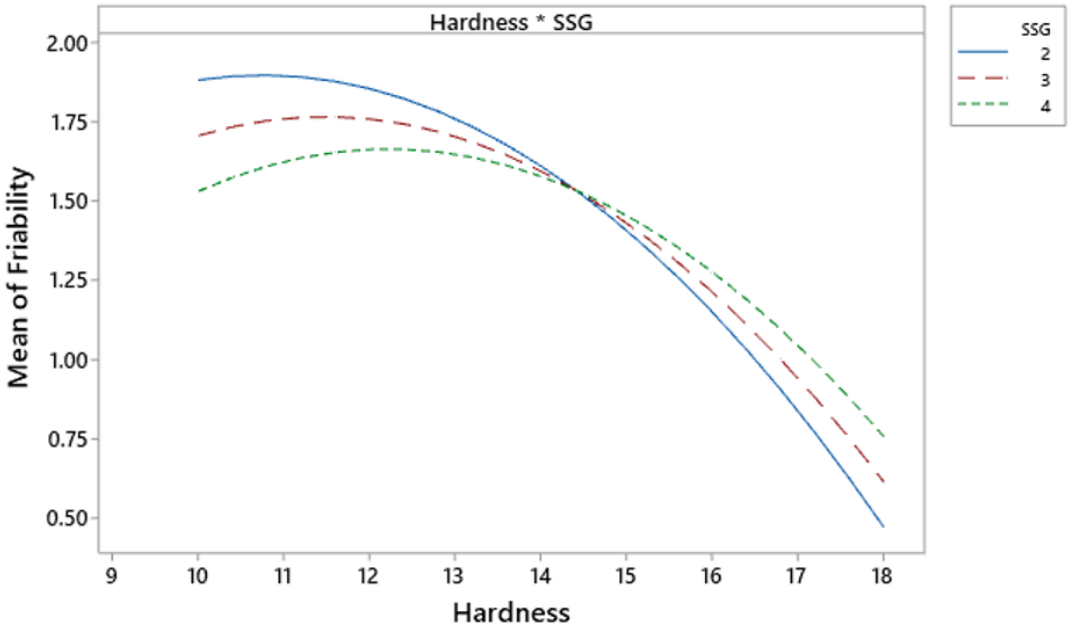

Figure 4.11: Interaction plot hardness-sodium starch glycolate for friability mass losses. SSG: sodium starch glycolate level. Note: consider hardness in kp, sodium starch glycolate in % (w/w) and friability mass losses in % (w/w).

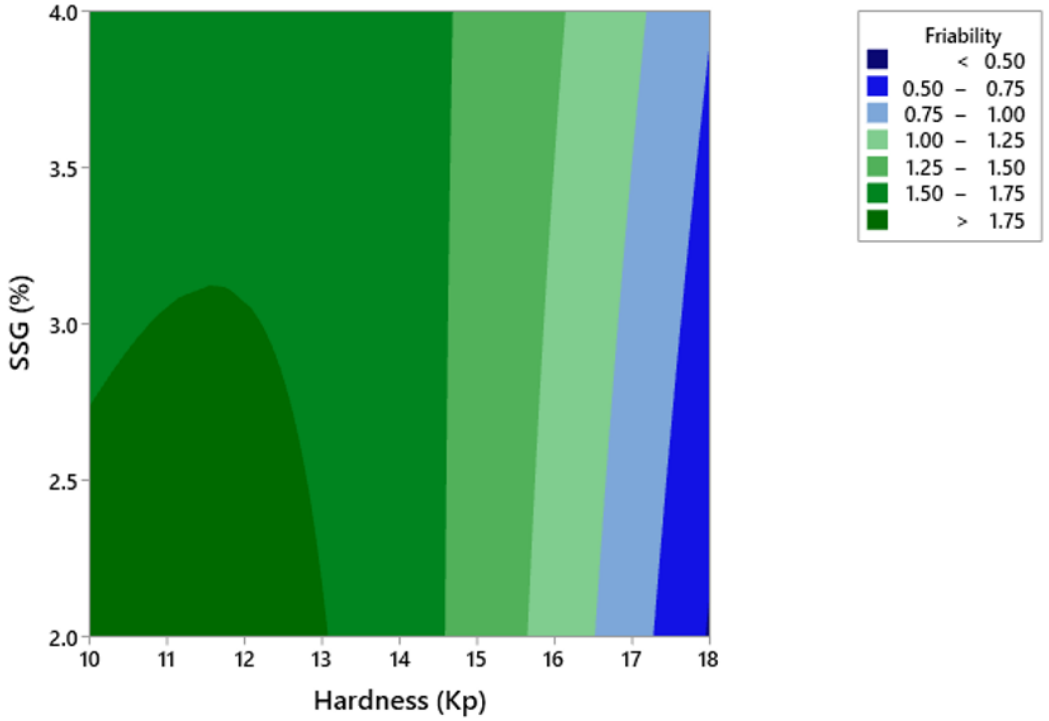

Figure 4.12: Contour plot regarding friability as function of sodium starch glycolate level and hardness variation. Note: consider friability mass losses in % (w/w).

Having both the SSG models and their results analyzed it is possible to run a response optimization. Through this analysis, SSG level and hardness settings can be defined in order to originate optimal responses values. With that purpose there were defined two response targets: i. 10 minutes of disintegration time and ii. $0.75\,\%w/w$ of friability. The model showed that a combination of the target responses can not be achieved in the present scenario, the nearest disintegration and friability values being 13.70 minutes and $0.76\,\%$, respectively. These values can be achieved through a formulation containing $4\,\%$ of SSG with a tablet hardness corresponding to $18\,kp$.

Concerning the croscarmellose sodium study, as already mentioned, it was performed a DoE in order to understand the impact of this disintegrant on the formulation performance (disintegration time and friability). The DoE was performed considering 2 factors, croscarmellose sodium percentage (Z_1) and hardness (Z_2), varying between two levels. Croscarmellose level was varied between 0.5 and $5.00\,\%\,(w/w)$ and tablet hardness between 10 and $18\,kp$. There were considered 2 center points corresponding to $2.75\,\%\,(w/w)$ of croscarmellose and $14\,kp$ of tablet hardness. Each experiment was repeated once, resulting in a total of 11 experiments, i.e., 11 formulations. The resulting formulations were tested for disintegration time and friability accordingly to what is described in the sub-chapters 3.2.7 and 3.2.8, respectively. In the Table 4.11 it is possible to find the DoE and the respective responses. As shown, the formulations with highest values of croscarmellose and lowest values of hardness, CC4 and CC10, present the lowest disintegration time values. Additionally, these formulations present the highest values of friability (1.68 and $1.74\,\%\,w/w$, respectively). Therefore, it is of interest to understand which factors have an impact on these responses and which factor has the greatest impact. With that purpose, two response surface designs were applied and analyzed in order to fit the experimental data, one for disintegration time data and another for friability data. The croscarmellose model for disintegration time (DT) in uncoded units is given by,

$$DT = 12.48 - 2.69Z_1 + 0.325Z_2 + 0.252Z_1^2 + 0.0081Z_1Z_2 + \varepsilon, \tag{4.4}$$

while the croscarmellose model for friability (FRI) corresponds to,

$$FRI = -0.122 + 0.0602Z_2 + 0.6053Z_1 - 0,00174Z_2^2 + \varepsilon, \tag{4.5}$$

where DT and FRI represent the measured responses related to each factor-level combination. The terms Z_1 and Z_2 represent the main effects of each factor on the response (croscarmellose level and tablet hardness, respectively). The term Z_1Z_2 represents the interaction effect, the polynomial terms Z_1^2 and Z_2^2 represent curvature, and ε is the error. The terms significance was evaluated through the ANOVA test (result not shown) and the Pareto charts of the standardized effects (presented elsewhere), in which was always considered a significance level of $\alpha = 0.05$. First, it will be presented the disintegration time

model analysis and further the analysis of the friability model.

Table 4.11: Disintegration time and friability values of the formulations resulting from the design of experiments varying croscarmellose and hardness. The hardness values correspond to the mean over 6 tablets.

ID	Croscarmellose (% w/w) (Z_1)	Hardness (kp) (Z_2)	Disintegration (min)	Friability (% w/w)
CC1	0.50	10.00	16.00	0.39
CC2	0.50	10.00	13.00	0.52
CC3	2.75	14.00	11.70	0.81
CC4	5.00	10.00	9.00	1.68
CC5	5.00	18.00	12.00	0.53
CC6	0.50	18.00	16.00	0.47
CC7	5.00	17.00	11.80	0.46
CC8	0.50	18.00	18.30	0.36
CC9	2.75	14.00	11.60	0.79
CC10	5.00	10.00	9.00	1.74
CC11	2.75	14.00	12.20	0.79

The disintegration time model obtained adequately fitted the data and presented a good predictive ability, since the R^2 and $R^2_{(pred)}$ values corresponded to $91.30\,\%$ and $65.57\,\%$, respectively. Considering the Pareto chart illustrating the effects on disintegration time presented in the Figure 4.13, it is possible to conclude that disintegration time is mainly impacted by croscarmellose level (B). Additionally, hardness (A) has a significant effect on disintegration time, as expected. Interestingly, the interaction between croscarmellose level and tablet hardness do not significantly impact disintegration time. This means that the relationship between croscarmellose level and disintegration time is not dependent on hardness variation, and *vice-versa*. This conclusion diverge from what was found before for SSG. In order to better understand the relationship between the factors and disintegration variability, a interaction plot and a contour plot are presented in Figure 4.14 and 4.15, respectively. As illustrated in both figures, disintegration time variability is mainly impacted by croscarmellose level variation. Moreover, considering Figure 4.14, it is possible to conclude that at a croscarmellose level $\geq 2.75\,\%\,(w/w)$ the disintegration values will always comply with the EP specification for disintegration time of immediate release tablets, regardless of tablet hardness values. Similarly, regarding Figure 4.15, it is possible to conclude that if croscarmellose level is $\geq 2\,\%\,(w/w)$ and tablet hardness is $\leq 16\,kp$ then the same specification will always be fulfilled.

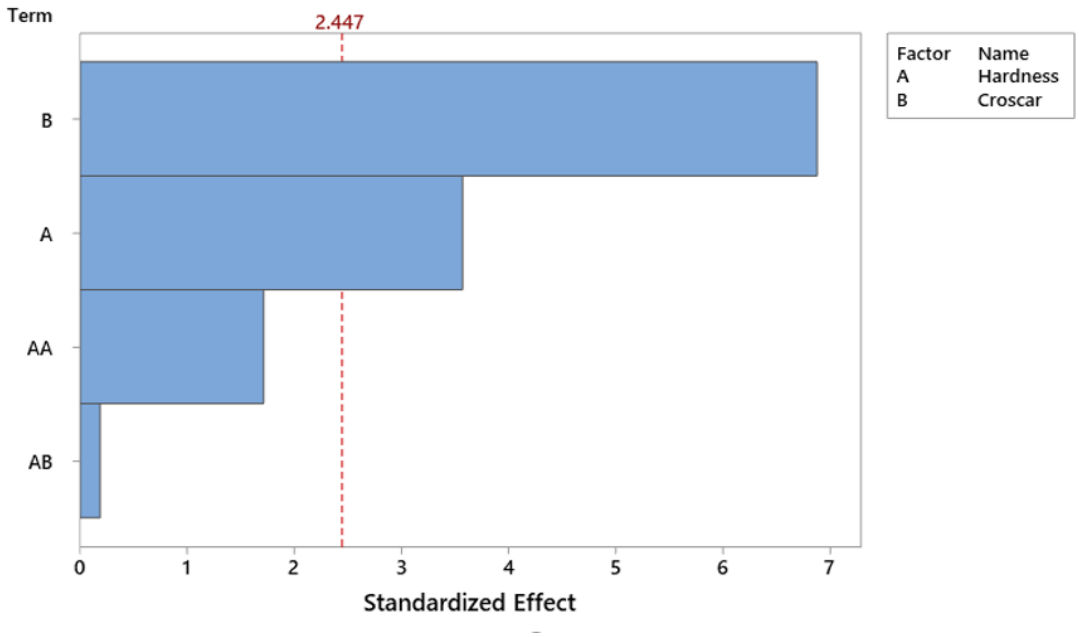

Figure 4.13: Pareto chart of standardized effects of croscarmellose level and hardness on disintegration time. Croscar: Croscarmellose level.

Taking in consideration the model of friability as function of croscarmellose level (see equation (4.5)), a $R^2 = 99.20\,\%$ was obtained, meaning that the model explains $99.20\,\%$ of friability variance. In addition, the $R^2_{(pred)}$ is equal to $96.84\,\%$. Summarizing, the model satisfactorily fits the friability data and simultaneously has an adequate predictive ability. The factor that mainly impacts friability mass losses seems to be croscarmellose level, as illustrated in the Pareto chart presented in the Figure 4.16. Considering the same figure, it is possible to conclude that hardness (A) and the interaction between hardness and croscarmellose (AB) also have a significant impact on friability values variance. The interaction plot, presented in Figure 4.17, allows a better understanding of the relationship between the factors interaction and the response variability. As illustrated by the mentioned figure, all the formulations tend to friability values $\leq 0.75\,\% \, w/w$ at hardness values $\geq 17\,kp$. Indicating that high values of tablet hardness enable low friability values, regardless the croscarmellose level considered. On the other hand, considering low values of tablet hardness ($\leq 12\,kp$), it is possible to notice that croscarmellose level has a great impact on friability mass losses, suggesting that high levels of croscarmellose lead to an increment on friability mass losses. The contour plot presented in Figure 4.18 displays such relationship for a wider range of croscarmellose values. As illustrated, high tablet hardness values are crucial in order to accomplish acceptable values of friability mass losses through formulations containing high levels of disintegrant.

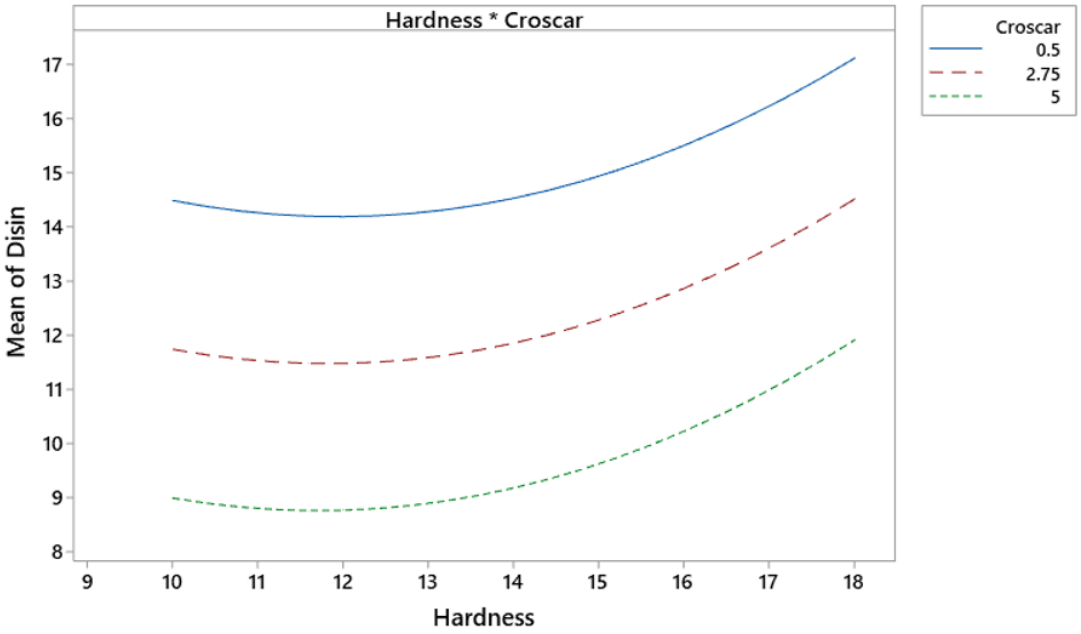

Figure 4.14: Interaction plot hardness-croscarmellose sodium for disintegration time. Disin: Disintegration time; Croscar: croscarmellose level. Note: consider disintegration time mean in minutes and croscarmellose level in % w/w.

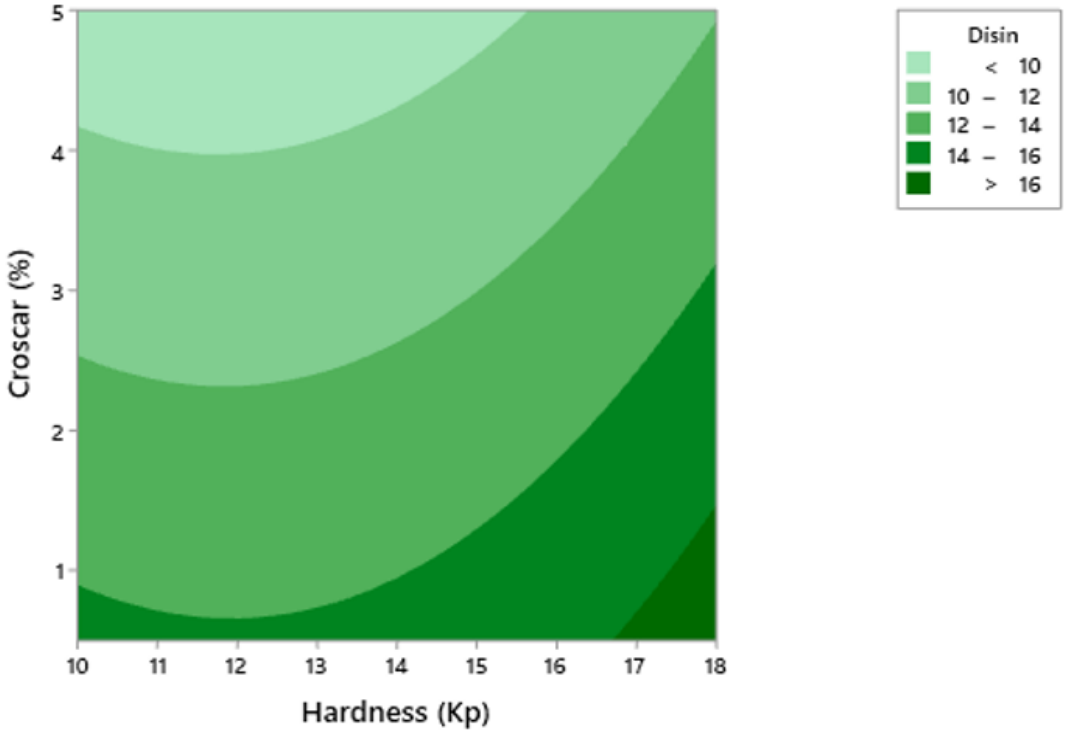

Figure 4.15: Contour plot regarding disintegration time as function of croscarmellose sodium level and hardness variation. Croscar: croscarmellose level. Disin: Disintegration time. Note: consider disintegration time in minutes.

Finally, with both croscarmellose data models analyzed, it is possible to define formulation settings that enable optimal disintegration and friability responses. With that purpose, a response optimization was performed considering two targets: i. disintegration time equals to 10.00 minutes and ii. friability mass losses equals to $0.60\,\%\,w/w$. Table 4.12 summarizes the suggested variable settings and the corresponding predicted values for disintegration time and friability mass losses. As demonstrated, a combination of the target responses is not possible. Specifically, the disintegration time target can not be met, the nearest predicted value corresponding to 11.30 minutes. Subsequently, the suggested formulation settings were experimental tested. The batch was produced accordingly to what is described in the sub-chapter 3.2.5.B. The resulting tablets were tested for disintegration time and friability (see sub-chapter 3.2.7 and 3.2.8, respectively). The results of such tests and the experimental settings considered are summarized in Table 4.12. The observed hardness is presented as a mean value of 6 tablets $\pm$ Standard Deviation (SD). As shown, the observed results differ from the predicted values. The observed disintegration time is 0.60 minutes less than its predicted value. On the other hand, the observed friability mass loss is superior to its predicted value by $0.06\,\%\,w/w$. Similarly, the tablet hardness observed is inferior to the required optimization setting (expected hardness). It is important to notice that this difference is not significant, nevertheless, the observed SD value is considerable, indicating reasonable tablet hardness variance. Such variance has an impact on the output responses, as shown before. So far, it was observed that a decrease of tablet hardness causes an increment of friability mass losses and a decrease of disintegration time. Therefore, it is not surprising that lower values of hardness resulted in lower disintegration time and greater tablet friability. It should also be considered the predictive ability of the models, specifically the disintegration model, in which a lower predictive ability was observed when compared with the friability model. Concluding, given that the disintegration time and friability values comply with the QTPP criteria, this formulation was considered adequate. Moreover, all the produced tablets complied with the appearance targets described in the QTPP (see Table 4.2).

Table 4.12: Optimal magnesium citrate formulation containing croscarmellose sodium. Cros$_{level}$ – Croscramellose level, H$_{exp}$ – Hardness expected, H$_{obs}$ – Hardness observed, DT$_{pred}$ – Disintegration time predicted, DT$_{obs}$ – Disintegration time observed, FRI$_{pred}$ – Friability predicted, FRI$_{obs}$ – Friability observed.

ID	Cros$_{level}$ $(\%\,w/w)$	H$_{exp}$ (kp)	H$_{obs}$ (kp) $\pm SD$	DT$_{pred}$ (min)	DT$_{obs}$ (min)	FRI$_{pred}$ $(\%\,w/w)$	FRI$_{obs}$ $(\%\,w/w)$
CO4	5	17.36	17.20 ± 0.53	11.30	10.70	0.60	0.692

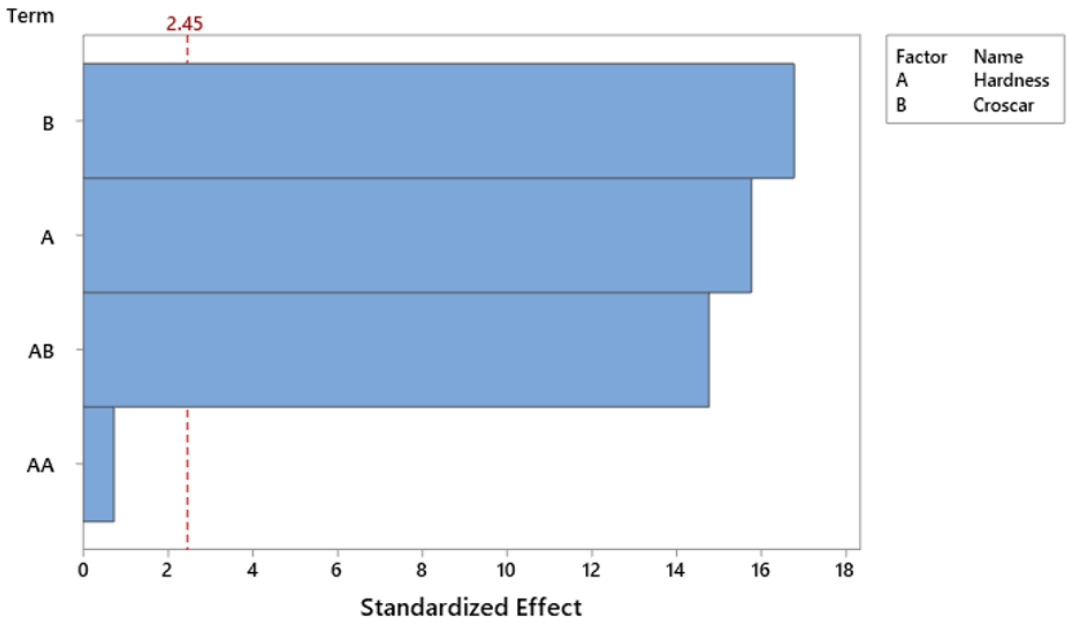

Figure 4.16: Pareto chart of standardized effects of croscarmellose level and hardness on friability mass losses. Croscar: Croscarmellose level.

Taking in consideration the results obtained so far, it is possible to conclude that the tablets disintegration time decreased with increasing levels of both croscarmellose sodium and SSG. This behavior was expected as it is largely described in literature [57]. The explanation for this behavior relays on the superdisintegrants mechanism of action. Superdisintegrants act mainly through swelling mechanisms. Swelling happens as a consequence of the hydrophilic nature of superdisintegrants. Particularly, superdisintegrants are insoluble hydrophilic colloids, therefore, they will absorb water from the tablet surrounding and as the water enters the tablet matrix the superdisintegrant particles will tend to swell [57,65]. The swelling will break-up the tablet matrix and culminate in tablet disintegration. The tablet swelling tends to be proportional to the superdisintegrant level, in the sense that, the more disintegrant particles available in the tablet matrix the greater quantity of water will be absorbed [57,65]. In literature it is also described a process of disintegration time stagnancy. This process happens for a given threshold disintegrant concentration, above which the disintegration time stagnates [57]. However, during the performance of this study this process was not observed.

Regarding the superdisintegrants performance, croscarmellose sodium seems to have a greater impact on disintegration time than SSG. In the sense that, croscarmellose appears to be more effective at lower formulation levels than SSG. Comparing the disintegration time contour plots for croscarmellose and SSG (Figures 4.15 and 4.9, respectively), it is possible to notice that the minimal disintegration time predicted for $2\,\%\,w/w$ of SSG is 13 minutes, whereas, the minimal disintegration time predicted for the same level of croscarmellose is 10 minutes. This fact may be explained by the differences found

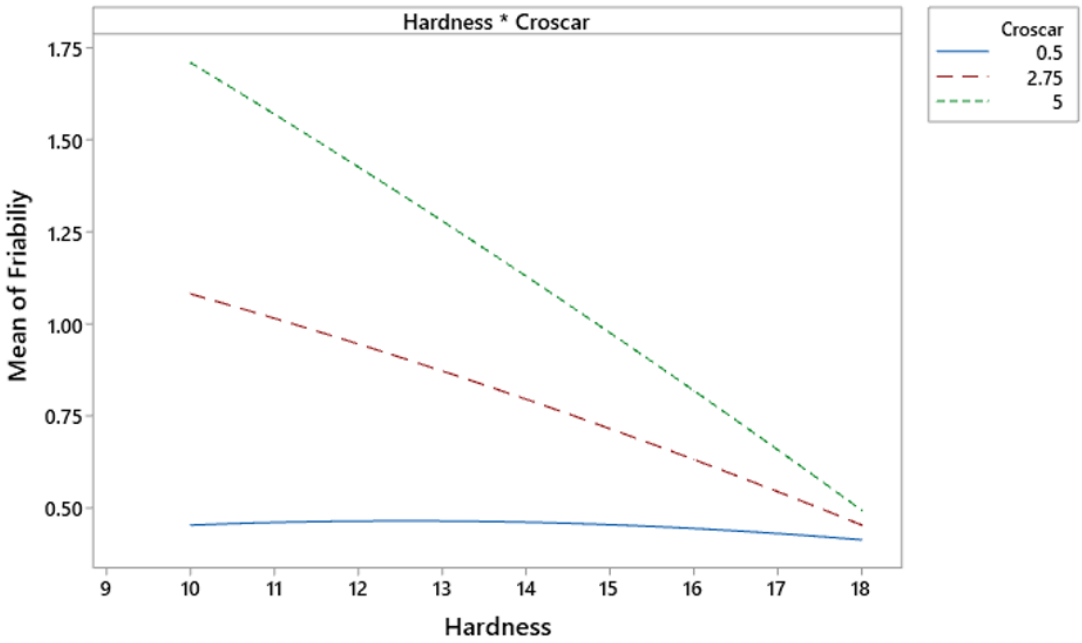

Figure 4.17: Interaction plot hardness-croscarmellose sodium for friability mass losses. Croscar: Croscarmellose level. Note: consider mean of friability mass losses and croscarmellose level in % (w/w).

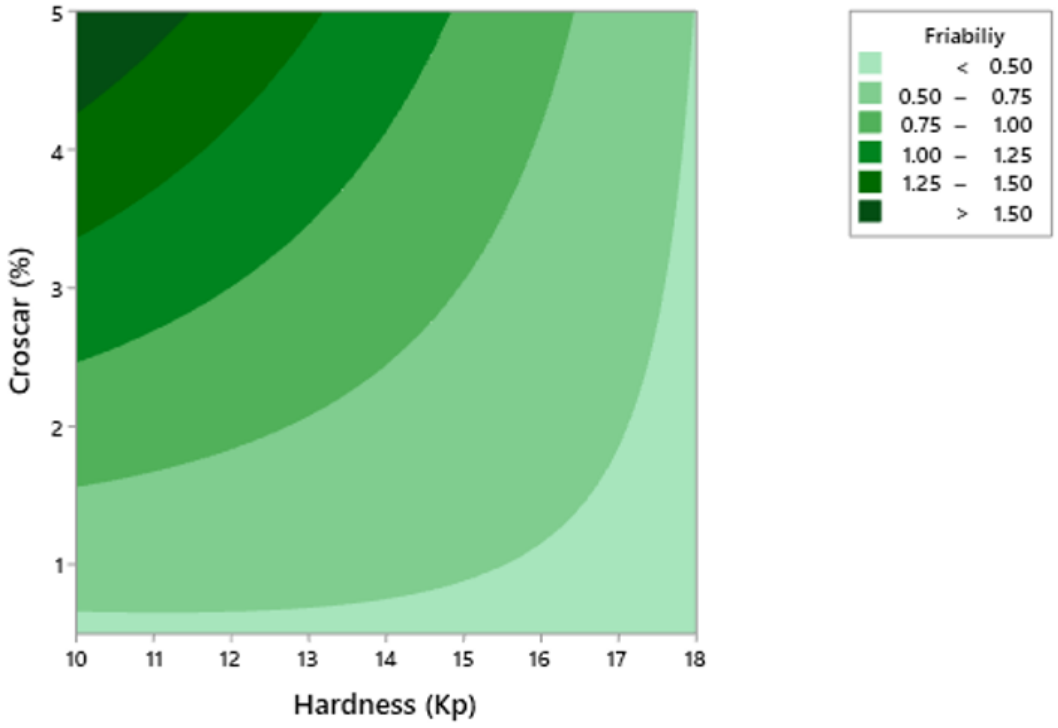

Figure 4.18: Contour plot regarding friability as function of croscarmellose sodium level and hardness. Croscar: croscarmellose. Note: consider friability mass losses in % (w/w).

between the mechanisms of action of SSG and croscarmellose. Specifically, SSG acts mainly through swelling, while, croscarmellose seems to work partly by swelling and, additionally, by wicking and recovery of energy of elastic deformation [57, 65]. The last two mechanisms will not be further discussed, given that these concepts fall out of the purpose of this work. Moreover, the superior effectiveness of croscarmellose is also commonly associated with its particles length [57]. Croscarmellose particles are fibers, while, SSG particles are spheres. Consequently, the length of croscarmellose particles enable a matrix breakage over a longer tablet distance than the SSG particles [57]. A combination of all the factors mentioned above seems to enable an efficient disintegration activity at low formulation values of croscarmellose sodium [57]. Nevertheless, it is notable that the optimal disintegration time (10 minutes) was not accomplished for optimal formulations, regardless the superdisintegrant used. Moreover, the addition of small amounts of superdisintegrants was not sufficient to significantly reduce disintegration time. Actually, significant disintegration time reductions were only observed at high values of superdisintegrant (5% of croscarmellose sodium and 4% of SSG). This behaviour may be a consequence of the high level of magnesium citrate per tablet (77% w/w) and its considerable water solubility. It has been strongly suggested in literature that superdisintegrants have a worse disintegration performance in tablet formulations that contain significant amounts of soluble substances [57, 67]. The explanation of this relays on the fact that superdisintegrants function mainly by breaking the tablet matrix (by a mechanism explained before). When a soluble substance is part of the tablet matrix, the matrix starts dissolving instead of disintegrating in smaller parts. As the matrix dissolves the superdisintegrant is no longer in contact with solid particles to physical force breaking, consequently reducing disintegration [57, 67]. This may be an explanation why low disintegration time values were never accomplished.

Another important aspect to consider is the relationship between disintegration time and hardness. During these studies, it was observed that as tablet hardness increases the disintegration time increases. Tablet hardness is a consequence of the pressure applied to the power during compression. Greater pressure, in normal conditions, results in greater tablet hardness [57]. It is suggested that high pressure reduces the tablet porosity by bringing the particles physically closer. Lower porosity retards the water penetration, thereby increasing disintegration time. In conclusion, tablet hardness is an important factor to control in order to obtain acceptable disintegration time [57]. Additionally, as shown before and discussed above, hardness has an important effect on friability. Thus, it is crucial to control and adjust this factor to meet disintegration time and friability requirements [68, 69].

Regarding the friability studies, it is possible to conclude that increasing levels of both superdisintegrants cause increasing friability mass losses. However, it appears that croscarmellose sodium has a less negative impact on friability when compared with SSG. Taking into account the friability contour plots for croscarmellose and SSG (Figures 4.18 and 4.12, respectively), it is possible to conclude that friability values under 0.75 % w/w are achievable for the maximum value of croscarmellose level, however,

for the maximum SSG value that is not true. Moreover, it was found that tablet hardness has an important impact on friability. For both disintegrants it was observed a decreasing effect on tablet friability caused by increasing tablet hardness. These results were found before in several studies [68, 69]. It was also found that friability is significantly impacted by the interaction of tablet hardness and superdisintegrant level.

In conclusion, croscarmellose sodium appears to be the superdisintegrant that allows a better formulation performance. As shown in the Figures 4.19 and 4.20, the range of croscarmellose level and hardness values that comply with the disintegration time and friability requirements is much wider than the same SSG range. This fact indicates that croscarmellose variance is a less significant risk factor for the accomplishment of the CQAs, disintegration time and friability, than SSG variance. Therefore, the formulation presented in the Table 4.13 was considered the final formulation and proposed for further studies, mostly importantly process understanding studies. It is important to highlight that the understanding of how process parameters impact the product CQAs is crucial to design a quality formulation, in particular, those identified as high risk factors.

Table 4.13: Formulation selected for immediate release magnesium tablets.

Components	Percentage (% w/w)	Amount per tablet (g)	Function
Magnesium citrate	77	1.16	Nutritional substance
Vitamin B6	0.047	0.0007	Nutritional substance
Sodium stearyl fumarate	0.7	0.0105	Lubricant
Aerosil 200	4	0.075	Glidant
Croscarmellose sodium	5	0.06	Superdisintegrant
Prosolv Nutra	13.25	0.20	Direct compression matrix
Total	100	1.5	

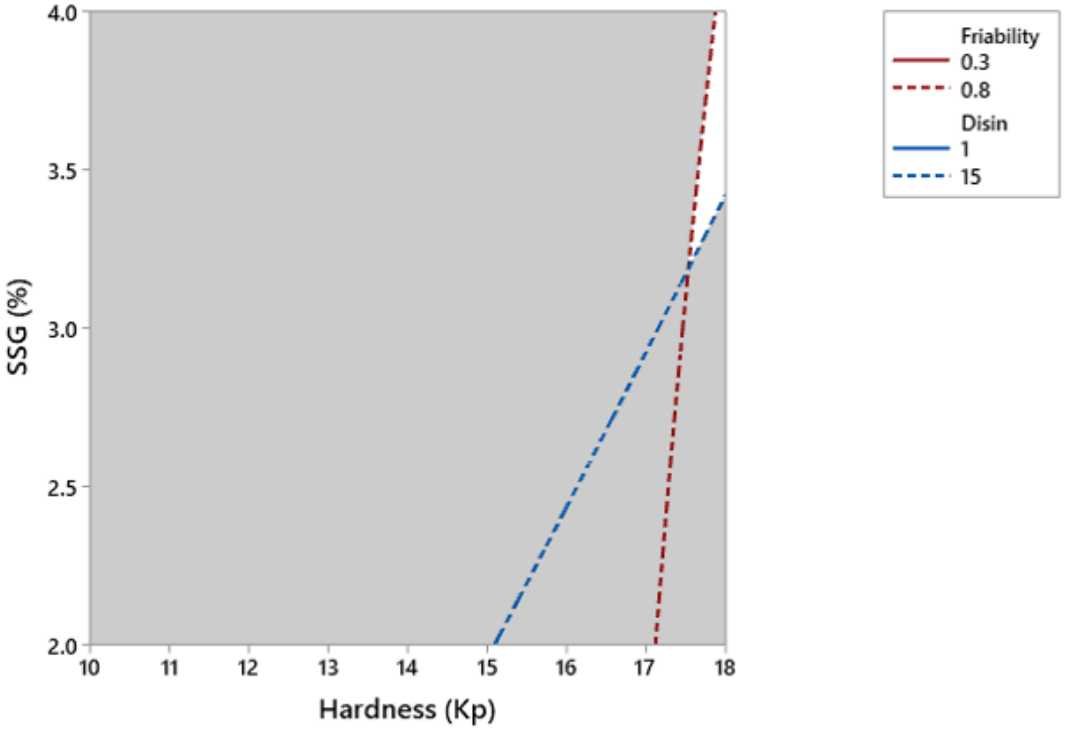

Figure 4.19: Overlaid contour plot regarding disintegration time and friability mass losses as function of sodium starch glycolate level and hardness variation. Note: consider disintegration in minutes and friability in % (w/w).

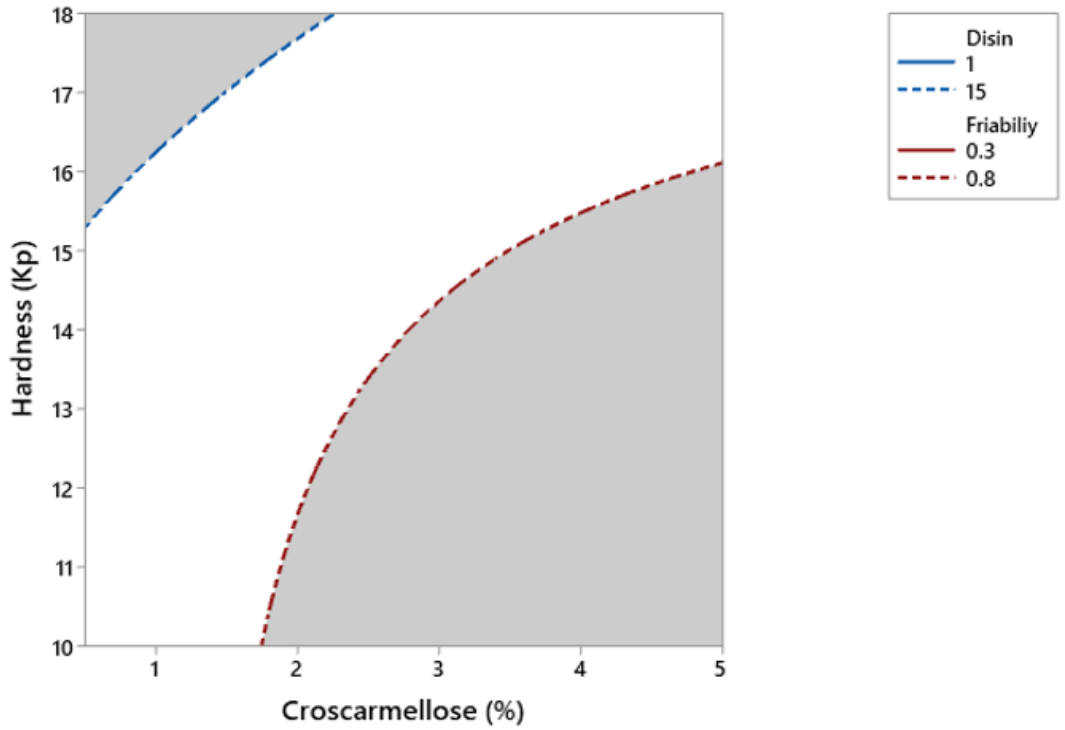

Figure 4.20: Overlaid contour plot regarding disintegration time and friability mass losses as function of croscarmellose sodium level and hardness. Note: consider disintegration in minutes and friability in % (w/w).

4.2 Oral solution formulation and process design

The development process carried during this research work aimed to develop an oral solution that complies with all the desired quality attributes. Therefore, the process was initiated with the establishment of the oral solution QTPP, as described in the sub-chapter 4.2.1. The oral solution development should be performed in such a way that all the quality targets are achieved. With the QTPP established, it is possible to develop the manufacturing process and formulation accordingly.

As mentioned before in the sub-chapter 1.4, an oral solution formulation may comprise several excipients. Nevertheless, in oral solutions, some classes of excipients are more used than others. Some examples of the commonly used excipients are solvents (e.g. purified water), microbial preservatives, antioxidants, sweeteners, buffer agents, dyes, flavours and flavour masking agents [48]. In order to select suitable excipients it is fundamental to study the behaviour of such excipients in the formulation and during the manufacture process. The same line of thought may be followed for the definition of process parameters. Only by performing formulation and process design studies it is possible to make a science based selection of excipients and process parameters set-ups. Therefore, posterior to the QTPP definition, manufacturing and formulation design studies were purposed and carried. The design studies had three main objectives:

- Select a suitable magnesium salt;
- Select a suitable combination of excipients;
- Select a reproducible manufacturing process.

In the next sub-chapters the results from the mentioned studies are described.

4.2.1 Quality target product profile

The oral solution QTPP was established in order to define the quality attributes desired for the final product, and in addition, supporting the identification of the CQAs of the product. Table 4.14 shows the quality attributes of the oral solution and the desired targets for each one. The development process should be performed in order to give a final product that meets all the criteria presented in the Table 4.14. In addition, the product shall comply with the company expectations. Thus, the oral solution is intended to be developed with a superior palatability score when compared with previous magnesium oral solutions developed by the Medinfar, be free of sugar and caloric excipients, and finally, contain low percentages of sodium. As mentioned before and shown in Table 4.14, there were identified seven initial CQAs: palatability, appearance, impurities, uniformity of dosage units, efficacy of antimicrobial preservation, microbial enumeration and pH. The justification for each is presented in the mentioned

table. The identification of the product CQAs was performed taking in consideration what is described in the literature (in particular ref. [48, 51, 70]) and prior knowledge.

Table 4.14: Oral solution quality target product profile. CQA – Critical Quality Attribute, NA – Not Applicable.

Quality attribute	Target	CQAs?	Justification
Product classification	Food supplement	No	NA
Dosage form	Oral solution	No	NA
Route of administration	Oral	No	NA
Dosage strength	250 mg	No	NA
Palatability	Without any immediate or belated sour or bitter taste, with mango aroma and a sweet taste.	Yes	Palatability influence decisively the patient compliance and should be appropriate for the target patient population. The formulation variables have a direct impact on the product palatability.
Appearance	Strong red color, homogeneous and clear solution, without precipitates.	Yes	Appearance influences the patient acceptance, compliance and satisfaction with the product. Appearance problems may be an indication of physical and chemical instability. The formulation and process variables have a direct impact on the product appearance.
Identity	Positive for magnesium	No	NA
Assay	Between 90% to 110%	Yes	Content variation impact product safety and efficacy. Process and formulation variables have a direct impact on the content uniformity.
Impurities	Meets the pharmaceutical criteria for oral solutions	Yes	Degradation products result from chemical and physical instability, and therefore impact product efficacy and safety. Both formulation and process variables impact the product stability.
Uniformity of mass	Meets the pharmaceutical criteria for oral solutions	No	NA
Uniformity of dosage units	Meets the pharmaceutical criteria for oral solutions	Yes	Variability in the dosage units impacts the product safety and efficacy. Both formulation and process variables impact the uniformity of dosage units.
Efficacy of antimicrobial preservation	Meets the pharmaceutical criteria for oral solutions	Yes	Inefficacy of the antimicrobial preservation leads to microbial growth, which may cause harm to the patient, cause chemical instabilities or appearance issues. Therefore an oral solution must have efficient antimicrobial preservatives. Both formulation and process variables impact the efficacy of antimicrobial preservation.
Microbial enumeration	Meets the pharmaceutical criteria for oral solutions	Yes	Microbial growth shows an ineffective antimicrobial preservative. Microbial growth may cause harm to the patient, cause chemical instabilities or appearance issues. Both formulation and process variables impact microbial enumeration.
Specified microorganisms	Meets the pharmaceutical criteria for oral solutions	No	NA
pH	4.5 - 6.5	Yes	pH variability may lead to precipitation, hydrolysis, oxidation and other chemical instabilities. Additionally, the pH values impact the antimicrobial preservation efficacy and, therefore, the microbial growth. Summarizing, variation of the pH values affects the product safety and efficacy. Formulation variables have a direct impact on pH.

4.2.2 Manufacturing process design

The manufacturing process of oral solutions is simple, encompassing a limited number of equipment and unit operations. The equipment necessary includes a vessel with a suitable capacity, an agitation system and a filtration system. The last, only being necessary to ensure the solution clarity [48, 51, 70].

The manufacture process consists of the addition of solid solutes to the solvent, while the system is constantly agitated. Considering solutes which solubility increases with temperature, heating the system may be necessary. Consequently, volatile substances should be added only when the system decreases its temperature to room temperature. Regarding the order of addition of solutes, it is common to start by adding the solutes present in high concentrations. Solutes present in low percentages are commonly dilute in small amounts of solvent and then added to the solution. Volatile substances (as vitamins and flavors) are added at the end of the process, in order to avoid losses by evaporation. After the volatile substances are dissolved, the solution volume is make-up to final volume, using the necessary amount of solvent [48]. Taking these aspects in consideration, an initial manufacturing process was established, as the Figure 4.21 demonstrates. Additionally, it is important to consider that at this point of the research work it was not possible to further define the manufacturing process procedure. In a stage where more information is available, the parameters should be adjusted and a final manufacturing process scheme designed.

In order to assess the risk that the variation in process parameters may encompass for the CQAs

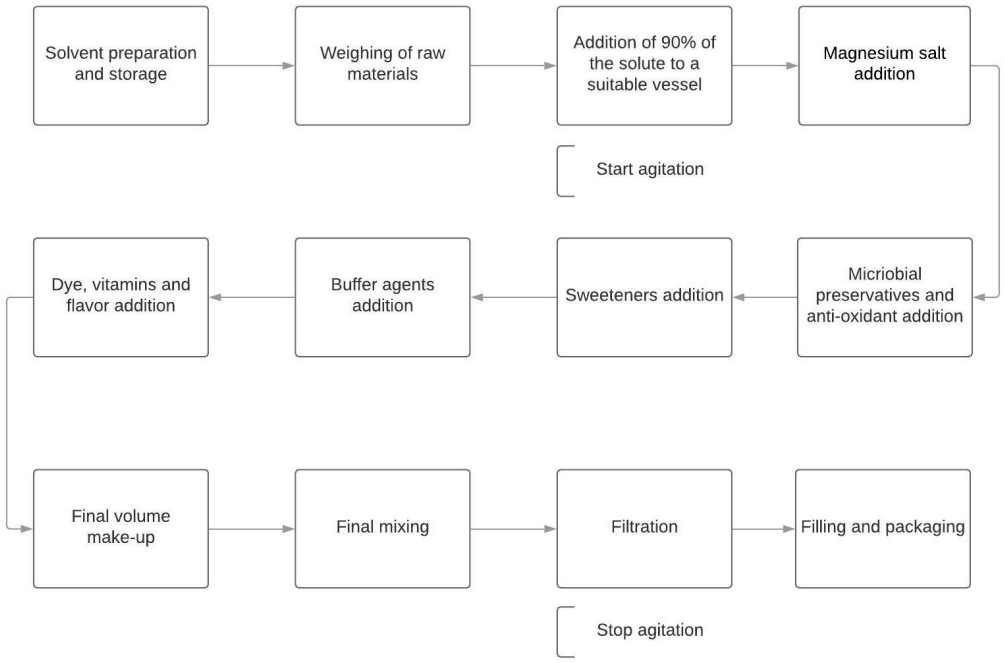

Figure 4.21: Oral solution initial manufacturing process scheme.

accomplishment, an initial risk assessment was performed. The process parameters do not have the same impact on each product CQAs, therefore it is important to understand which process variables encompass a higher risk. The process parameters that encompass higher risk for the CQAs should be special studied, in order to better understand and control them. As the Table 4.15 demonstrates, the process variables that represent a higher risk for the accomplishment of the product CQAs are solvent preparation and storage, solubilization of the API and the microbial preservatives, pH adjustment, final volume make-up, and final mixing time and speed. Such process stages should be special studied and when more information about it is available, the process parameters should defined and a final risk assessment should be established.

In the next sub-chapter the results from the formulation design studies carried to define the oral solution formulation are described.

4.2.3 Formulation design

The formulation design studies were carried in order to identify the combination of excipients and magnesium salt that result in a formulation that meets all the QTPP criteria presented. Taking in consideration the magnesium salts review presented in the sub-chapter 3.2.9, three magnesium salts were considered and tested: trimagnesium citrate, magnesium chloride and magnesium L-pidolate.

In order to characterize the palatability and stability in water of the selected salts, water solutions of each salt comprising a magnesium dosage of $250\,mg$ were prepared accordingly to what is described in sub-chapter 3.2.9. Each solution was qualitatively tested for taste and the appearance of the solution recorded over time. As presented in the Table 4.16, the salt that provokes the worst taste feeling is magnesium chloride. All the solutions were clear and transparent after the solubilization time, but after one day the trimagnesium citrate solution presented a white precipitate, similar to sand (in shape and size). After seven days, the white precipitate in the trimagnesium citrate solution increased in volume and the remaining solutions maintain a clear appearance. Concluding, magnesium citrate revealed itself as the salt with the highest pleasant taste, however, the solution developed a precipitate, suggesting that this salt is not stable in aqueous solutions. Thus magnesium citrate was considered as an unsuitable

Table 4.15: Initial risk assessment for oral solution process variables.

	Process variables					
Product CQAs	Solvent preparation and storage	API + Preservatives solubilization	Organoleptic additives addition	Buffer pH adjustment	Final volume make-up	Final mixing time and speed
Palatability	Low	Low	High	Medium	Medium	Medium
Appearance	Low	Low	Low	Low	Medium	High
Assay	Low	High	Low	Low	High	High
Impurities	High	High	Low	Low	Medium	Medium
Uniformity of dosage content	Low	High	Low	Low	High	High
Efficacy of microbial preservative	Low	Medium	Low	High	Low	Low
Microbial growth	High	Medium	Low	High	Medium	Low
Solution pH	High	Low	Medium	High	Medium	Low

Table 4.16: Magnesium salts taste and appearance characterization. Y_S – Salt amount needed to achieve 250 mg/10 ml of magnesium.

Magnesium salt	Y_S	Taste description	Appearance description
Trimagnesium citrate	1.55 g	Overall pleasant taste, with a weak sour aftertaste.	After the stabilization time, the solution appeared clear, transparent and with no signals of precipitation. After 24 hours the solution develop a white precipitate, similar to sand. After 7 days, the precipitate increased in volume, creating a hard white layer on the base of the vessel.
Magnesium chloride	2.09 g	Very strong sour taste, making the solution very unpleasant.	After the stabilization time, the solution appeared clear, transparent and with no signals of precipitation. After 24 hours no changes were found in the solution appearance. The same was found after 7 days.
Magnesium L-pidolate	2.89 g	Strong sour taste, solution unpleasant but more acceptable than magnesium chloride and less than magnesium citrate.	After the stabilization time, the solution appeared clear, transparent and with no signals of precipitation. After 24 hours no changes were found in the solution appearance. The same was observed after 7 days.

magnesium source for this formulation. On the other hand, both magnesium chloride and magnesium L-pidolate salts presented a sour taste, with magnesium chloride having a superior sour taste. Moreover, magnesium chloride has a superior percentage of magnesium, meaning that a fewer amount of salt could be used in order to achieve the desired magnesium concentration. This feature may represent a stability advantage, given that the solution will be furthest from the saturation point, preventing precipitation. However, it was decided to give priority to the oral solution taste. Thus, magnesium L-pidolate was selected as the magnesium salt for further formulation studies.

Regarding the excipients selection, a literature review (ref. [48, 51]) was performed in order to understand which excipients are commonly used in oral solutions. Table 4.17 lists the selected excipients. It is important to highlight that the final formulation is expected to be composed by three sweeteners, two antimicrobial preservatives and one flavoring agent. Therefore, through formulation design studies, the best combination of sweeteners, antimicrobial preservative and flavoring agents will be selected form the presented list (Table 4.17). The next sub-chapter describes the results from the studies perform in order to select the combination of excipients that better meets the QTPP criteria.

4.2.3.A Oral solution excipient selection

In order to select the best combination of excipients to constitute the final oral solution formulation, two QbD tools were initially used: risk assessment and DoE. As the Table 4.18 exhibits, a risk matrix was used in order to qualitatively assess the risk that each formulation variable may represent for the given oral solution CQAs. Considering the palatability attribute, it is possible to conclude that variation in the majority of the formulation variables represents high or medium risk for the accomplishment of this product CQAs. Thus, and given that no other CQAs may be equally affected by the formulation variables, palatability was chosen as a selection attribute. Concluding, formulations were primordially evaluated for palatability and only the solutions with pleasant taste were further studied. In order to facilitate the comprehension of the excipient selection process (also mentioned as formulation selection) a flow chart

Table 4.17: Oral solution excipients.

Excipient	Function	Level (% w/w)
Sodium ciclamate	Intense sweetener	0.170
Aspartame	Intense sweetener	0.370
Sodium saccharine	Intense sweetener	0.240
Neohesperidin	Intense sweetener	0.0120
Stevia	Intense sweetener	0.200
Potassium acesulfame	Intense sweetener	0.125
Potassium sorbate	Antimicrobial preservative	0.150
Benzoic acid	Antimicrobial preservative	0.100
Sodium benzoate	Antimicrobial preservative	0.240
Citric acid	Antioxidant and preservative	0.850
Monosodium glutamate	Flavoring agent	0.200
Sodium gluconate	Flavoring agent	0.200
Ponceau coloring agent	Dye	0.030
Mango flavor	Flavor	0.020
Purified water	Solvent	q.s to 100

Table 4.18: Initial risk assessment for oral solution formulation variables.

	Formulation variables							
Product CQAs	Magnesium salt	Solvent	Anti-microbial	Anti-oxidant	Colors	Flavor	Sweeteners	Buffer agents
Palatability	High	High	Medium	Low	Low	High	High	Medium
Appearance	High	High	Low	Low	High	Low	Low	Low
Assay	High	High	Low	Low	Low	Low	Low	Low
Impurities	Medium	High	Medium	High	Low	Medium	Medium	Low
Uniformity of dosage content	High	High	Low	Low	Low	Low	Low	Low
Efficacy of microbial preservative	Low	High	High	Low	Low	Low	Low	Medium
Microbial growth	Medium	High	High	Medium	Medium	Low	Low	Medium
Solution pH	Medium	High	Low	Low	Low	Low	Low	High

was elaborated, as the Figure 4.22 demonstrates. The flow chart was established based on the initial risk assessment results, giving priority to the CQAs that are at greater risk. Nevertheless, the flow chart was also adapted to the feasibility of the CQAs analysis and to the time each one costs. For example, pH measurements were performed after the palatability and appearance evaluation given that this measurement is easy and fast to perform. Moreover, pH values may affect other product CQAs, such as efficacy of microbial preservatives and microbial growth, so it seems reasonable to primordially evaluate these CQAs. As describe above, another QbD tool was employed during the excipients selection process: DoE. The DoE was performed in order to establish different formulations with the excipients presented in the Table 4.17. From this process resulted twelve different formulations, each with a different combination of sweeteners, antimicrobial preservatives and flavoring agents, as shown in Table 4.21. The manufacture of these formulations followed what is described in sub-chapter 3.2.10.

As previously mentioned, each formulation was initially tested for palatability. For this test, ten volunteers were asked to blind taste each formulation and score them for sweet and sour taste. The scale score can be found in the Chapter 3. The higher the score for sweet, the more pleasant the solution

is and the same line of thought should be followed for sour score interpretation. It was defined that an acceptable solution should score 5 or higher in both evaluations. Figure 4.23 illustrates the average score for sweet and sour taste for each formulation. As shown, there are six formulations that scored 5 or higher in both taste evaluations: 2.A, 2.B, 5.A, 5.B, 6.A and 6.B. From this set of formulations, the formulations 6.A and 6.B were discarded, given that both have sodium saccharin in its composition. As mentioned before, it is a company expectation to develop a product free of caloric sweeteners, therefore no further studies were performed on the solutions 6.A and 6.B. Another aspect demonstrated in Figure 4.23, is the constant score superiority of the solutions A when compared with the solutions B. The difference between A and B solutions is only one excipient, the flavoring agent. Solutions A have sodium gluconate, while solutions B have monosodium glutamate in its composition. The flavoring agents were added with the intention to mask the typical sour taste of magnesium salts and given the results obtained, sodium gluconate seems to have a superior masking taste capacity than monosodium glutamate. Concluding, from the previous set of formulation the formulations B where discarded, remaining the solutions 2.A and 5.A. An important factor to consider regarding the solutions 2.A and 5.A is their similarity in sweeteners composition. Both formulations contain sodium cyclamate and aspartame, this may suggest that this combination of sweeteners have a synergistic action that improves the sweet feeling caused by the formulation. On the other hand, the solutions 1.B and 3.B were the solutions with the lowest scores, as the Figure 4.23 shows. This formulation have sodium saccharin and acesulfame potassium in its composition, suggesting that this combination of sweeteners is not sufficiently efficient.

Considering the solutions appearance, it was observed that it did not significantly varied between the different formulations. All the solutions presented an uniform red color, with homogeneous and translucent appearance. Therefore it was concluded that the combination of excipients did not impact the solution appearance, at least immediately. Summarizing the formulations 2.A and 5.A complied with both appearance and palatability targets. Therefore pH measurements were performed in both formulations. Has shown in Table 4.19 and 4.20, the formulations had, at t = 0 months, pH values of 4.86, complying with the pH target. Thus, no pH adjustments procedures were needed. As the flow chart in Figure 4.22 shows, the next step in the formulation selection would be to test the microbial preservative efficacy. However, at this point of the research work there faced some resources constrains, therefore both solutions were submitted to stability storage conditions, as described in the sub-chapter 3.2.12.

The stability studies performed aimed to evaluate the stability of the product through time. There were established 8 points in time in which the solutions would be evaluated after being subjected to real time and accelerated conditions. The points were set apart by 1 month (30 days), therefore the stability studies will be complete after 8 months. Whenever a sample is collected from storage, it should be immediately analyzed. As the Figure 4.22 demonstrates, the sample should be analyzed in terms of palatability, appearance, pH, assay, impurities, microbial growth and uniformity of content. These

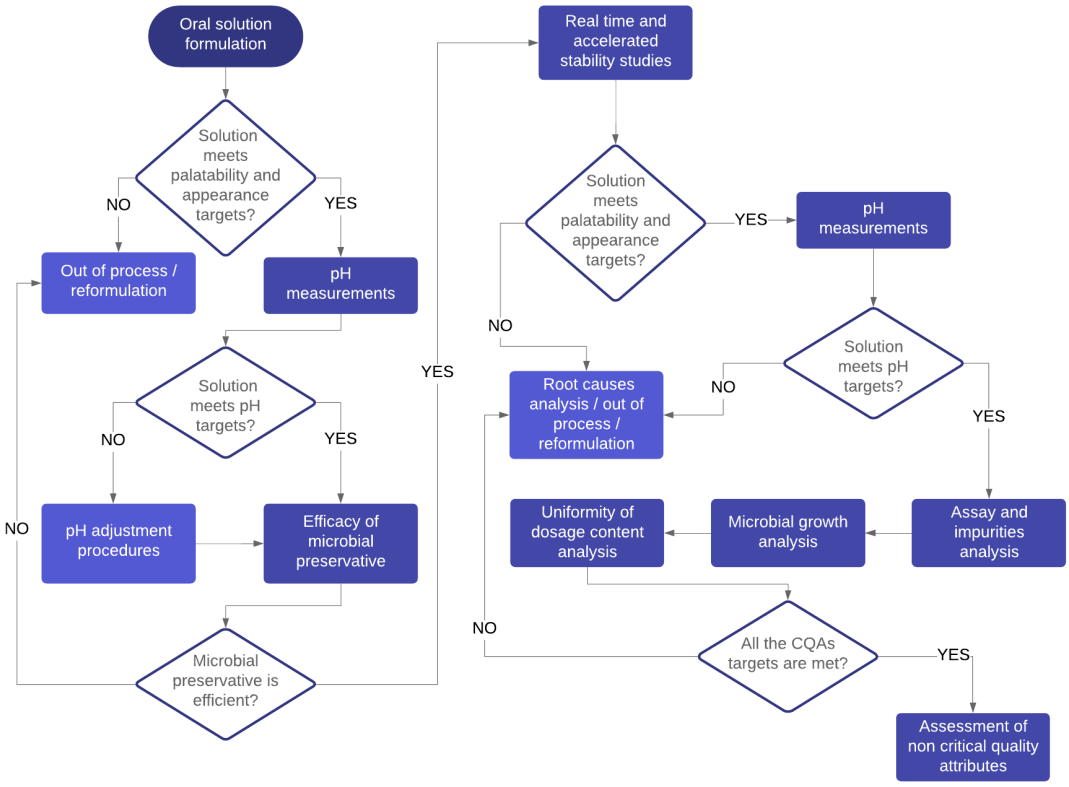

Figure 4.22: Flow chart representing the selection process of oral solution formulations.

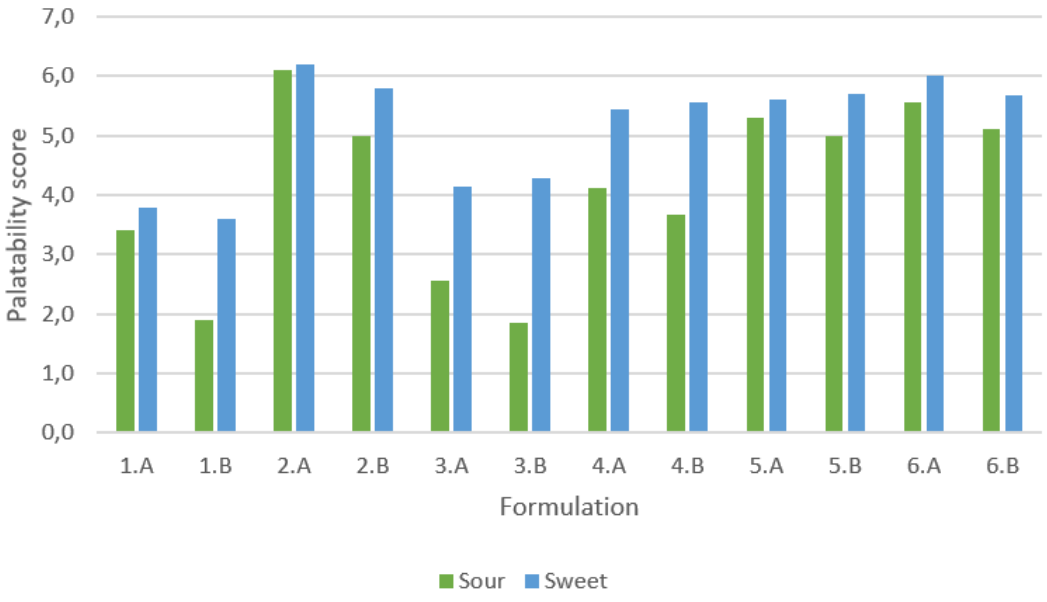

Figure 4.23: Bar chart of the palatability score of each oral solution formulation.

attributes should be primarily studied given that they correspond to the product CQAs. Moreover, special attention should be given to the impact of formulation and process variables identified as high risk factors. If any of the critical attributes is not fulfilled, a root causes analysis should be performed in order to understand the source of the problem. If the formulation fulfills the CQAs, then the remaining quality attributes should be studied. When the process presented in the Figure 4.22 is completed, one solution should be selected as the final product. Such solution should be the one that better fulfills all the quality attributes presented in the QTPP. In the Tables 4.19 and 4.20 the results from 0 months and 1 month regarding pH, appearance and palatability are summarized. As illustrated, no significant changes were found on those attributes. In conclusion, both formulations kept the mentioned attributes stable through 30 days regardless the storage conditions.

Table 4.19: Oral solution 2.A pH values, taste score and appearance description over time. RC - Real Conditions; AC - Accelerated Conditions.

Time (months)	RC pH	AC pH	Appearance
0	4.86	4.86	Translucent and limpid liquid with a strong red color, without precipitates. Weak sour and sweet taste.
1	4.85	4.85	No changes registered.

Table 4.20: Oral solution 5.A pH values, taste score and appearance description over time. RC - Real time Conditions; AC - Accelerated Conditions.

Time (months)	RC pH	AC pH	Appearance
0	4.86	4.86	Translucent and limpid liquid with a strong red color, without precipitates. Weak sour aftertaste and moderately sweet taste overall.
1	4.83	4.85	No changes registered.

Table 4.21: Different formulations of magnesium L-pidolate oral solutions. 1 - In composition; 0 - Not in composition.

	Excipients														
Solution ID	Cyclamate	Aspartame	Na saccharin	Neohesperidin	Stevia	Acesulfame	K sorbate	Benzoic acid	Na benzoate	Citric acid	Na gluconate	Na glutamate	Dye	Flavor	Purified water
1.A	0	0	1	0	1	1	1	1	0	1	1	0	1	1	1
1.B	0	0	1	0	1	1	1	1	0	1	0	1	1	1	1
2.A	1	1	0	0	1	0	1	0	1	1	1	0	1	1	1
2.B	1	1	0	0	1	0	1	0	1	1	0	1	1	1	1
3.A	0	0	1	1	0	1	1	0	1	1	1	0	1	1	1
3.B	0	0	1	1	0	1	1	0	1	1	0	1	1	1	1
4.A	0	0	0	1	1	1	0	1	1	1	1	0	1	1	1
4.B	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1
5.A	1	1	0	1	0	0	1	1	0	1	1	0	1	1	1
5.B	1	1	0	1	0	0	1	1	0	1	0	1	1	1	1
6.A	0	1	1	0	0	1	0	1	1	1	1	0	1	1	1
6.B	0	1	1	0	0	1	0	1	1	1	0	1	1	1	1

5

Conclusion

The development of magnesium dosage forms carried during this research work complied with the main objectives initially proposed. The development process was carried through a combination of literature review and research work based on the several QbD concepts suggested in the ICH guidelines Q8 and Q9. The main conclusions resulting from this research will be presented and discussed throughout this chapter. Initially, the main findings resulting from the tablets development will be described. Secondly, there will be presented the conclusions resulting from the oral solution development. Finally, suggestions for future work and the main limitations encountered during this project are discussed.

In this project, immediate release tablets, containing a considerable bioavailable magnesium source, were satisfactorily manufactured through direct compression method and taking in consideration the QbD approach. Initially, it was established a QTPP that enabled the early identification of product CQAs. Following, a risk assessment tool was applied and the variables that may have a considerable impact on the tablets CQAs were identified and proposed for further studies of formulation and manufacturing design.

The formulation design studies allowed the selection of a suitable magnesium source and the establishment of a combination of excipients with an output performance in accordance with the QTPP. In particular, magnesium citrate was selected as the nutritional substance, Aerosil 200 as glidant, SSF as lubricant and croscarmellose sodium as superdisintegrant. During the magnesium salt selection, it was found that the presence of glidant and lubricant in the formulation was crucial for the accomplishment of the QTPP appearance criteria. Therefore, the impact of Aerosil 200 and SSF level on the formulation performance was studied, applying DoE tools. Through this study it was possible to conclude that increasing levels of Aerosil 200 have a beneficial impact on disintegration time. On the other hand, it was found that high SSF levels have a negative impact on tablets disintegration. These findings are in agreement with several studies found in literature. Considering hardness, it was found that variation in this particular term did not significantly impact disintegration time. This behaviour was unexpected, given that in literature a significant negative impact of hardness in this quality attribute is commonly described. The analysis of the resulting regression model allowed the identification of two nearly optimal tablet formulations (CO1, CO2). The formulations were experimental tested and it was found that the predicted and observed values were not in close agreement. This observation may be a consequence of the limited experimental data used to build the model. In particular, more variation levels of Aerosil 200 and SSF should have been considered in order to ensure a better predictive ability to this model. Additionally, resource constrains prevented the performance of a correct model validation. Nonetheless, considering the disintegration times observed, it was possible to drawn two main conclusions: i. The nearly optimal disintegration values are considerably lower when compared to the prototype formulation C3, thus, the DoE analysis contributed for the optimization of this particular quality attribute; ii. The nearly optimal values are still very close to the specification limit for immediate realise tablets, thereby,

the addition of a superdisintegrant may result in lower disintegration time.

In light of the above, the impact of superdisintegrants on the formulation performance was study through two different DoE. The first, considered SSG level and hardness as factors, while the second considered croscarmellose level and hardness. Disintegration time and friability were taken as responses for both experiments. Four mathematical models were established to evaluate the variability of the responses as function of the factors. These models satisfactorily fitted the data and had an adequate predictive ability. From this study it was possible to conclude that variation in SSG level, croscarmellose level and hardness have a significant impact on disintegration time and friability. In particular, it was found that the disintegration time of tablets containing SSG was more sensitive to tablet hardness variation than to SSG level variation. Whereas, the disintegration time of tablets containing croscarmellose was substantially impacted by its concentration, being less sensitive to hardness variation. It was also found that croscarmellose sodium had a greater impact on the tablets disintegration time. Specifically, croscarmellose was more effective at lower formulation amounts when compared to SSG, suggesting a higher disintegration capacity. Increasing levels of both superdisintegrants appeared to cause increasing tablet friability. However, from both superdisintegrants, croscarmellose had the less negative impact on this attribute. Furthermore, it was found that tablet hardness increment, had a negative impact on disintegration time but a positive impact on tablet friability. Thus, it seams crucial to control this attribute in order to jointly satisfy disintegration and friability requirements. Finally, the models analysis resulted in the identification of two nearly optimal formulations (one containing SSG and another containing croscarmellose). As result of the predicted and observed responses, the optimal formulation containing croscarmellose sodium ($5\,\%w/w$) was chosen as the final formulation.

In conclusion, this research allowed the optimization of formulation settings, thereby resulting in a final formulation that complies with the QTPP criteria for appearance, friability and disintegration time. Furthermore, it may be concluded that mathematical models represent a useful tool to predict and understand the relationship between the formulation attributes and the input variables, supporting a more efficient product development.

Some of the limitations encountered during tablet development form the basis for future work. In particular, it was not possible to perform studies of process understanding. Thus, it seems relevant to study the impact of process parameters variation on the formulation performance (specially of those assessed as high risk factors). Only through these studies it would be possible to develop a manufacturing process that consistently gives an output product with the desired quality attributes. Moreover, time and resource constraints prevented the complete study of the tablets performance. Therefore, the impact of formulation variables on the tablets dissolution and uniformity of content shall be further studied. Additionally, the extent of the experimental data was also found as a project limitation. If more variation levels were considered during experimentation, the models would have a better predictive ability and the risk of

false predictions would be lower. The poor model experimental validation is another important limitation to considered. Such experimental validation shall be performed to increase the confidence level on the optimal formulation settings identified. Finally, a particular limitation of this research is the investigation of hardness as an independent variable (i.e. model input). Hardness is impacted by formulation and process variables, being dependent on the experimental design. However, compression force could not be precisely adjusted and controlled due to limitations within the compression machine system. Therefore, in a scenario where this parameter can be adjusted and controlled, hardness shall be studied as a response of compression force variation. Additionally, it also seems relevant to study the efficiency of different types and levels of superdisintegrants when incorporated in tablets containing highly soluble components.

Regarding the oral solution development, it is possible to conclude that the designated QTPP was properly established based on literature review and prior knowledge. Thereby, allowing the identification of possible product CQAs. In addition, this quality profile served as basis for the formulation design studies. The design studies enable the selection of magnesium L-pidolate as the oral solution nutritional substance (along with vitamin B6). Moreover, it was possible to select combinations of excipients that comply with the desired palatability, thus, reducing the risk associated with the accomplishment of this product CQAs. The real time and accelerated stability studies showed that the solutions 2.A and 5.A were stable through 30 days, in what concerns palatability, appearance and pH. Summarizing, the oral solution development fulfilled the overall aim initial proposed. However, time and resource constrains prevented the study of several product CQAs.

The limitations found during the oral solution development lay the foundation for future work concerning the impact of process parameters and formulation variation on the product CQAs. In particular, it was not possible to study the impact of process variables (e.g. solvent preparation or mixing time and speed) in the identified product CQAs. Thus, it is important to further study the effect of process parameters variation on the product CQAs and, thereby, define a robust manufacturing process that consistently gives a quality product. Furthermore, it should be interesting to study the impact of formulation variability in the following product CQAs: assay, impurities, microbial growth and uniformity of content. These studies would jointly contribute for the understanding of how the formulation attributes and process parameters impact the output product CQAs. This knowledge is crucial for the establishment of meaningful specifications. Notwithstanding the above, the present research may serve as groundwork for the accomplishment of such knowledge.

Overall, it may be conclude that the QbD tools applied throughout this work contributed for an efficient development process of magnesium dosage forms. The systematic approach to food supplements development enabled the overcoming of many formulation design challenges. Furthermore, as result of this project, immediate release tablets of magnesium and vitamin B6 were develop complying with the

appearance, disintegration and friability targets. Similarly, two magnesium oral solutions complying with dosage strength, appearance, palatability and pH targets were formulated.

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Allowed magnesium substances

The allowed substances which may be used in the manufacturing of food supplements as sources of magnesium, in the European Union, are the following:

- Magnesium acetatemagnesium L-ascorbate;
- magnesium bisglycinate;
- Magnesium carbonate;
- Magnesium chloride;
- Magnesium salts of citric acid;
- Magnesium gluconate;
- Magnesium glycerophosphate;
- Magnesium salts of orthophosphoric acid;
- Magnesium lactate;

- Magnesium L-lysinate;
- Magnesium hydroxide;
- Magnesium malate;
- Magnesium oxide;
- Magnesium L-pidolate
- Magnesium potassium citrate;
- Magnesium pyruvate;
- magnesium succinate;
- Magnesium sulphate;
- Magnesium taurate;
- Magnesium acetyl taurate.