UNIVERSIDADE DE LISBOA
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FACULDADE DE FARMÁCIA

INVESTIGAÇÃO E DESENVOLVIMENTO DE UMA FORMULAÇÃO INALATÓRIA DE CICLODEXTRINAS COMO BASE DE UM INSTRUMENTO DE GESTÃO DE RISCO

RESEARCH AND DEVELOPMENT OF A CYCLODEXTRIN INHALATION FORMULATION AS THE BASIS OF AN APPLIED RISK MANAGEMENT TOOL

Nuno Miguel da Conceição Marigesa Resende Arriagas

MESTRADO EM FARMACOTECNIA AVANÇADA

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MESTRADO EM FARMACOTECNIA AVANÇADA
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ABSTRACT

Asthma is a chronic disease affecting a significant number of patients worldwide, causing symptoms on the respiratory tract.

Corticosteroids like ciclesonide are used to manage the disease. However, its physicochemical properties – namely water solubility – and, consequently, pharmacokinetics and pharmacodynamics could be object of improvement. One way to achieve this is by the use of cyclodextrins, a well known group of molecules able to include insoluble molecules, solubilising them. The aim of this work is to verify if is possible to obtain a dry powder for inhalation containing ciclesonide included within Crysmeb (a commercial type of methylated beta-cyclodextrin) applying the simple and inexpensive technique of kneading. The complex formed was analysed using ultraviolet spectroscopy, differential scanning calorimetry and infrared spectroscopy. Inhalation powders characteristics were assessed by time-of-flight of the particles (particle size analysis), and twin stage liquid impinger (aerodynamic assessment of fine particles). It was concluded that the kneading method is feasible for obtention of a ciclesonide-Crysmeb inclusion complex, although further studies would be desirable to optimize the process.

Some science projects involve massive funds, time and human resources, having, sometimes, a relatively high risk of being unsuccessful. Therefore, using this experimental work as a starting point, an online questionnaire was publicized to science-related people from the top ten universities in the world. It was asked what was considered to be the most critical factors when engaging in scientific research. This data allowed narrowing the inputs that can be applied to ISO 31000:2009, hence directing an otherwise generic risk management standard to this spectrum of activity. The defined Risk Severity Index can also be used as a standalone tool, namely for individual and small organisations that do not have a risk management plan in place – as it was the case with this work. The methodology appears appropriate and with room for improvement.

Keywords: Cyclodextrins, Crysmeb, ciclesonide, inhalation, risk management, ISO 31000:2009.

RESUMO

A asma é uma doença crónica que afecta um número significativo de doentes a nível mundial, causando sintomas que a nível do tracto respiratório.

Corticosteróides como a ciclesonida são utilizados no controlo da doença. Contudo, as suas propriedades físico-químicas – nomeadamente a solubilidade aquosa – e, consequentemente, a farmacocinética e farmacodinamia podem ser objecto de melhoria. Um dos modos de atingir este objectivo é através da utilização de ciclodextrinas, um grupo de moléculas bastante conhecido capaz de incluir no seu interior moléculas insolúveis, solubilizando-as. É o objectivo deste trabalho verificar a exequibilidade da obtenção de um pó seco para inalação contendo ciclesonida complexada com Crysmeb (uma forma comercial de beta-ciclodextrina metilada) através de um método simples e económico, a malaxagem. A formação de complexo foi analisada através de espectroscopia ultravioleta, calorimetria diferencial de varrimento e espectroscopia por infravermelhos. As características dos pós para inalação foram avaliadas pelo tempo-de-vôo (análise do tamanho de partícula) e impactador em cascata de vidro (avaliação aerodinâmica de partículas finas). Foi concluído que através do método de malaxagem é possível a obtenção de um complexo ciclesonide-Crysmeb, apesar de serem desejáveis mais estudos de modo a optimizar o processo.

Alguns projectos científicos envolvem avultados montantes monetários, tempo e recursos humanos, tendo, por vezes, um risco de insucesso relativamente elevado. Assim, utilizando este trabalho experimental como ponto de partida, um questionário *online* foi publicitado junto de pessoas relacionadas com a ciência das dez universidades de topo, a nível mundial.

Era questionado sobre quais os factores que consideravam ser os mais críticos quando iniciavam pesquisa científica. Estes dados permitiram focar os *inputs* que podem ser aplicados à ISO 31000:2009, direccionando assim uma norma de gestão de risco que doutro modo seria genérica a este espectro de actividade. O Índice da Gravidade do Risco definido pode igualmente ser utilizado isoladamente, nomeadamente no caso de indivíduos e pequenas organizações que não possuem

um plano de gestão de risco – situação que se verifica com este trabalho. A metodologia utilizada parece ser apropriada e com margem para melhorar.

Palavras-chave: Ciclodextrinas, Crysmeb, ciclesonida, inalação, gestão do risco, ISO 31000:2009.

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PART A: DOSAGE FORM – PHARMACEUTICAL DEVELOPMENT

I – Introduction and background

1. OBJECTIVE

This part of the work has the objective of verifying the feasibility to obtain a drug-cyclodextrin complex, using the kneading method, formulated as a dry powder for inhalation.

Specifically, the work aims to analytically confirm the formation of a drug-cyclodextrin complex recurring to a simple physical method: kneading; and its ability to be formulated as dry powder for inhalation, including the *in vitro* delivery efficiency.

2. INTRODUCTION

Asthma is a chronic disease affecting the respiratory tract. *Circa* 235 million people worldwide suffer from this disease, being the most common chronic disease among children (1).

This disease represents a considerable health burden due to its consequential morbidity, mortality, and cost (2).

Corticosteroids are widely used to manage asthma (3,4). In Portugal, ciclesonide is pharmacotherapeutically classified under group 5.1.3.1 – Respiratory system/ Antiasthmatics and bronchodilators/ Anti-inflammatories/ Glucocorticoids (5). Its approved therapeutical indication is the management of persistent asthma in adults and adolescents over twelve years old (6). Elsewhere ciclesonide is also approved for the therapeutic management of allergic rhinitis (7–10).

It is well known that highly water-insoluble molecules can not be absorbed by the aqueous mucous layer of the lung, hence needing to be solubilized so they can exert their pharmacological effect (11–13). Therefore, the use of cyclodextrins appears to be adequate in order to improve the physicochemical properties of the drug and its absorption.

3. PATHOPHYSIOLOGY

Asthma is a chronic disease involving inflammation of the pulmonary airways as well as bronchial hyperresponsiveness resulting in, usually reversible, lower airway obstruction. Factors which can trigger airway obstruction include cold air, cigarette smoke, respiratory allergen and upper respiratory infection of viral nature. Active asthma displays *lamina reticularis* thickening, mucus gland hypersecretion, mucosal edema, lung hyperinflation, smooth muscle hypertrophy, epithelial cell sloughing, cilia cell disruption. At a microscopic level, asthma is characterized by the presence of increased numbers of neutrophils, eosinophils, lymphocytes, and plasma cells in the bronchial tissues, bronchial secretions, and mucus. Activated T-lymphocytes direct the release of inflammatory mediators from lymphocytes, eosinophils and mast cells. The cross-linkage of two IgE molecules by allergen causes mast cells to degranulate, releasing leukotrienes, histamine, and other mediators that maintain the airway inflammation. The activated mast cells and eosinophils also generate their cytokines that help to perpetuate the inflammation. Regardless of the trigger, the repeated cycles of lung inflammation with injury to the pulmonary tissues followed by repair may produce long-term structural changes of the airways (14).

4. ACTIVE PHARMACEUTICAL INGREDIENT

4.1. Physical and chemical properties

Physicochemical information on ciclesonide is very scarce. However, the following has been reported (15):

- It is a non-halogenated glucocorticoid having the chemical name pregna-1,4-diene-3,20-dione,16,17-[[Rcyclohexylmethylene]bis(oxy)]-11-hydroxy-21-(2-methyl-1-oxopropoxy)-,(11β,16α)-;
- Is a white to yellow-white powder;
- Solubility: practically insoluble in water and freely soluble in ethanol and acetone;
- The R-epimer is used in pharmaceutical preparations;
- Its molecular weight is 540,7 Da;
- The empirical formula is $C_{32}H_{44}O_7$;
- Has the following structural formula:

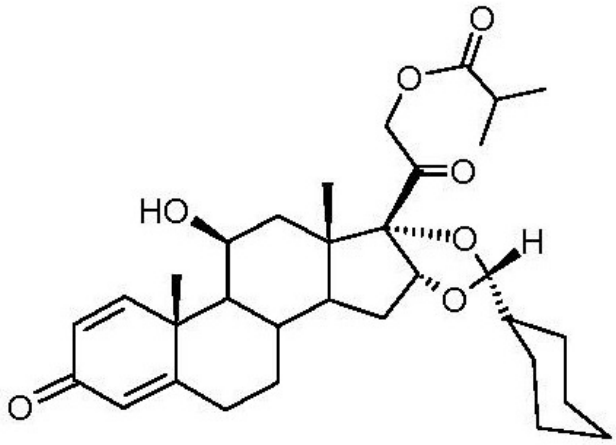


Fig. 1 – Structural formula of ciclesonide (15)

4.2. Pharmacology

Ciclesonide is a pro-drug. It is enzymatically hydrolyzed in the lung via esterases to its pharmacologically active metabolite, C21-desisobutyryl-ciclesonide (des-ciclesonide or RM1), which has anti-inflammatory activity. Des-ciclesonide has

120 times higher affinity for the glucocorticoid receptor than the parent compound ciclesonide (2,4,6,15–18).

Treatment with inhaled corticosteroids reduces the number of inflammatory cells in the bronchi. Among those cells are mast cells and eosinophils. They also reduce the proportion of alveolar macrophages and of type 2 helper T-lymphocytes and their related proinflammatory cytokines in asthmatic airways (3,19).

It is thought that inhaled corticosteroids may down regulate T-cell activation by allergen-presenting cells, inhibiting T-cell differentiation toward the stimulation of IgE production as well as promotion of inflammation in general (3,20,21). This activity has been broadly demonstrated *in vitro* (3,22–25). Reasonably low doses of inhaled corticosteroids allow attaining not only these cellular effects, but also the respective therapeutic effects in asthmatic patients, representing a low risk/benefit ratio (3). Despite these data showing a good safety profile, the existence of some local and systemic side effects must still be taken into account, as some of them, namely oropharyngeal candidiasis, are common (3,26).

The potency of an inhaled corticosteroid is assessed in terms of its relative receptor affinity when compared to the drug dexamethasone, which is assigned a value of 100 (2,27). An inhaled corticosteroid which possesses higher relative receptor affinity will induce a greater anti-inflammatory effect. Des-ciclesonide has a relative receptor affinity of 1200, similar to that of beclomethasone monopropionate, 1345. For a matter of comparison, mometasone fuorate has a relative receptor affinity of 2300, fluticasone propionate of 1800 and budesonide of 935 (2,18).

Ciclesonide and des-ciclesonide have a reported half-life of 0,36 hours and 3,4 hours, respectively. Des-ciclesonide possesses a 21-hydroxyl group and, therefore is able to form fatty acid esters that will lead to a longer retention time in the lungs allowing for a longer efficacy. The formation of these lipid conjugates has not been related to adverse effects (18,28).

After inhalation of a corticosteroid, a part of it is deposited in the mouth and oropharynx, driving to side effects such as oral candidiasis. Inhalation of a pro-drug may reduce the incidence of these local side effects in the mouth and oropharynx region, as the inactive drug that deposits in that region will be swallowed before activation can occur. This has been verified with ciclesonide (18).

5. CYCLODEXTRINS

5.1. Overview

Carbohydrates, *exempli gratia* starch, cellulose and sucrose, are likely to be the most abundant organic compounds in nature and from very ancient time they have been used as food, for shelter and for clothing. Throughout times, carbohydrates have been processed via fermentation, as their enzymatic degradation was observed. These processes, we know now, originate mixtures of monosaccharides, disaccharides and oligosaccharides, such as linear and branched dextrins. It is also known that, when subjected to certain conditions, these degradation processes also originate small amounts of cyclodextrins (29,30). These are also known as cyclomaltoses, cycloamyloses or Schardinger dextrins (31,32). **Table 1** presents a short chronological summary on the development of carbohydrate chemistry, focusing on cyclodextrins (30).

Table 1 – Cyclodextrins and the development of carbohydrate chemistry (30)

Year	Event
1808	Malus develops plane polarized light and observes optical rotation by carbohydrates.
1811	Acid-hydrolyzed starch is shown to produce sweet crystalline sugar.
1821	Production of dextrins by heating starch is discovered.
1838	The sugar from honey, grapes, starch and cellulose is found to be identical and is called glucose by Dumas.
1858	The empirical formula of glucose is determined to be $C_6H_{12}O_6$.
1870	Bayer and Fittig propose that the formula of glucose is HO-CH_2-CH(OH)-CH(OH)-CH(OH)-CH(OH)-CHO.
1888	Glucose is shown to be a six-carbon polyhydroxy aldehyde.
1888–1891	Fisher determines the structure of several carbohydrates, including glucose, fructose, mannose and arabinose.
1891	Villiers publishes his discovery of cellulosine (cyclodextrin).
1903	Schardinger publishes his first paper on α- and β-dextrins (cyclodextrins).
1920–1930	English carbohydrate research group, lead by Norman Haworth, definitely demonstrate the size of carbohydrate rings to be primary six-membered pyranoses and propose a six-membered hexagon to represent the carbohydrates.
1924	Methylation of cyclodextrins first described, later both Freudenberg and Meyer-Delius (1938) and Szejtli (1980) prepared different grades of methylated cyclodextrins.
1928–1932	The ability of cyclodextrins to form complexes with various organic compounds discovered.
1935	Freudenberg and Jacobi discover γ-cyclodextrin.
1938–1952	The chemical structure of α-, β- and γ-cyclodextrin elucidated by Freudenberg, Cramer, Borchert, French and Rundle.
1948–1951	Formation and structure of cyclodextrin inclusion complexes discovered.
1953	The first cyclodextrin patent entitled "Method for preparation of inclusion compounds of physiologically active organic compounds" was issued in Germany to Freudenberg, Cramer and Plieninger.
1954	Cramer's book on inclusion complexes (Einschlussverbindungen) published.
1957–1965	French describes the existence of large natural cyclodextrins with up to 12 glucose units.
1965	Higuchi and Connors publish their article on classification of complexes based on their phase-solubility profiles.
1976	The parent α- and β-cyclodextrin officially approved as food additives in Japan.
1976	The worlds first pharmaceutical product, prostaglandin E_2/β-cyclodextrin (Prostarmon E™ sublingual tablets), marketed in Japan by Ono Pharmaceutical Co.
1981	The first International Symposium on Cyclodextrins was organized and held in Budapest by Szejtli.
1983–1985	Brauns and Müller (Europe) and Pitha (USA) file for patents on 2-hydroxypropyl-β-cyclodextrin.
1988	Piroxicam/β-cyclodextrin tablets (Brexin®) marketed by Chiesi Farmaceutici (Italy).
1990	Stella and Rajewski file for a patent on sulfobutyl ether β-cyclodextrin.

Cyclodextrins were first discovered in 1891, when in addition to reducing dextrins a small amount of crystalline material was obtained from starch digest of *Bacilus amylobacter*. This crystalline product was named 'cellulosine'. Later, in 1903,

Schardinger isolated two crystalline products, dextrins A and B. These were described with regard to their lack of reducing capacity (31).

There are three types of cyclodextrins: alpha-cyclodextrin, beta-cyclodextrin and gamma-cyclodextrin, which are referred to as first generation or parent cyclodextrins. Alpha-, beta- and gamma-cyclodextrins are composed of six, seven and eight alpha-(1,4)-linked glycosyl units, respectively (31,33,34).

They result from an intramolecular transglycosylation reaction from degradation of starch by cyclodextrin glucanotransferase (CGTase) enzyme (31,34).

Depending on the type of cyclodextrin and guest compound, cyclodextrins can crystallise in two major types of crystal packing: cage structures and channel structures (31,34).

Generally being the most useful, beta-cyclodextrin is the most accessible and the lowest-priced as well. Types of cyclodextrins properties are laid down in **Table 2** (31)**.**

Table 2 – Cyclodextrins properties (31)

Property	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin
Number of glucopyranose units	6	7	8
Molecular weight (g/mol)	972	1135	1297
Solubility in water at 25 °C (%, w/v)	14.5	1.85	23.2
Outer diameter (Å)	14.6	15.4	17.5
Cavity diameter (Å)	4.7–5.3	6.0–6.5	7.5–8.3
Height of torus (Å)	7.9	7.9	7.9
Cavity volume ($Å^3$)	174	262	427

There are now available multiple cyclodextrin derivatives other than these naturally occurring cyclodextrins, that have been synthesised. The chemical reactions more often leading to the obtention of these derivatives are etherifications, aminations or esterifications of the primary and secondary hydroxyl groups of the cyclodextrins. These derivatives will usually alter the properties of the cyclodextrin, namely their solubility, stability and the volume of the hydrophobic cavity. The changes in the host may also play a role helping to control the activity of the guest molecule and the entrapment ability (31).

Up to 20 substituents have been linked to beta-cyclodextrin in a regioselective manner. To allow this to occur in a consistent manner, there is a need to use regioselective reagents, while optimising reaction conditions, as well as a good separation of products (31,34).

Natural cyclodextrins and their hydrophilic derivatives are, usually, only able to permeate lipophilic biological membranes with considerable difficulty. Although the somewhat lipophilic randomly methylated beta-cyclodextrins interact more readily with membranes than the hydrophilic cyclodextrin derivatives, not even these permeate lipophilic membranes with ease (31,35). Toxicity studies have demonstrated that orally administered cyclodextrins are practically non-toxic, due to lack of absorption from the gastrointestinal tract (31,36).

It has also been shown that short-term exposure to inhaled hydroxylpropyl-beta-cyclodextrin, gama-cyclodextrin and randomly methylated beta-cyclodextrin formulations in terms of lung and renal integrity and function, as those cyclodextrins were non-toxic after assessing bronchoalveolar lavage, lung and kidney histology, bronchial responsiveness to methacholine and blood urea (37,38).

It is also reported that sparingly methylated beta-cyclodextrin (Crysmeb) presents properties allowing it to be used for inhaled drug delivery. When inhaled, Crysmeb has a high solubilisation capacity, transient absorption promoting properties and a low toxicity to the lungs (39).

5.1.1. Inclusion complex formation

When a drug molecule (guest) is enclosed within a cyclodextrin (host), it forms a drug-cyclodextrin complex or inclusion complex. The basic principle is that if the guest molecule is of appropriate size and shape to fit into the cavity of the host molecule. Therefore, stereochemistry and, probably, polarity of both the drug and the cyclodextrin molecules determine if the complex will occur. Thus, the complexation occurs in a stereospecific fashion. Due to its spatial arrangement requirements, the formation of the inclusion complex resembles the “lock and key” mechanism described for enzyme-substrate. However the guest molecule may not be included completely in

the cyclodextrin, leaving some part(s) of the drug molecule exposed while other are enclosed. When the complex is formed, the guest molecules are isolated from each other and are dispersed on a on a molecular level in an oligosaccharide matrix and cyclodextrins may be regarded as nano-encapsulating agents (40).

The complex formation is composed by the steps shown in **Fig. 2** (40).

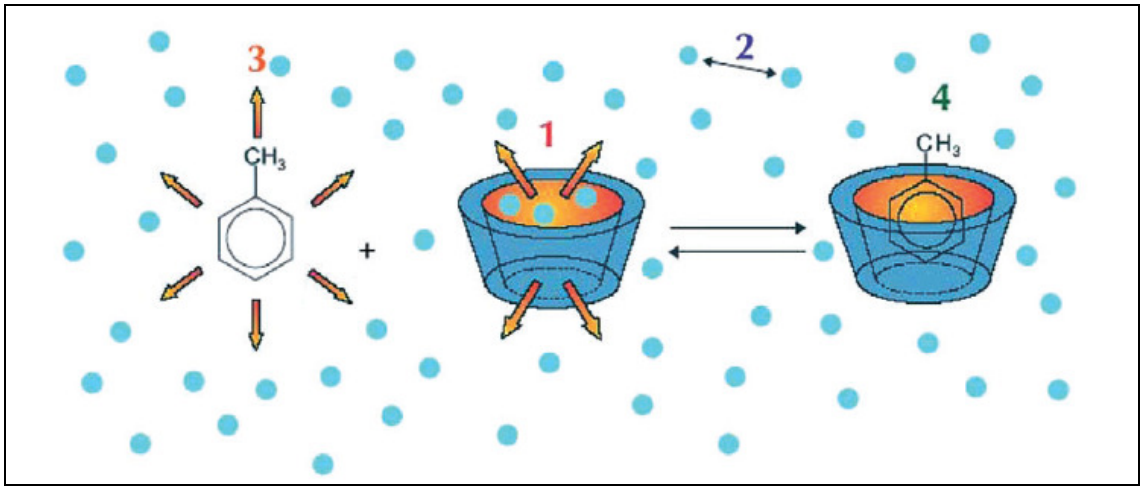


Fig. 2 – Formation of an inclusion complex between a drug and a cyclodextrin: 1. Displacement of polar water molecules from the apolar cyclodextrin cavity; 2. An increasing number of hydrogen bonds is formed as the displaced water returns to the pool; 3. Reduction of the repulsive interactions between the hydrophobic guest molecule and the aqueous environment; 4. Increase in hydrophobic interactions as the guest inserts itself into the apolar cyclodextrin cavity (40).

In overview, complexing activity increases as the guest molecule size is smaller, minding that interactions keep occurring between guest and host. Similarly, for large molecules, their activity depends whether there is a suitable chemical group capable of entering the cavity of the cyclodextrin, *exempli gratia*, one or two benzene rings or larger ones carrying an aromatic moiety, a side chain or other groups of comparable size (40).

The greatest percentage increase in solubility *versus* the concentration of cyclodextrin is shown for compounds with the lowest water solubility. By opposition, little or no effect was observed for molecules that are water soluble (40).

However, besides inclusion complexes, cyclodextrins are also able to form non-inclusion complexes. Also, cyclodextrins and their complexes can form water soluble aggregates in aqueous solutions, which are able to solubilize water insoluble

active pharmaceutical ingredients, via non-inclusion complexation or micelle-like structures (41).

Beta-cyclodextrin appears to be, among the naturally occurring cyclodextrins, the most useful complexing agent due to its size and availability (40). A representation of a beta-cyclodextrin is shown in **Fig. 3** (42).

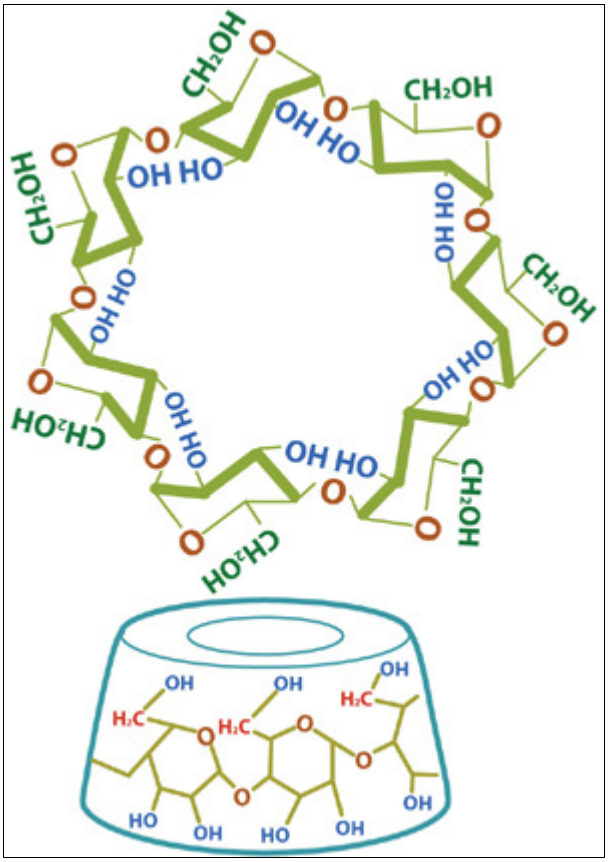


Fig. 3 – Beta-cyclodextrin (42)

The utilisation of cyclodextrins also carries the advantage of its economical cost as it is simpler and more economical than the majority of other available methods of encapsulation (40).

When solubilised, inclusion complexes not always present themselves with an identical structure to that of the crystalline state. When in solution, the entire or part of the guest molecule is enclosed in the cyclodextrin with the entire complex being surrounded by a multilayer hydrate shell; however, when crystallised, besides the guest molecules that are within the cavity of the cyclodextrin, there are guest

molecules between the cyclodextrin rings as crystallattice inclusions, that may form non-inclusion complexes (40).

Diverse factors may influence the formation of inclusion complexes, such as cavity size, temperature, method of preparation and pH and ionization state (40).

5.1.2. Evaluation of Inclusion Complex Formation

Ks, the stability or equilibrium constant, characterises the extent of complexation in an aqueous medium, *id est*, the stability of the complex formed **(Eq. 1)**:

$$K_S = \frac{k_r}{k_d} = \frac{[\text{complex}]}{[\text{CyD}][\text{guest}]}$$

Eq. 1 – Equation for the calculation of the stability constant

where Kr ($M^{-1}.s^{-1}$) is the recombination rate constant and Kd (s^{-1}) is the dissociation rate constant. The greater the magnitude of this ratio the greater is the stability of the complex (40).

In solution, the multiple parameters relevant for the inclusion complex formation, such as stability constant or stoichiometry can be accurately obtained and the equilibrium of **Eq. 1** can be altered in order to shift in the desired direction. To obtain this effect, some changes can be made to the environmental conditions such as temperature, pH, addition of a competitive molecule, concentration, polarity of the solvent, or even by choosing other (most) appropriate cyclodextrin or cyclodextrin derivative (40).

According to Higuchi and Connors (43) the solubility profiles shown in **Fig. 4** can be considered.

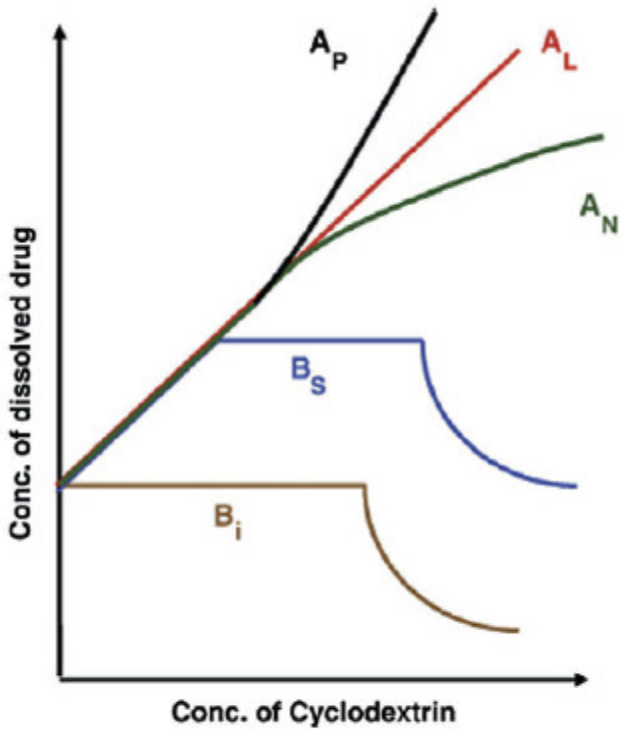


Fig. 4 – Phase solubility profiles and classification of complexes according to Higuchi and Connors. S_0 is the intrinsic solubility of the substrate (the dissolved guest/ drug) in the aqueous complexation medium when no ligand (cyclodextrin) is present (43).

Summarising, A-type curves point to the formation of soluble inclusion complexes. B-type indicates the formation of inclusion compounds with poor solubility, where B_S-type indicates complexes of limited solubility and a B_I-type curve means the formation of insoluble complexes (40).

On the other hand, A-type curves can be classified in A_L-type, showing a linear increase of drug solubility as a function of cyclodextrin concentration, or; A_P-type (positively deviating isotherms) and A_N-type (negatively deviating isotherms) (40).

K_S may be obtained from the linear portion of the phase solubility diagrams by the following equation **(Eq. 2)** (40,43):

$$K_S = \frac{\text{slope}}{S_0(1-\text{slope})}$$

Eq. 2 – Stability constant calculation, from phase solubility diagrams

where S_0 is the intrinsic solubility of the substrate on the medium.

However, methods other than phase solubility studies have been reported for the determination of K_S, namely, conductometry, ultraviolet–visible spectrophotometry, polarography, potentiometry, microcalorimetry, circular dichroism, nuclear magnetic ressonance, optical rotatory dispersion, high-performance liquid chromatography and thin layer chromatography (40).

Usually, complexes with stability constants from about 100 to 5000 M^{-1}, seem to be suitable for practical applications (40).

When the formed complex is very labile, the guest molecule will be released from the cyclodextrin due to the weak interaction and, therefore, not having an improved solubility. Conversely, very stable complexes will result in a retarded or incomplete release of the guest molecule, hence hindering its absorption (40).

Despite what is stated above, in some cases, even with small K_S values, the complex formation confers the active pharmaceutical ingredient better characteristics regarding its pharmaco-technical, physicochemical and biopharmaceutical properties. Usually, the inclusion complexes formed in solution have a molar ratio of host to guest molecules of 1:1 (40).

5.2. Crysmeb

5.2.1. General information

Crysmeb is stable both in solution as well as in solid state; being possible to develop solid formulations on its basis. Crysmeb solutions, even at high concentrations do not originate insoluble precipitates; have a low support for microbial growth and is very stable under different conditions such as neutral or alkaline solutions. In acidic solutions Crysmeb is slowly hydrolyzed. It is stable when exposed to atmospheric oxygen (44).

Crysmeb has a more specific and narrower substitution pattern than any other industrial derivative of beta-cyclodextrin. Whereas beta-cyclodextrin forms very stable crystals, reason for which has relatively low water solubility, the same does not

happen with Crysmeb. An approach to the beta-cyclodextrins water solubility is the use of its derivative hydroxypropyl-beta-cyclodextrin, where the existence of multiple large hydrophilic hydroxypropyl groups into all positions suitable for substitution prevents crystal formation. Conversely, Crysmeb has a random distribution of a few sterically small methyl groups into a small number of chosen positions, increasing its water solubility, without impairing the formation of crystals. Furthermore, since the methyl substituents are small they do not act as guest molecules when at high concentrations. (44).

Crysmeb (**Fig. 5** shows its crystal structure) is a mixture of methyl-beta-cyclodextrins, which contains from one to seven methyl groups. It has, on average, 4 methyl groups per native cyclodextrin molecule (44).

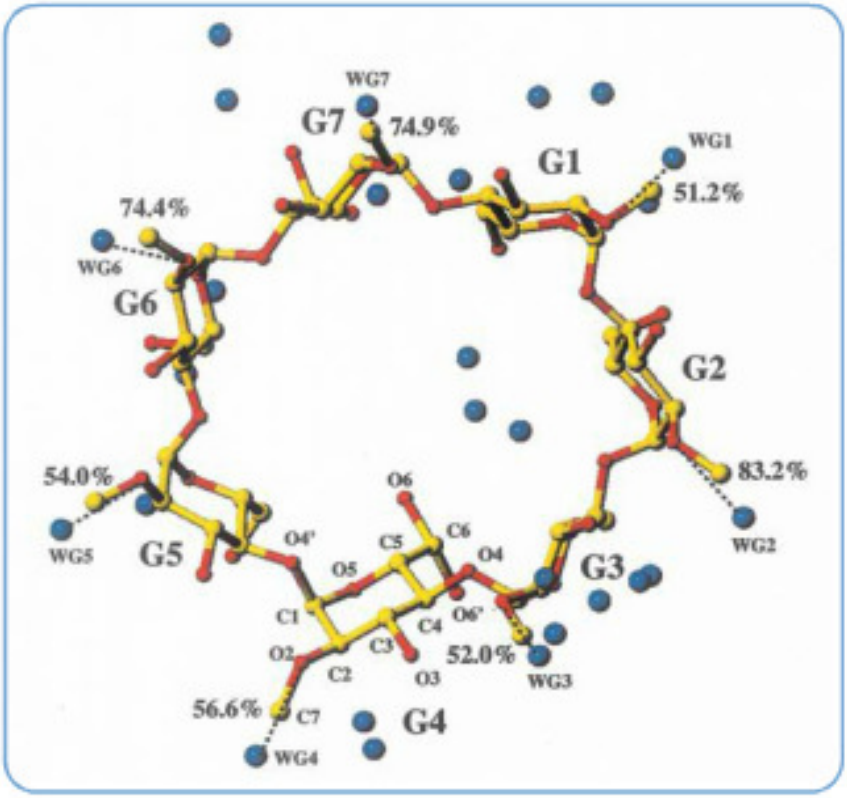


Fig. 5 – Crystal structure of crystallized Crysmeb (44)

5.2.2. Physical and chemical properties

Table 3 – Crysmeb physical and chemical properties (44)

Generic name	2-O-methyl-beta-cyclodextrin
Organoleptic characteristics	Anhydrous white powder
Molecular weight (average)*	1191 Da
Aqueous solubility (g/100 mL) at 20ºC	20%
Aqueous solubility (g/100 mL) at 70ºC	~65%
Decomposition temperature	>300ºC
Moisture content	≤5%

* - The average molecular weight is calculated with basis on the degree of molar substitution (MS) – average degree of molar substitution is 0,57 – of the mixture and it can be calculated as follows **(Eq.3)**:

Average Molecular Weight (Da) = 1135 + 7 x MS x 14

5.2.3. Toxicity

Regarding its toxicity, when compared to other methylated derivatives of beta-cyclodextrin, the low substitution degree of Crysmeb diminishes its affinity for cholesterol and phosphatidylcholine. Methylated-beta-cyclodextrins toxicity depends on a higher degree on the number and slightly on the position of the methyl groups. Nonetheless it still maintains good solubilizing properties for other substances. This selective solubilizing properties of Crysmeb for active pharmaceutical ingredients, rather than for lipid may be of interest, as cyclodextrins toxic consequences towards biological membranes, chiefly their haemolytic and cytotoxic effects, are generally recognised as being related to interaction with lipids (complexation and depletion) (45,46).

6. DRY POWDER INHALERS

The thriving integration of medicinal products with devices able of delivering them to the respiratory tract in the correct doses has shown that inhalation is a valuable route of administration. This route allows limited systemic exposure and provides local topical delivery. Multiple orally inhaled medicinal products have been successfully developed over the last 50 years, giving symptomatic relief to millions of patients with respiratory tract diseases such as chronic obstructive pulmonary disease and asthma (47,48).

As large doses of corticosteroids for the treatment of asthma are required for oral administration, these are associated with an unacceptably high adverse event profile (49,50). As a result of the availability of inhaled formulations, with the introduction of beclometasone dipropionate in 1972, inhaled corticosteroids are now part of the cornerstone of asthma treatment (49).

During the last decades, the focus has been in the pharmaceutical formulation of the drug as well as in device design, aiming to respond the need for more efficient inhalers that can deliver larger doses to the lung while diminishing extrathoracic drug deposition (47,48,51).

Once it is deposited in its target, the lungs, drug disposition (dissolution, absorption, distribution, metabolism and elimination) as well as the influence of pulmonary pharmacokinetics on drug efficacy and safety are the critical determinants of clinical outcomes (48).

Despite their achievements, inhaled therapies for lung diseases are still restricted to a small number of classes, notably β2 agonists, antimuscarinic and corticosteroid drugs (48,52).

Similarly to what was earlier stated, the health and economic burden of respiratory disease provide a massive market for inhalation drugs, and, thus the constant need to develop new and better inhaled medicines (48,53).

6.1. Drug particles deposition and absorption

Although the mechanism by which inhaled particles are absorbed in the lung is not completely known, some general ideas are evident: small lipophilic molecules are very quickly absorbed (within tens of seconds), typically with little metabolism; small hydrophilic molecules are absorbed fast (within tens of minutes), equally with minimal metabolism; drugs with very low water solubility can have their absorption retarded; peptides are rapidly absorbed and suffer significant metabolism, except if protected against peptidases; larger proteins are more slowly absorbed with variable bioavailabilities. For the intent of local lung disease applications, many small molecules are absorbed too quickly to produce the desired clinical outcome, the same happening with some systemic applications as well. A better understanding of the determinants of pulmonary drug dissolution, absorption, metabolism, and how to target specific regions and/or cells in the lung will enable safer and more effective inhaled medicines in the future (12).

Inhaled dosage forms that deliver drug aerosols onto the lung surface in a solid or semisolid form are becoming increasingly common, as these systems hold the potential to improve the duration and effectiveness of inhaled drugs and vaccines. Although we have decades of experience delivering drugs systemically via the lungs (54), we typically describe blood levels of a drug following its inhalation. Rarely do we measure drug/aerosol particle persistence and location within the lungs. Ensuing processes after particle deposition, such as dissolution in the lung lining fluid, lateral spreading over the lung surface, and/or penetration within the fluids, facilitate a drug access to various locations in the respiratory tract. However, each of these processes is affected by fluid and cellular barriers and natural clearance mechanisms that operate in the lungs, which ultimately limit the availability and persistence of drugs within the lung **(Fig. 6)** (12). A physical structure of the tracheobronchial tree of humans **(Fig. 7)** has been proposed (48).

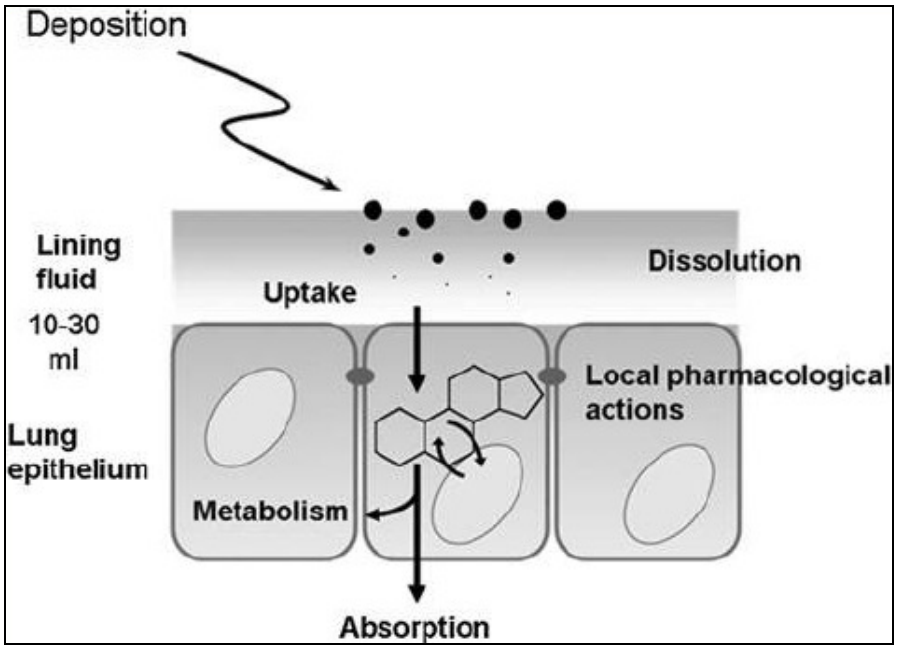


Fig. 6 – General concept of aerosol drug deposition, dissolution and absorption for local or systemic actions (12)

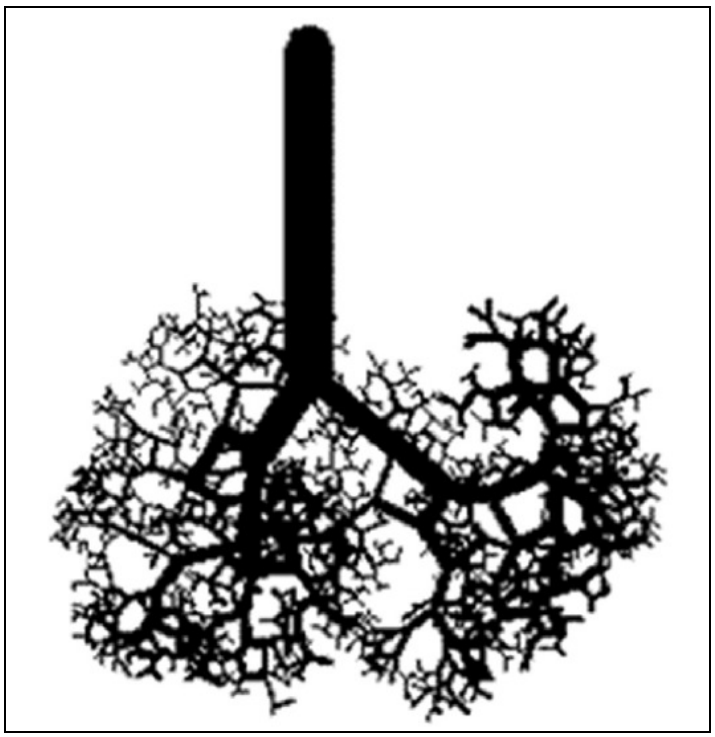


Fig. 7 – Idealised geometry of the tracheobronchial tree of humans (48)

Upon deposition in the lungs, the first interaction of drug particles is with the lung lining fluids (which have a total volume limited to approximately 10-30 mL), and

act as a protective barrier for the underlying epithelium. There, the particles must dissolve for subsequent absorption and/ or cellular uptake, hence the importance of the drug solubility (12,55,56).

If the drug particles are not dissolved without delay in the lung lining fluids after depositing, it is possible they move through the fluids and further interact with other lung structures (12,57–59).

6.2. Inhalers

It is necessary an adequate and efficient delivery system – the inhaler – so the drug to reach the lungs.

After aerosolisation, powders must be deagglomerated to a size small enough for deposition in the lungs alveolar region.

Typically, an aerodynamic diameter between 0,5 and 5 micrometers is required for appropriate lung deposition. Larger particles are deposited in the upper airways, being swallowed or expelled. On the other hand, smaller particles are usually exhaled or deposited by Brownian diffusion upstream of the alveoli. The mechanisms that allow powder deagglomeration and dispersion are explained in **Fig. 8**. Here there are two types of formulations of dry powders for inhalation (60,61).

These powders are usually formulated for inhalation as either the active pharmaceutical ingredient alone or as a mixture with a carrier. The carrier chief purpose is to reduce the susceptibility of the fine drug particles to form strongly cohesive agglomerates and to increase the flowability of powders previously to aerosolisation (60).

Lactose is a preferred carrier, as it does not present less advantages than, for example, trehalose and is inexpensive, accessible and widely used for this purpose (37).

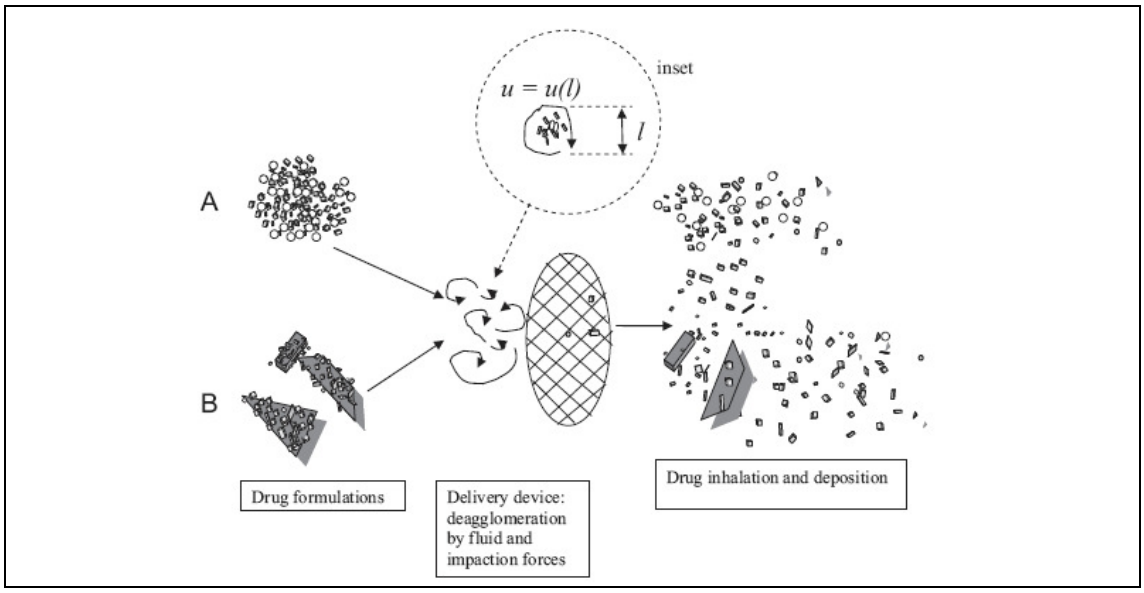


Fig. 8 – Schematic diagram of dry powder inhaler formulation and dispersion process: A. Drug-only formulation (drug agglomerates); B. Carrier-based formulation. (60)

The ideal dry powder inhaler should comprise the criteria described in **Fig. 9** (49).

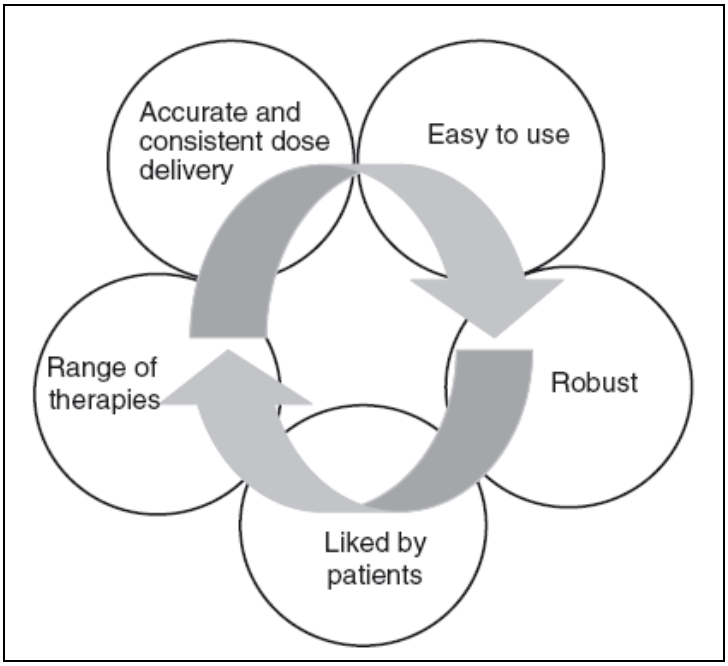


Fig. 9 – Criteria for an ideal inhaler (49)

According to a survey conducted in 169 patients with respiratory disease (asthma or chronic obstructive pulmonary disease), the most important criterion when

selecting a dry powder inhaler is its ease of use. Even if a dry powder inhaler demonstrates an outstanding pharmaceutical performance regarding drug output, if it is not correctly used then it can be rendered ineffective. If patients are unable to use the inhaler as directed, their treatment is compromised which may lead to severe consequences (49,62).

When compared to pressurised metered-dose inhalers, dry powder inhaler devices have an intrinsic advantage over the former, given that the dose of drug is only expelled from the inhaler as the patient inhales it. As a result, it is not necessary the patients coordination between the inspiration and the actuation of the device. Nonetheless, the patient still has to provide a sustained and adequate inspiratory flow rate through the inhaler in order to get the complete drug dose to be released from the device (49).

II – Materials and methods

1. OBJECTIVE

The objective was to develop a ciclesonide-Crysmeb complex by kneading and, analytically, confirm its formation and characteristics.

2. MATERIALS, REAGENTS AND EQUIPMENTS

All reagents used were of analytical grade.

Crysmeb: Roquette Pharma as *KLEPTOSE CRYSMEB*.

Ciclesonide: Hovione.

All materials, reagents and equipments used in this work were provided to the author by the Faculty of Pharmacy of the University of Lisbon.

The 0,22 micrometer pore syringe filters used were from *Millipore*.

Ultraviolet spectroscopy was performed with a *Hitachi U-2000* spectrophotomer, using quartz cells. The *DSC Q200, TA Instruments* was used to obtain the thermograms. Infrared spectroscopy analysis was made with an *IRAffinity, Shimadzu*. Particle size analysis was carried out using an *Aerosizer LD – Amherst process instruments, Inc.*

3. METHODS

3.1. Calibration curve and detection limits

A calibration curve is used so that an unknown concentration of a given solution can be determined. The method used for the construction of the calibration curve was ultraviolet spectroscopy, since this method will be used throughout the work to perform assays (40).

Firstly, a wavelength scan was performed to evaluate the peak absorption for ciclesonide, using an hydroalcoholic solution (equal volumes of water and ethanol), once the molecule of interest, ciclesonide, is highly insoluble in water but soluble in ethanol. Then the calibration curve followed – for the same reason as stated previously, an hydroalcoholic solution was used. Ciclesonide solutions were prepared with concentrations ranging from 0,0025 mg/mL to 0,05 mg/mL, at regular intervals.

3.2. Phase solubility studies

Aiming to identify the solubility profile of the complex, phase solubility studies were performed in accordance with the method proposed by Higuchi and Connors (43).

Briefly, to aqueous solutions of increasing concentrations of cyclodextrin an excess of drug was added and the suspension was shortly vortexed. The mixtures were then kept under agitation at 25ºC and the drug excess was assured until equilibrium was attained. Cyclodextrin concentration ranged from 0 to 0,125 M and aliquots were retrieved at 30 minutes, 2 hours, 8 hours, 24 hours, 48 hours and 72 hours. The aliquots were then filtered through a 0,22 µm pore filter, so that the suspended drug was not measured in the ultraviolet spectroscope, but only the molecules included in the cyclodextrin. The filtrate was then diluted with an equal volume of ethanol and measured at 242 nm – the reference cell of the spectroscope was an hydroalcoholic solution of cyclodextrin, with equal cyclodextrin concentration of that of the diluted sample. This was performed to ensure that no signal from the cyclodextrin was being measured, as wavelength scans of cyclodextrin solutions showed interference at higher concentrations.

3.3. Preparation of the powders for inhalation

The powders were prepared by kneading.

Four different powders were attained, varying the amount of water used for kneading and molar ratio of drug-to-cyclodextrin:

Powder A – 1:1 M (drug:cyclodextrin), 50 % (m/m) water

Powder B – 1:1 M (drug:cyclodextrin), 90 % (m/m) water

Powder C – 1:2 M (drug:cyclodextrin), 50 % (m/m) water

Powder D – 1:2 M (drug:cyclodextrin), 50 % (m/m) water

For powders A and B, equimolar quantities of cyclodextrin and drug were mixed in a porcelain mortar, by hand, adding successively the respective amounts of water (50% m/m for powder A and 90% m/m for powder B).

The same procedure was followed for powders C and D, varying only the molar proportion of drug-to-cyclodextrin, which is 1:2.

From this point on all powders were treated equally.

Powders were thoroughly kneaded for 15 minutes, time at which they presented as an apparently dry mass. They were then kept over night in a dessicator at room temperature. Subsequently, the masses were scrapped from the porcelain mortar and transferred to a glass mortar where they were pulverized during 5 minutes and passed through a 63 micrometers sieve.

Physical mixtures (1:1 M and 1:2 M) of drug:cyclodextrin were also prepared.

The powders were then analyzed regarding their physicochemical properties by ultraviolet spectroscopy, differential scanning calorimetry and infrared spectroscopy. A portion of the powders were also mixed with sieved lactose 3,5%:96,5% powder:lactose (m/m) – these proportions were based on previous data (37).

After prepared according to the description above, the powders went through a set of analytical procedures, so that their physicochemical properties could be assessed.

The following techniques were applied:

3.4. Ultraviolet spectroscopy

This method was used for the assay of every powder/ mixture.

Every solution tested was measured against the appropriate reference, namely concerning their concentration in cyclodextrin.

3.5. Differential scanning calorimetry

The powders were subject to calorimetric analysis using the ramp method for temperature increase, ranging from 35ºC to 300ºC (at a rate of 10ºC/minute). The analysed powders were A, B, C, D, physical mixtures drug:cyclodextrin (1:1 M and 1:2 M), cyclodextrin alone and ciclesonide alone.

Also washed powders were analysed: A', B', C' and D'. In brief, the washing process consists of an amount of powder that is resuspended in water, after which the mixture is filtered and the filtrate is dried at 60ºC during 24 hours to recover the solid inclusion complex. This allows the removal of the insoluble ciclesonide particles that were not included and remained in suspension.

The cyclodextrin and the drug, separately, were also tested, under the same conditions, in order to be used as a reference.

3.6. Infrared spectroscopy

Disks of potassium bromide with the analyte, were prepared; and analysed at a wavelength between 0 and 4000 cm^{-1} (63).

Powders assessed were: A', B', C' and D', ciclesonide and cyclodextrin.

3.7. Particle size profile analysis

Particle size analysis was carried out by the method of time-of-flight. In this method particles are accelerated their times-of-flight are measured across two laser beams taking into account the sample density (64).

Powders A, B, C and D were measured. Were also measured powders A, B, C and D mixed with sieved lactose (63 µm sieve) at a ratio of 3,5% powder to 96,5%

lactose (mass percentage) – Mixtures A, B, C and D. Powders A, B, C and D were also measured, before sieving, to evaluate the need for that final action.

These samples did not require any prior special preparation and were directly measured.

3.8. Aerodynamic assessment

Samples consisted of gelatine capsules manually filled with the equivalent amount of 160 µg of active pharmaceutical ingredient (corresponding to 3,5% of the active substance to 96,5% of lactose (m/m)), as follows:

Powder A – 26,15 mg/ capsule;

Powder B – 26,20 mg/ capsule;

Powder C – 36,29 mg/ capsule;

Powder D – 35,71 mg/ capsule.

Five capsules of each powder were, cumulatively, used and the experiment was performed according to the current edition of the European Pharmacopoeia. Shortly, the twin stage liquid impinger device is put under an air flow of 60 $L.min^{-1}$ and the powder was discharged from the capsule via a dry powder inhaler (the Microhaler® was used for these experiments). The discharge of each capsule is maintained for a period of 5 seconds. At different stages of the apparatus hydroalcoholic solution [50:50 (V/V)] was used. The apparatus was then disassembled, washed thoroughly with the appropriate solvent and those solutions were assayed. Every stage, except the lower one with a final volume of 50 mL, were washed up to a final volume do 20 mL (65).

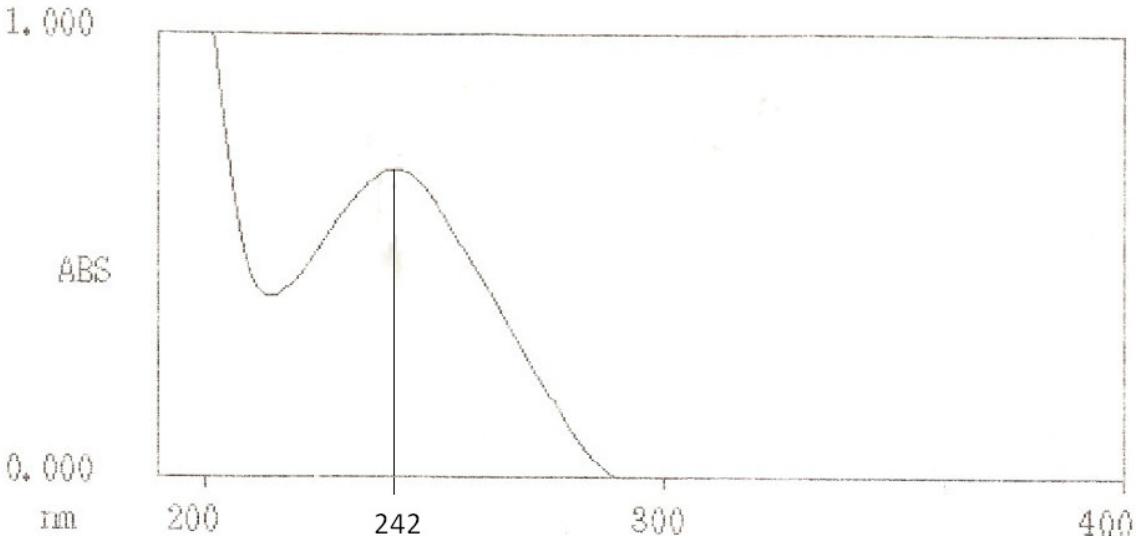
4. RESULTS AND DISCUSSION

4.1. Calibration curve and detection limits

The chosen technique for assaying every preparation was ultraviolet spectroscopy. Ultraviolet spectroscopy has been extensively used for this and multiple other purposes. It is based on the absorption of a specific radiation wavelength by a given molecule (40,66–68). Hence, a calibration curve for ciclesonide was firstly obtained.

Being that the drug is highly insoluble in water, hydroalcoholic standard solutions of ciclesonide were prepared.

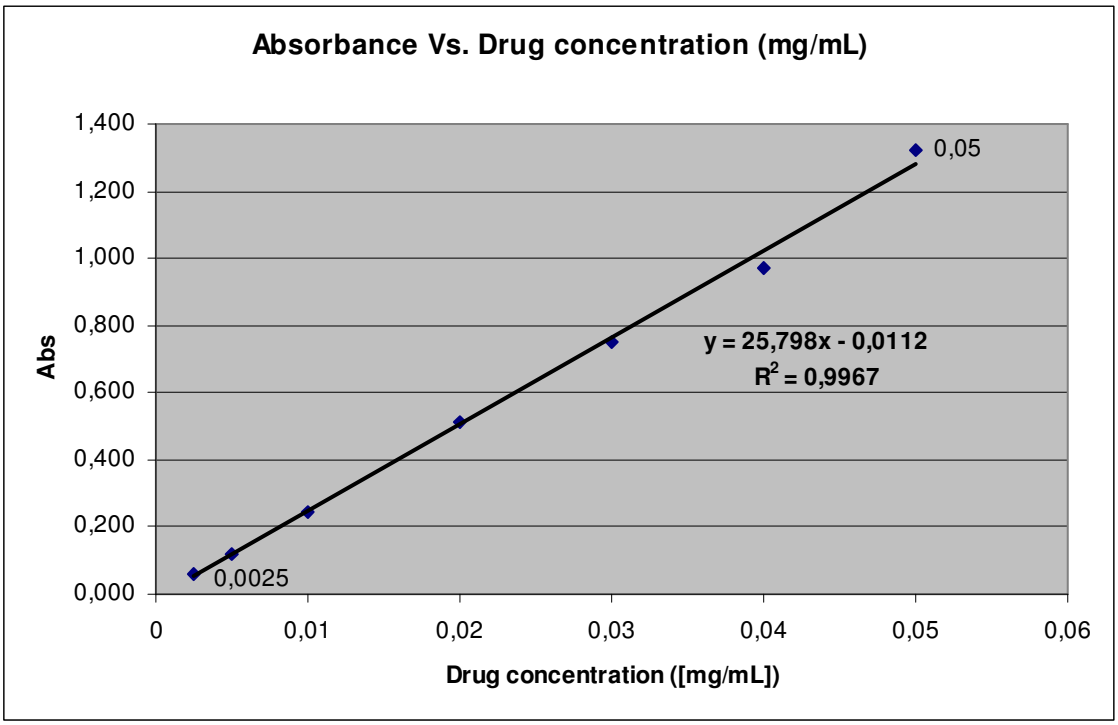
Performing a wavelength scan, the peak for the ciclesonide hydroalcoholic solution was found to be 242 nm **(Graph 1)**.



Graph 1 – Wavelenght scan for ciclesonide solution

Following the determination of absorvance for known concentrations of ciclesonide in hydroalcoholic solutions, a calibration curve was obtained (**Graph 2**). The correlation coefficient (R^2) determined, close to the unit, indicates a good linearity, showing that absorbance values within that interval can be adequately applied to the model.

The segment where linearity was obtained represents a lowest measurable drug concentration limit of 0,0025 mg/mL and a highest concentration limit of 0,05 mg/mL of ciclesonide hydroalcoholic solution. These values revealed adequate for the assays performed.

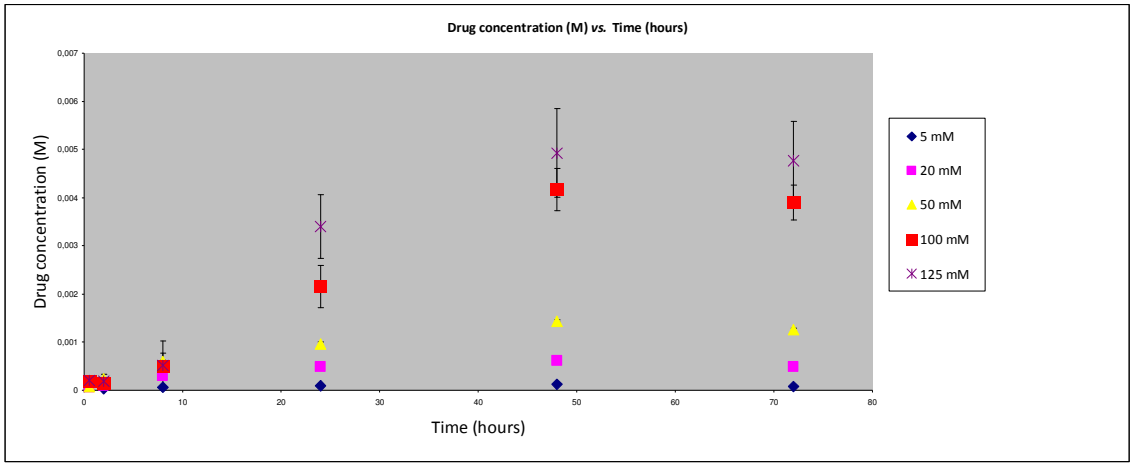


Graph 2 – Calibration curve for ciclesonide

4.2. Phase solubility studies

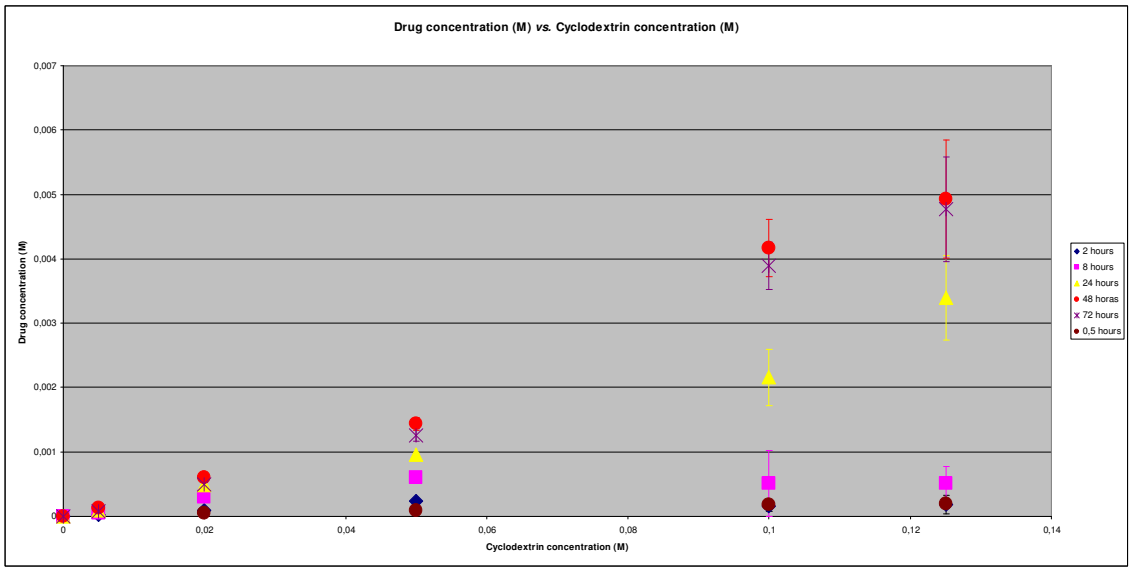
Phase solubility studies were performed to determine at what time equilibrium was found and to calculate the complex stability constant as well as the stoichiometry for the inclusion process.

Subsequently to the determination of drug concentration in each sample, **Graph 3** was plotted. From its analysis it is possible to verify that dynamic equilibrium was achieved at 48 hours, and the drug concentration in solution stabilised from that point on.



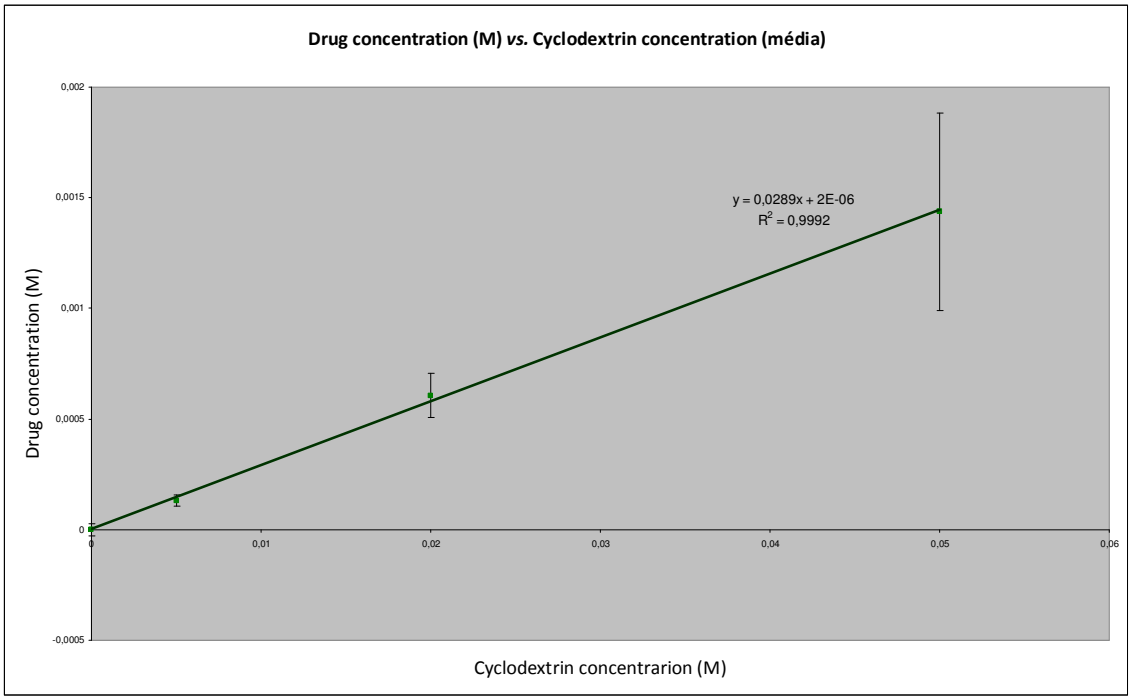
Graph 3 – Phase solubility studies at different times

Graph 4, relating drug concentration *versus* cyclodextrin concentration was also plotted.



Graph 4 – Drug concentration *versus* cyclodextrin concentration

Considering the linear portion of data at 48 hours (dynamic equilibrium achieved) from **Graph 4**, a new graph was plotted **(Graph 5)**, for the determination of the solubility constant.



Graph 5 – Ks and complexation stoichiometry determination

The linear regression model showed to fit the real data (R^2 = 0,9992), therefore suggesting an A_L-type curve.

Ks was calculated to be 14880,033 M^{-1}, applying the before mentioned formula **(Eq. 2)**:

$$K_S = \frac{\text{slope}}{S_0(1-\text{slope})}$$

, with slope = 0,0289; $S_0 = 2x10^{-6}$ M

Eq. 2 – Stability constant calculation, from phase solubility diagrams

The ultraviolet method was unable to detect the drug low concentrations when Crysmeb concentration was null. Also, the literature did not provide a definitive answer regarding what was the intrinsic solubility of ciclesonide. Hence, S_0 was calculated from the interception of the curve from the linear regression with the *yy* axis of Graph 5.

The linear relation obtained supports that complexation occurred in a 1:1 M (drug:cyclodextrin) stoichiometric fashion. The obtained stability constant value of 14880,033 M^{-1} points to a very stable inclusion complex, which might hinder drug release from the complex. However, this might be of interest if aiming, for example, at a modified release dosage form.

Despite phase solubility studies point towards a 1:1 M (drug:cyclodextrin) stoichiometry, powders were prepared at both 1:1 M and 1:2 M (drug:cyclodextrin) stoichiometry.

4.3. Preparation of the powders for inhalation

The kneading process, including sieving, had the following yields **(Table 4)**:

Table 4 – Powder yield

Powder	Yield (% m/m)
A	**40,9**
B	**41,3**
C	**49,1**
D	**42,5**

This method shows the same yield as other methods, having several advantages, such as being more economic and less time consuming to obtain the complex (69).

4.4. Ultraviolet Spectroscopy

4.4.1. Inclusion efficiency and drug content determination

Subsequent to their obtention powders A, B, C and D were assayed both before and after washing, to allow determining the inclusion efficiency.

Results are shown in **Table 5**:

Table 5 – Inclusion efficiency

	Assay (mg/mL)		Inclusion efficiency (%)
	Regular	*Washed*	
Powder A	**0,0926**	**0,0154**	**16,65**
Powder B	**0,0924**	**0,0098**	**10,59**
Powder C	**0,0708**	**0,0091**	**12,84**
Powder D	**0,0715**	**0,0121**	**16,87**

The same data was also used to determine drug content of each powder, *id est*, what amount of active pharmaceutical ingredient is present in each mixture, either included within the cyclodextrin or free – **Table 6**.

Table 6 – Drug content determination

	Solution concentration (mg/mL)	Assay (mg/mL)	Drug content (mg/g)	Drug percentage (% m/m)
Powder A	**0,53**	**0,0926**	**174,72**	**17,5**
Powder B	**0,53**	**0,0924**	**174,34**	**17,4**
Powder C	**0,56**	**0,0708**	**126,43**	**12,6**
Powder D	**0,56**	**0,0715**	**127,68**	**12,8**

The first parameter of the powders verified was the yield **(table 4)**. In fact, this parameter is not very reliable nor conclusive as, at laboratorial level, working with minimal amounts, there are great percentages of mass losses. Visible mass losses occur mainly when transferring the kneaded mass to the pulverizing mortar and, afterwards, when sieving the powder.

The determined inclusion efficiency **(Table 5)** is not very high. This might be due to the need of more kneading time as fifteen minutes seem not to be sufficient to achieve higher inclusion efficiency. Further studies on this matter can be conducted to evaluate if the kneading process or the host/ guest characteristics are the limitative steps themselves for the achieved inclusion efficiency or if the kneading parameters might be changed towards a better inclusion efficiency. However, bearing in mind that

the proposed formulation includes not only the complexed active substance, but also free ciclesonide, it might not be considered a draw back, as no active pharmaceutical ingredient is discarded. This characteristic may even be of interest for controlling the pharmacokinetic profile of the drug. Taking in consideration the steps taken before the measurement, as described previously, these determinations were also a first pointer (although indirect) that an inclusion complex was obtained by kneading, as the active substance was identified even after samples were suspended in water alone and then filtered. Being a nearly water-insoluble drug, if not included within the cyclodextrin, the active pharmaceutical ingredient could have not been detected by ultraviolet spectroscopy or, even if some free drug molecules might dissolve, considering the drug water solubility, the concentration would be below the detection limit. Corresponding drug content of the powders were also determined **(Table 6)**.

4.5. Proof of complex formation

4.5.1. Differential scanning calorimetry

Cyclodextrin complex formation can be verified differential scanning calorimetry, as an endothermic peak is observed for the molecule (equivalent to its melting or boiling point) and for the physical mixture but will be absent for the complex (40). Thermograms obtained by differential scanning calorimetry were used to determine if an inclusion complex by kneading was indeed formed.

Thermograms obtained are presented in **Fig. 10** and **Fig. 11**:

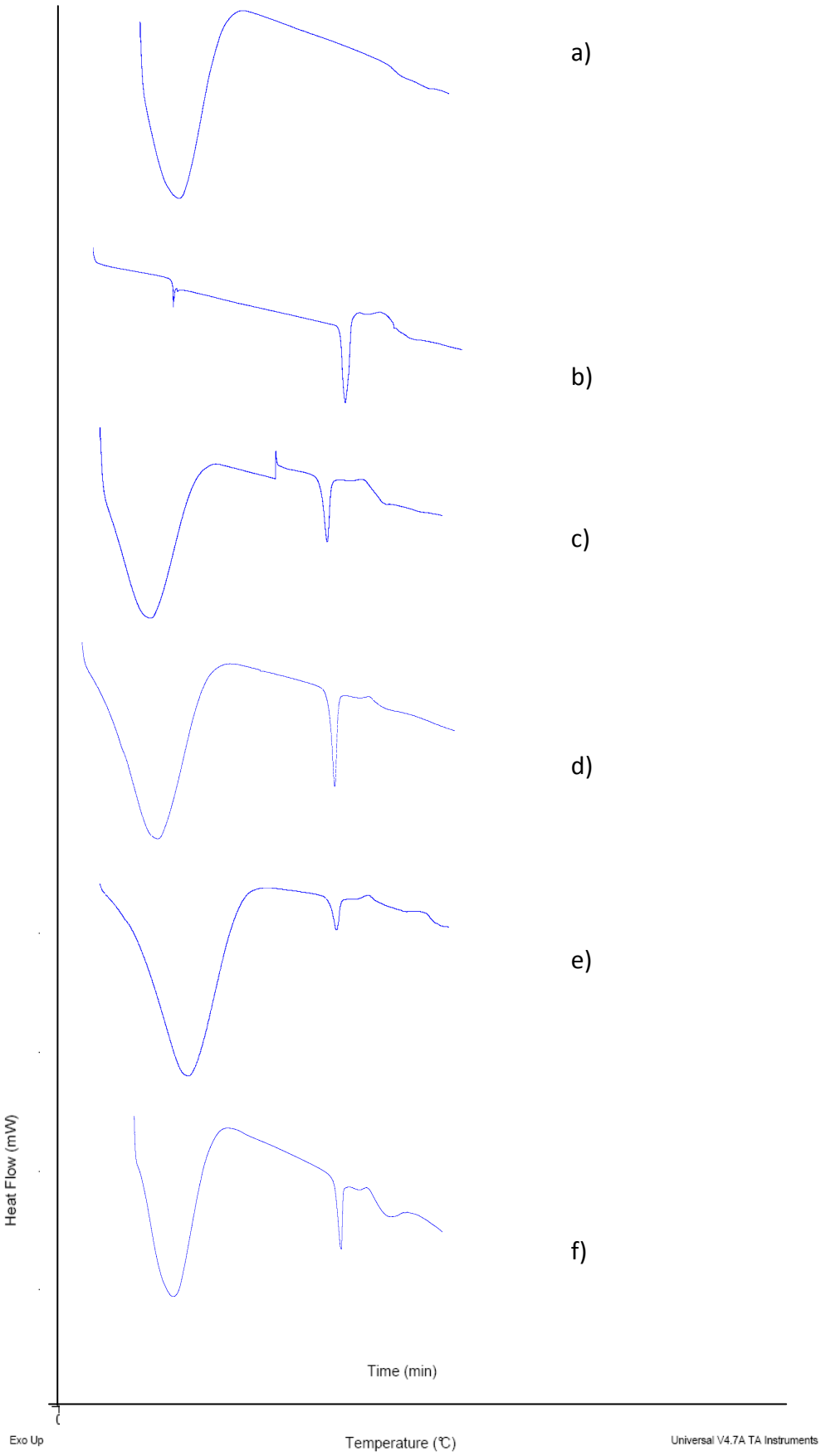


Fig. 10 – Thermograms for: a) Crysmeb; b) ciclesonide; c) powder A; d) powder B; e) powder C; f) powder D.

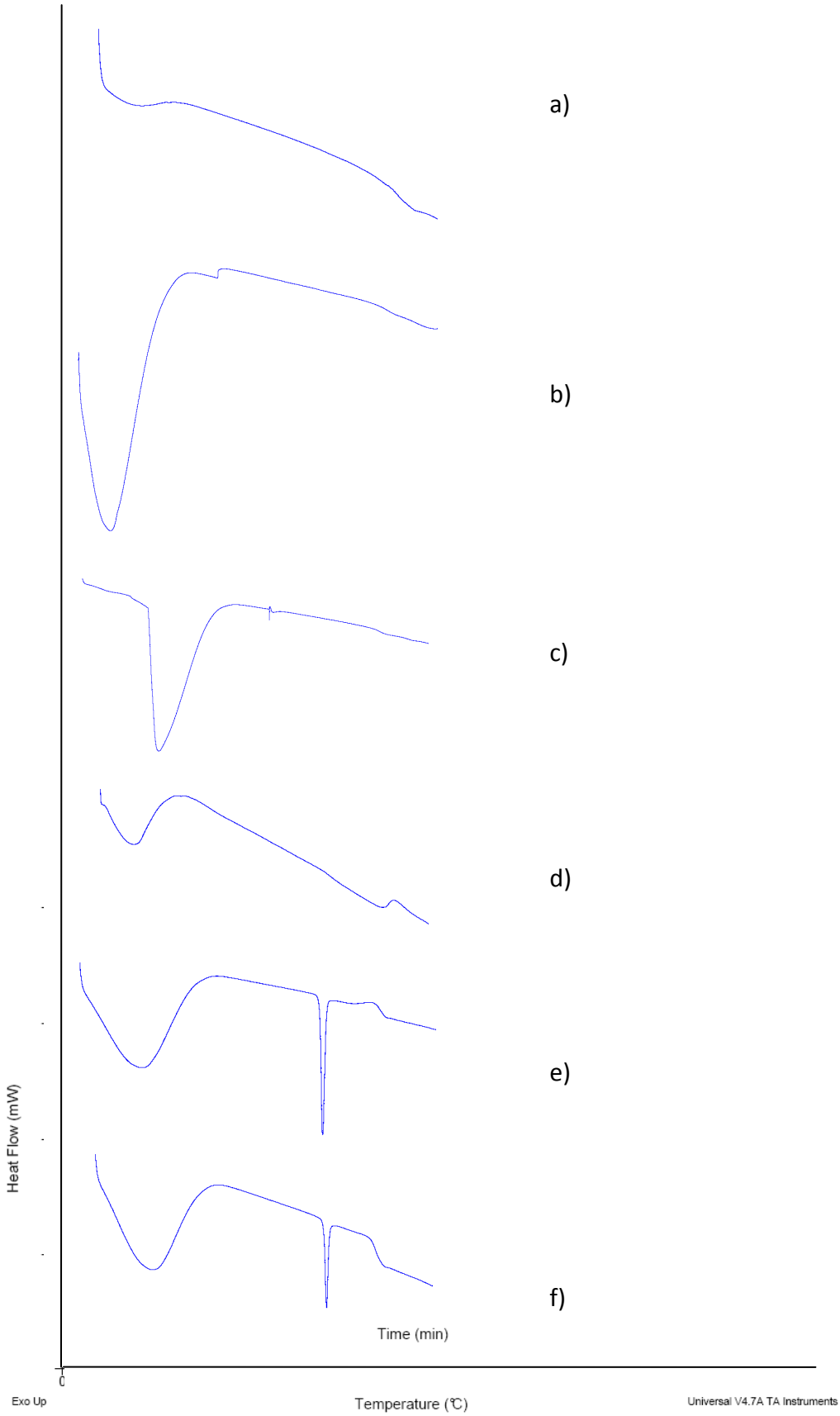


Fig. 11 – Thermograms for: a) powder A'; b) powder B'; c) powder C'; d) powder D'; e) physical mixture 1:1 M (drug:cyclodextrin); f) physical mixture 1:2 M (drug:cyclodextrin).

The characteristic endothermic peak for ciclesonide (melting point *circa* 210 ºC) is present in the thermograms of the powders A, B, C and D **(Fig. 10, c) to f))** – *id est*, it seems there is still some amount of free drug; but this peak is absent in the washed powders **(Fig. 11, a) to d))** – *id est*, only inclusion complex is present and no free ciclesonide.

In the physical mixtures the same peak is visible **(Fig. 11, e) and f))**.

Crysmeb has a characteristic endothermic peak at approximately 210ºC **(Fig. 10 a))**, while ciclesonide presents its characteristic endothermic peak at around 75 ºC **(Fig. 10 b))**. Tested samples results also point to the formation of an inclusion complex by the kneading method, as the kneaded and washed samples (A' B', C' and D') **(Fig. 11, a) to d))** presented only the endothermic peak corresponding to cyclodextrin (although slightly shifted or with different intensity), while the physical mixtures **(Fig. 11, e) and f))** and unwashed powders **(Fig. 10, c) to f))** showed both endothermic peaks. As discussed previously, these kneaded and washed samples (A' B', C' and D') were assayed, confirming that there was indeed included active substance within the cyclodextrin.

4.5.2. Infrared Spectroscopy

Some types of complex formation may be demonstrated by infrared spectroscopy. Bands, due to the included portion of the guest molecule, are generally shifted or their intensities altered (40).

Following the obtention of thermograms, which already shown the inclusion complex formation, as discussed above, this analysis was performed to further verify the occurrence of drug:cyclodextrin complex formation. Powders A, B, C and D were not used, as the intent was to confirm the inclusion complex formation, that is masked in these powders, as they contain free drug as well.

The above mentioned spectra are presented as follows:

Graph 6 – Crysmeb;

Graph 7 – Ciclesonide;

Graph 8 – powder A';

Graph 9 – powder B';

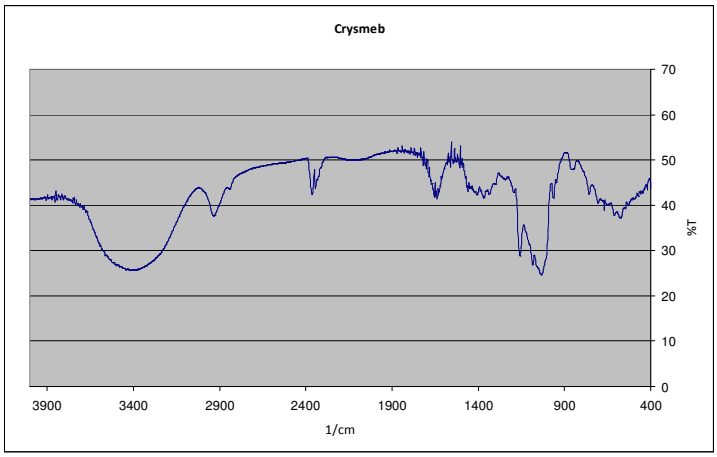
Graph 10 – powder C';

Graph 11 – powder D';

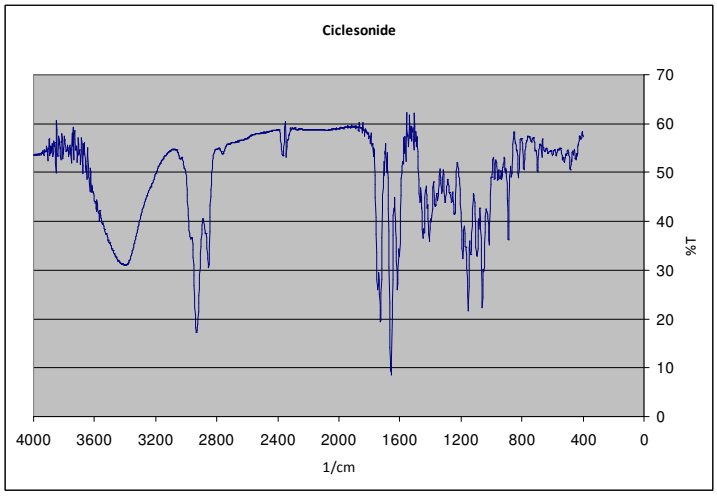
Graph 12 – powders A', C', drug, Crysmeb;

Graph 13 – powders B', D', drug, Crysmeb;

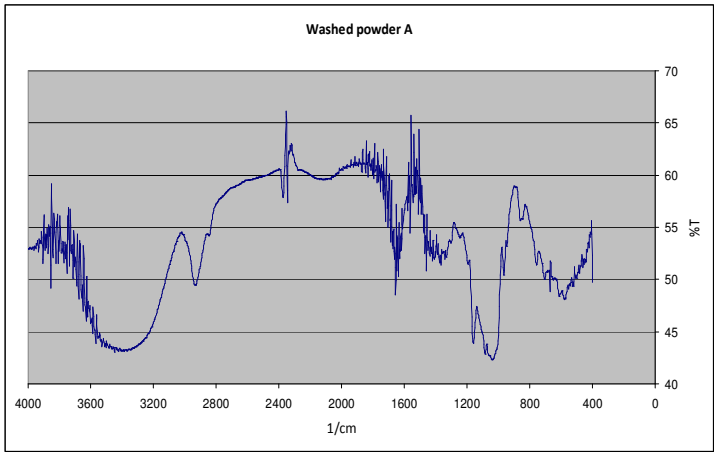
Graph 14 – powders A', B', C' and D'.



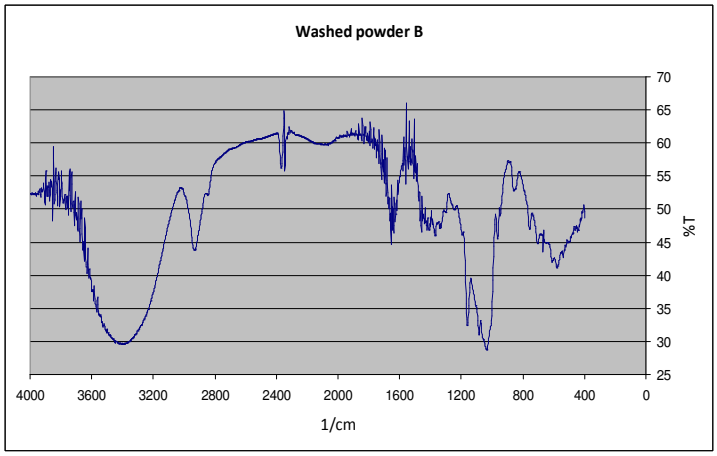
Graph 6 – Infrared spectrum of Crysmeb



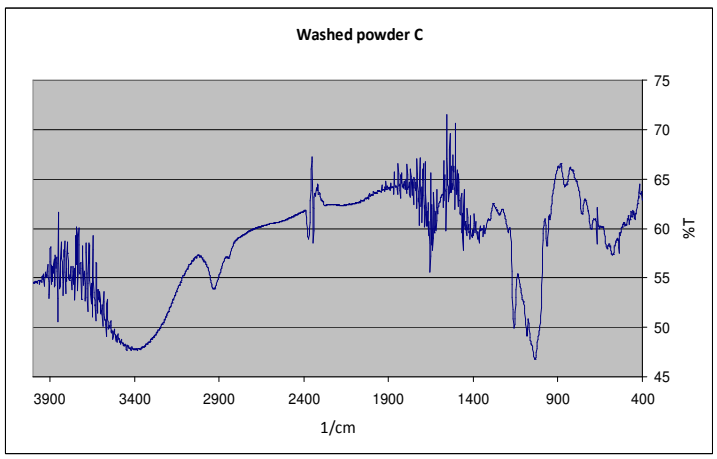
Graph 7 – Infrared spectrum of ciclesonide



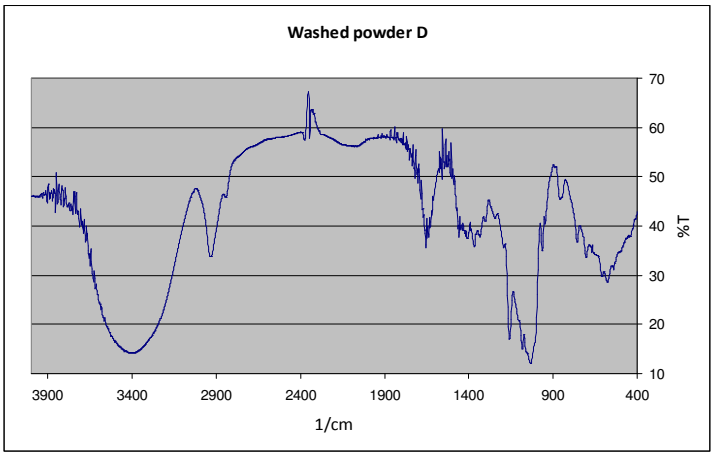
Graph 8 – Infrared spectrum of powder A'



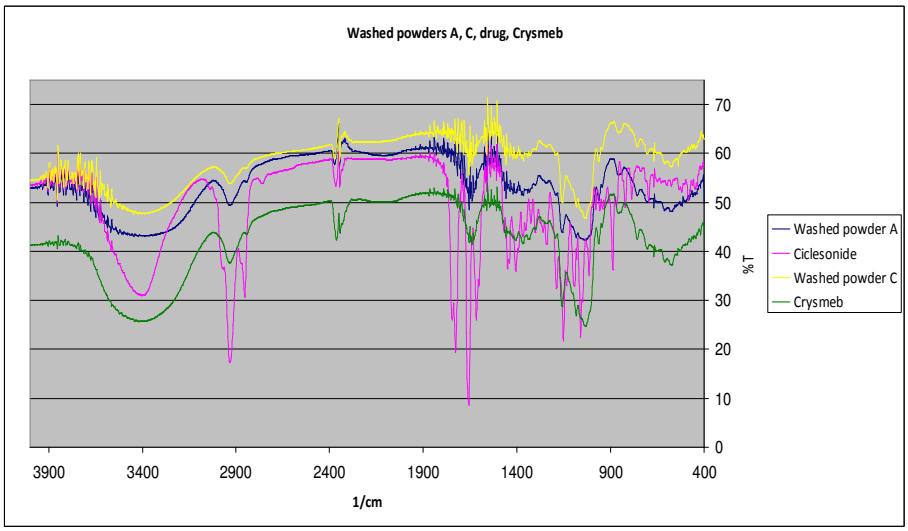
Graph 9 – Infrared spectrum of powder B'



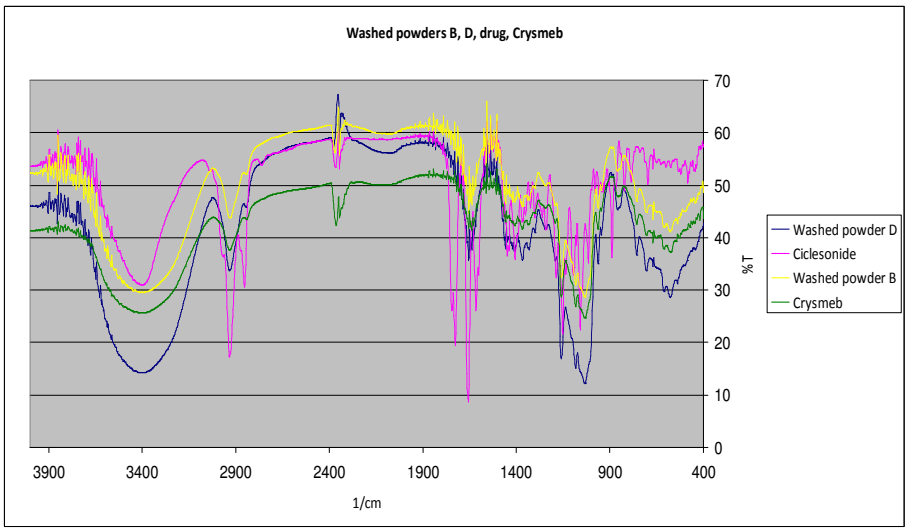
Graph 10 – Infrared spectrum of powder C'



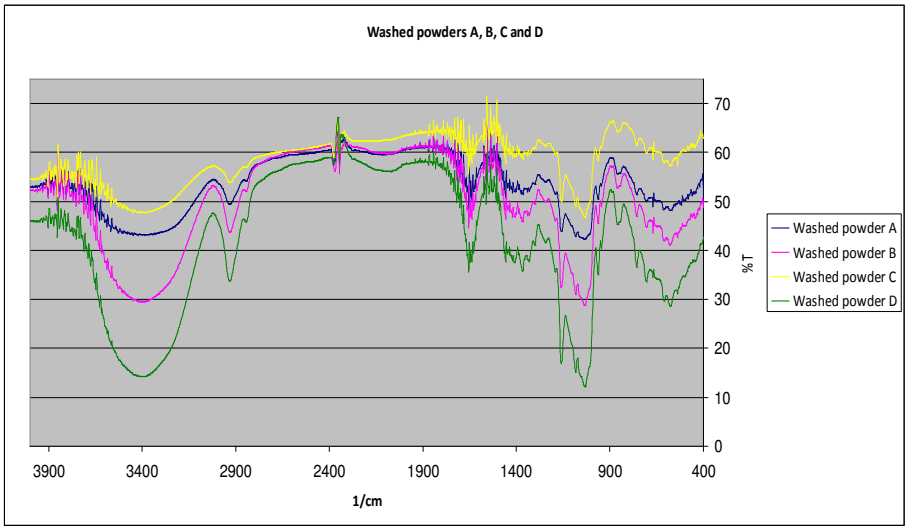
Graph 11 – Infrared spectrum of powder D'



Graph 12 – Infrared spectra of powders A', C', ciclesonide, Crysmeb



Graph 13 – Infrared spectra of powders B', C', ciclesonide and Crysmeb



Graph 14 – Infrared spectra of powders A', B', C' and D'

One possible interpretation for the infrared spectrum of ciclesonide **(Graph 6)** is adapted from the work proposed by Feth *et al.* (70) – **table 7**:

Table 7 – Interpretation of the infrared spectrum for ciclesonide

Peaks (cm^{-1})	Proposed assignment
3387 (strong, broad)	O-H stretch vibration in secondary alcohols
2968-2853 (strong, sharp)	-CH2- and CH3- asymmetric and symmetric stretch vibrations
1746, 1728 (strong, sharp)	C=O stretch vibrations of ketones and esters
1653 (strong, sharp)	C=O stretch vibration of α, β unsaturated carbonyls
1616, 1603 (medium, sharp)	C=C stretch vibrations of α, β unsaturated carbonyls
1470-1362 (medium, sharp)	-CH2- and CH3- asymmetric and symmetric deformation vibration of methyl, isopropyl and cyclohexyl groups
1240 (medium, sharp)	C-O stretch vibrations in esters
1188-1059 (short, sharp; medium, sharp)	C-O stretching in secondary alcohols and asymmetric C-O-C-O-C stretch vibrations in acetates
821	C=C-C=O out of plane deformation vibration
787, 696	-CH2- rocking vibration

Analysing graphs 12 to 14 it is noticeable that none of the identified characteristic peaks from the drug (ciclesonide) is visible in powders A', B', C' and D' – the peaks from the said powders correspond to those from Crysmeb.

As predictable from the thermograms results, the obtained spectra reveal the formation of an inclusion complex. As visible in Graphs 12 to 14, characteristic peaks of ciclesonide – Table 7 – are not present in any of the samples, except for the ciclesonide sample itself (Graph 6). Powders A', B', C' and D' showed a transmittance pattern similar to those of cyclodextrin, only with some changes in the peaks intensities. Thus, these results provide confirmation that an inclusion complex of drug-cyclodextrin is formed.

4.6. Particle size profile analysis

Particle size analysis was performed to evaluate particle size distribution – a major aspect for inhalation powders.

Analysed samples are shown in **Fig. 12** and **Fig. 13**:

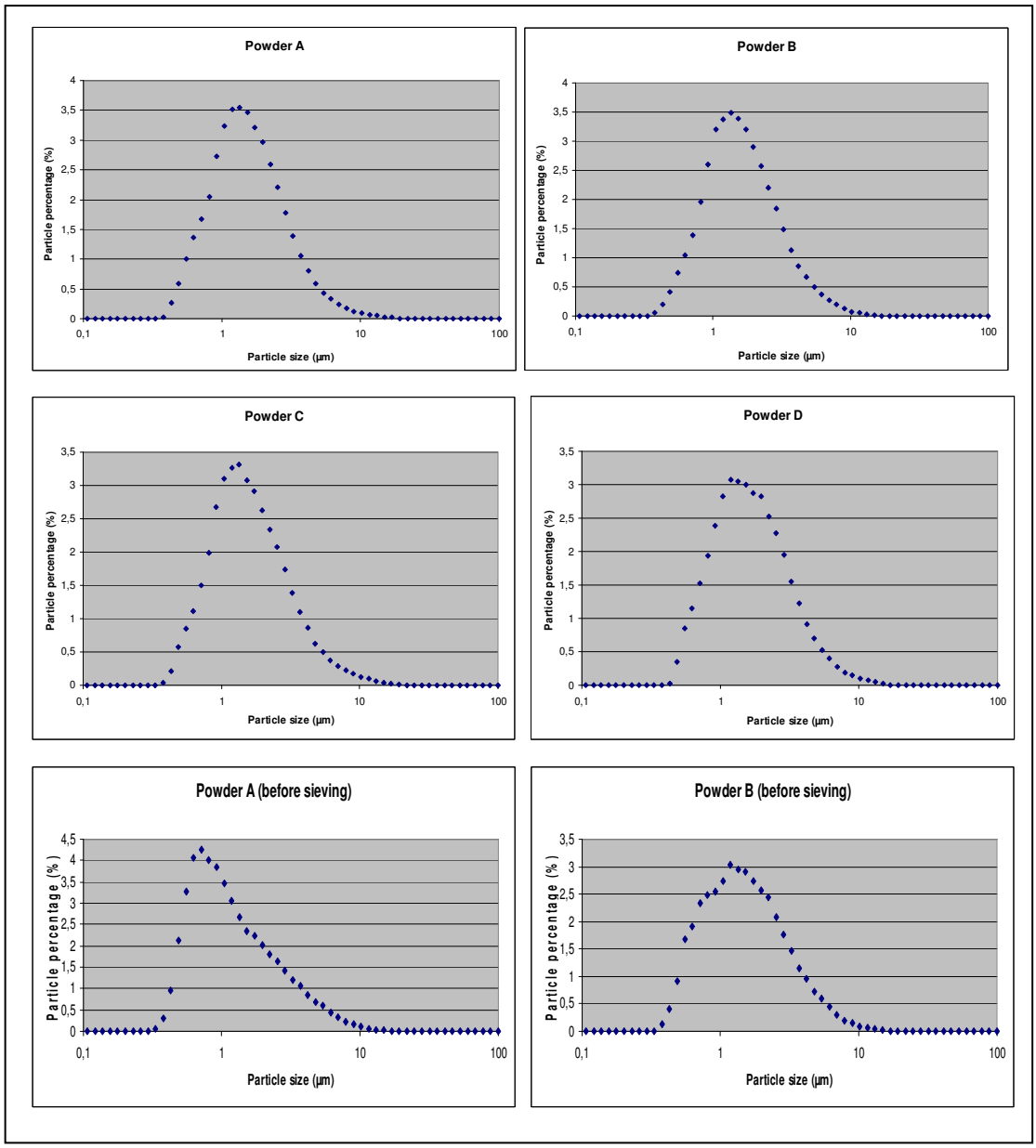


Fig. 12 – Graphs for particle size analysis for the different samples

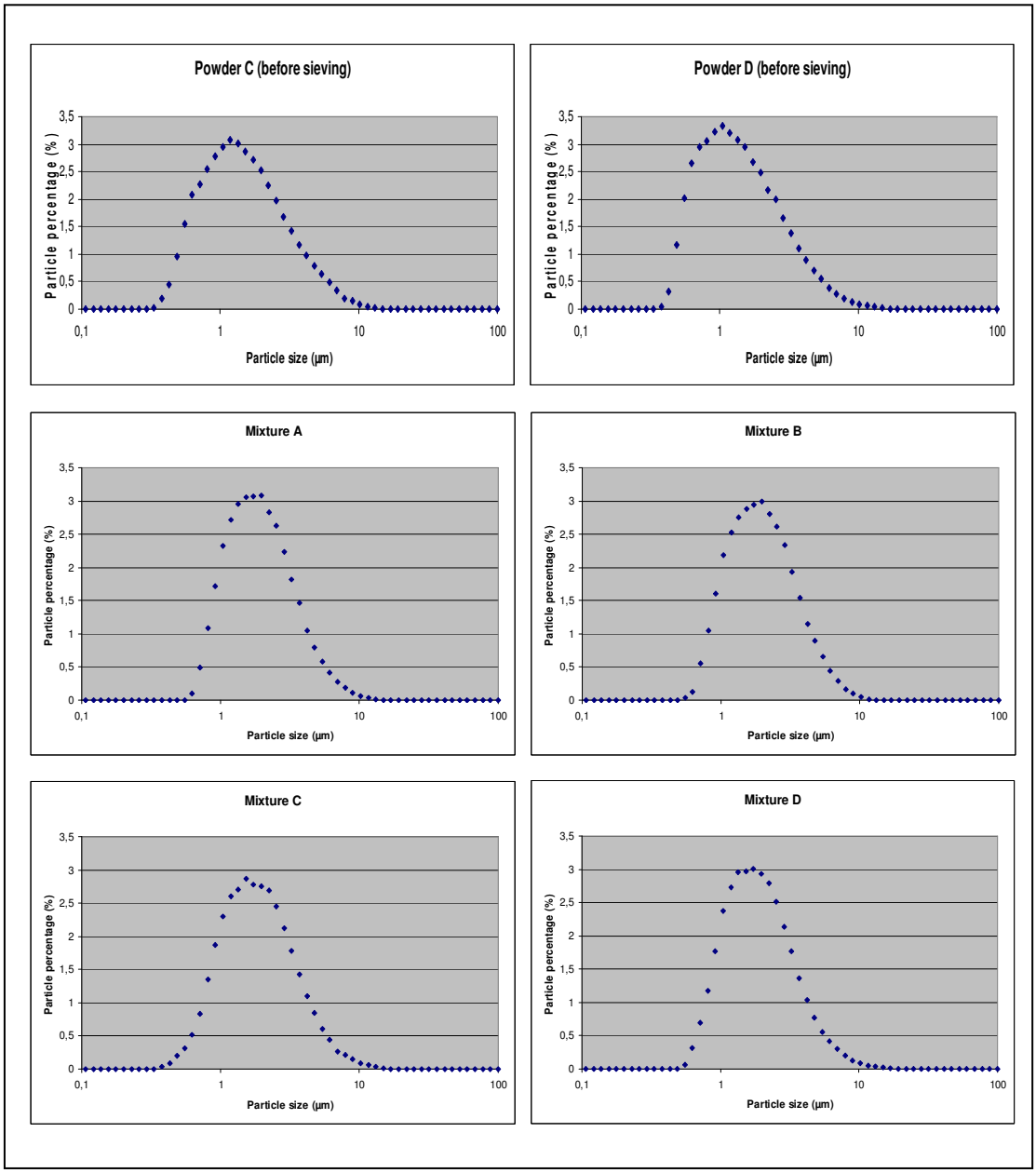


Fig. 13 – Graphs for particle size analysis for the different samples

Table 8 shows the parameters determined for the powder samples.

Table 8 – Particle size analysis results

	Mean size (µm)	% under 5 µm	$D_{\%\ under\ 5\ \mu m}$ (µm)	D_{90} (µm)	D_{50} (µm)	D_{10} (µm)	Span (µm)
Powder A	**2,38**	**80**	**4,24**	**6,27**	**2,24**	**1,01**	**2,35**
Powder B	**2,42**	**80**	**4,35**	**6,14**	**2,32**	**1,04**	**2,20**
Powder C	**2,59**	**80**	**4,94**	**7,53**	**2,44**	**1,03**	**2,66**
Powder D	**2,55**	**80**	**4,60**	**6,56**	**2,47**	**1,05**	**2,23**
Powder A (before sieving)	**2,19**	**80**	**4,75**	**6,60**	**2,19**	**0,73**	**2,68**
Powder B (before sieving)	**2,44**	**80**	**4,64**	**6,37**	**2,41**	**0,93**	**2,26**
Powder C (before sieving)	**2,41**	**80**	**4,67**	**6,26**	**2,40**	**0,92**	**2,23**
Powder D (before sieving)	**2,29**	**80**	**4,44**	**6,23**	**2,27**	**0,85**	**2,37**
Mixture A	**2,65**	**80**	**4,41**	**5,88**	**2,59**	**1,23**	**1,80**
Mixture B	**2,67**	**85**	**4,97**	**5,69**	**2,67**	**1,25**	**1,66**
Mixture C	**2,70**	**80**	**4,65**	**6,28**	**2,67**	**1,18**	**1,91**
Mixture D	**2,70**	**80**	**4,60**	**6,36**	**2,61**	**1,21**	**1,97**

4.7. Aerodynamic assessment

The last test performed was the aerodynamic assessment of fine particles of mixtures A, B, C and D – the twin stage liquid impinger provides information regarding the expected amount of powder deposited along the respiratory tract. The equivalent mass of the powders to 160 µg of active pharmaceutical ingredient was determined by assaying the above mentioned powders. The powders used were A, B, C and D instead of A', B', C' and D' (washed powders), so that no active pharmaceutical ingredient was lost, even if not included within the cyclodextrin – cost minimisation of the formulation.

Capsules containing a nominal dose of 160 µg of ciclesonide were tested accordingly to the European Pharmacopoeia.

Results are shown on **table 9:**

Table 9 – Aerodynamic behaviour results

	Twin Liquid Impinger stages	Average filling weight (mg/ capsule)	Total active substance content (mg)	Active substance recovery (% m/m)
Mixture A	**Lower stage**	**26,3**	**0,8**	**26,18±2,84**
	Middle stage			**50,03±3,79**
	Upper stage			**18,77±2,84**
	Capsule + inhaler device			**9,06±3,07**
	Total			104,03±12,54
Mixture B	**Lower stage**	**26,4**	**0,8**	**25,76±2,84**
	Middle stage			**42,89±3,20**
	Upper stage			**18,95±2,98**
	Capsule + inhaler device			**12,04±3,47**
	Total			99,65±12,49
Mixture C	**Lower stage**	**36,2**	**0,8**	**17,63±3,08**
	Middle stage			**54,35±3,21**
	Upper stage			**11,96±2,85**
	Capsule + inhaler device			**14,15±3,13**
	Total			98,09±12,27
Mixture D	**Lower stage**	**35,7**	**0,8**	**17,38±3,09**
	Middle stage			**54,48±3,23**
	Upper stage			**12,01±2,86**
	Capsule + inhaler device			**14,21±3,14**
	Total			98,19±12,33

It is clear the marked decrease (around 35%) of drug deposition in the lower stage of the liquid twin impinger in mixtures C and D when compared to the remaining powders.

Mixtures A and B, which have less cyclodextrin, present higher amount of powder deposition in the lower stage, corresponding to the lung level. On the other hand, formulations C and D display roughly less 9% of drug deposition in the lower stage. Considering the data from the particle size analysis, although mixtures A and B have presented a slightly higher percentage of fine powders (< 5 micrometers), as well as an almost marginal lower mean particle size, these factors do not seem to differ enough to justify lower stage deposition percentage variations. It can, however, be hypothesized that mixtures C and D, having twice the amount of cyclodextrin of mixtures A and B, can have a poorer flowability. This because Crysmeb molecules are large, having a molecular weight of 1,191 kDa, consequently with higher adhesion and cohesion forces between them forming agglomerates that hamper the mixtures route until the lower stage (71). Also, being a carbohydrate, when in contact with humidified surfaces it may originate a viscous solution, which adheres to the upper glass surfaces of the apparatus. The obtained results, namely for the lower stage, positively differ from those presented, for example, elsewhere by Marques and Schmidt (72), representing a good aerodynamic behaviour profile of the mixtures.

No major difference was found among formulations beside the deposition percentage of mixtures A and B *versus* C and D; thus, mixture A, stands out as the better choice, since it has the best aerodynamic behaviour and is better or comparable to the remaining ones regarding the other parameters tested, with the lowest amount of excipients.

5. CONCLUSION

It can be concluded that is feasible to produce a drug:cyclodextrin complex with ciclesonide and Crysmeb, recurring to the simple and inexpensive method of kneading. This method allowed to obtain a drug:cyclodextrin complex with a reasonable inclusion efficiency and a good aerodynamic behaviour, suitable for lung administration via inhalation. However, further studies to enhance the kneading process, so that parameters like yield and inclusion efficiency can be enhanced.

PART B: RISK MANAGEMENT

Directing a risk management model to scientific research

OBJECTIVE

The objective was to develop a tool that could be used integrated within a risk management plan or by itself, to support decision-making when facing risks during scientific research projects.

This one, like any other scientific research and development project, presents a risk for every stakeholder.

While initially outlining this work, soon became obvious that it was susceptible be affected by both internal and external factors that could hinder the work progress. Therefore, the need to evaluate which factors and how they could affect Part A of the work arose. This requirement led to the design of a more comprehensive approach. Hence it was decided to add Part B to this thesis, where a more in-depth characterization of the factors that affect scientific research was made.

This approach resulted in an investigation that expanded itself beyond this particular work, although including it, as it is a science research project. It was so decided that the focus should not be exclusively on this work. Instead, we proposed to develop a tool that could provide not only information to guide our research, but to be an input for existing risk management plans and international standards.

Taking into account the high monetary costs involved, time spent and social relevance, research projects – namely the drug-related ones – should be built upon a risk management plan.

Although tools like ISO 31000:2009 already exist (73), these end up being of general application, meaning they can be applied to such a range of activities that span from, *exempli gratia*, aircraft manufacturing to stockbroking, hence not specific for life sciences research and development. Thus, a tool specially designed for this purpose, taking into account the input of the main stakeholders, presents itself as a more appropriate approach.

In research and development oriented organisations, ensuring that multiple projects are executed simultaneously and timeliness, meaning the development of the right products at the right times, is a subject of serious consideration. Taking into account that research and development is a cardinal to the organisation, and their interaction within the remaining activities of the institution are dynamic, it is highly challenging to undertake such projects (74).

Technological innovation is not only one of the important key strategies for high technology firms but also a social imperative for scientific research and development institutions, as their work usually reflects, either directly or indirectly, on peoples lives and quality of life (75–77).

However, different types of innovation entail different degrees of uncertainty that may originate unsuccessful research and development projects (77–82).

Taking the pharmaceutical industry as an example, the success rate of a drug development project, since the first human testing to its marketing authorisation, is under 10%. Bearing in mind the financial burden these and other research and development projects carry, it is important to have planned ahead how to manage risks for these projects throughout all the development stages, so their success rates are improved (77,83–85).

Generally, risk management is a structured approach which has been widely applied in many fields, such as management, engineering, insurance, finance, environment and politics for the identification, assessment, and prioritization of risks. Following these steps there should be a planning of activities and resources to minimize, monitor, and control the likelihood of undesirable events and, if they occur, their impact on the organisation and its stakeholders (77,83). In the setting of research and development projects, the main objective of risk management is to raise success rate of the project, which will, ultimately, lead to the institution success (77,81,84,86–89). The main problem in risk management is that if the risks are improperly identified and prioritized, that can reflect in time and cost wasted in dealing with risk of losses (77,81,83,84,87,89,90).

Risk has multiple definitions which vary according to the different application domains. In project management, there is no consistent definition for risk (77,90,91). Although some, consider risk as ‘‘an uncertain event or condition that, if it occurs, has a positive (opportunity) or negative (threat) impact on project objectives.’’, others, mostly practitioners and researchers in project management, continue to consider risk being more related to adverse effects on project performance (77,83,91–93). From this point of view, risk management appears to be related to the identification and

management of threats to the project. Moreover, in the literature, uncertainty is also defined as randomness of the environment, inability to foresee the impacts of environmental change, and powerlessness to predict the consequences of choice (77,80,94,95). Risk is, as well, frequently defined as undesired project outcomes and exposure to uncertainty (77,81,83,84,86,87,89).

Managing the risk in research projects to enhance its success rates has been subject studies for many years and widely applied in practice, showing that applying risk management techniques to innovative research and development projects is able to improve their success rates (77,80,81,83,84,86,93,96–99).

The principles and guidelines for effective risk management and the importance of active risk management for accelerating projects and improving their success rates have previously been described. It has also been shown that risk management practice is more applicable for higher-risk projects (77,81,86).

Some studies have developed risk management approaches to select the proper projects for increasing success rates of product launch and to capture the business opportunity and attain profit for the company (77,100–102). However is still noticeable a lack of research on providing an integrated framework that takes into account operational risk management and provides a methodical approach for risk identification, assessment, response planning, and control (77).

In this context, ISO 31000:2009 plays a major role outlining the main requirements which a risk management plan should comprise to be effective for any organisation or activity and thus providing a backbone for the development of specific risk management policies.

ISO 31000:2009 approaches risk management in view of several aspects that, although generically applicable, are well described within the document itself. In summary, the text describes the dialectics involved in identifying, analysing, evaluating and mitigating the risk inside an organisation in a transparent and robust manner.

This International Standard recognizes that every sector or application has its own individual needs and criteria. Hence, it places the establishment of context as a key feature to help revealing and assess the nature and complexity of the risks, by capturing the organisation objectives, the environment in which these objectives are pursued, its stakeholders and the specific risk criteria.

The context in which an organisation operates is a key factor for the performance of their operation.

Establishing the context comprises the following items, that jointly allow to establish the framework for any organisation (73):

1) External context – it is based on the organisation context as a whole. Regarding scientific research, major external factors may include legal, regulatory, technological and financial/ economic issues;

2) Internal context – risk management should be aligned with the organisation culture, structure, processes and overall strategy. Internally, factors to take into account when establishing the context may encompass governance, structure, policies, objectives and human and financial capabilities;

3) Context of the risk management process – it should be established to which activities, objectives and parameters risk management is applied. Objectives, responsibilities, scope, depth and methodologies for the risk management process should be clearly defined;

4) Risk criteria definition – this item relates to the significance of risk evaluation. Factors to achieve this purpose may include characterization of consequences, definition of acceptable risk and definition of likelihood.

The following steps of a risk management plan, according to ISO 31000:2009, relate to risk assessment. Namely, risk identification, risk analysis and risk evaluation. Risk identification concerns to the recognition of risks that might affect the organisation or project; risk analysis purpose is to provide an input for risk evaluation, as this step analyses the causes of the risk, their consequences and likelihood of those consequences; on the other hand, the object of risk evaluation is to compare the level of different risks, prioritize them and come to a decision of whether or not there is the need to take action.

Identifying specific risks for scientific research and defining which ones are more likely to occur and which ones have more impact allows directing an otherwise generic set of risk management standards to the practice of science. Therefore, this is the focus of our work: introducing knowledge regarding the specificities of scientific research. This will allow a narrower application of the International Standard with verifiable practical effects on risk management.

Remaining steps of ISO 31000:2009, such as risk treatment, monitoring and review or recording of the risk management process will not be dealt with on this work.

METHOD AND TOOLS

In order to assess what is considered to have more impact and to be more likely in this setting, an online form **(Annex I)** was created.

In short, the questionnaire inquired about the respondents formation, place of work and what factors they considered critical when starting a new research project. The factors available for choice were based on a first questionnaire **(Annex II)** sent only to a reduced number of investigators, who gave their input. Similar responses were then categorized and grouped, and each group was presented in the public form as a distinct option.

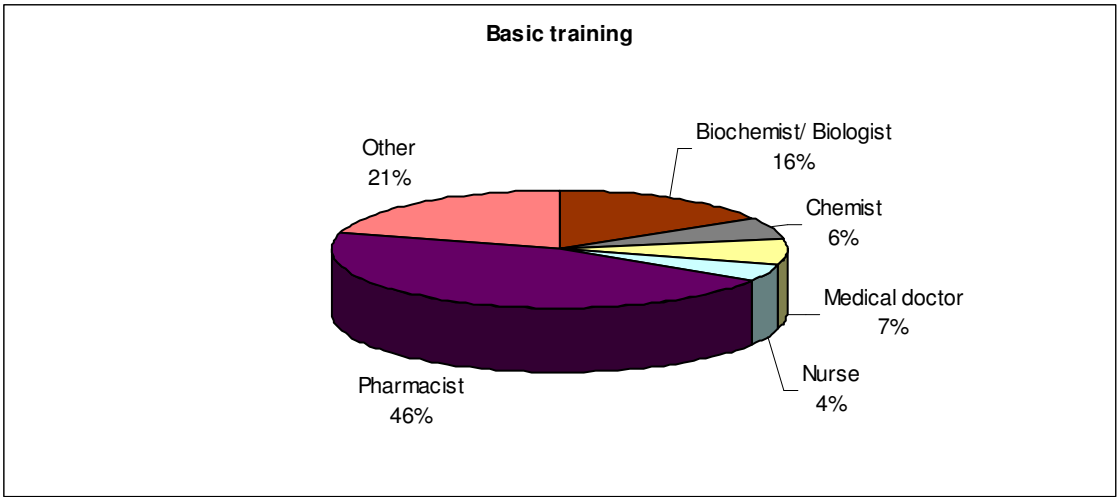
Impact evaluation was made directly from raw data, as it was asked for the respondents to rank what they considered to be the critical factors, *id est*, the ones with more impact. To avoid a lengthy questionnaire, it was chosen not to ask directly about the probability of each factor, which was estimated from the frequency of each option.

The online form was sent to individual emails collected from the websites of the world top ten universities (103): University of Cambridge (United Kingdom), Harvard University (United States of America), Massachusetts Institute of Technology (United States of America), Yale University (United States of America), University of Oxford (United Kingdom), Imperial College of London (United Kingdom), University College London (United Kingdom), University of Chicago (United States of America), University of Pennsylvania (United States of America) and Columbia University (United States of America). The form was also published in some relevant groups (professional, *alumni* and institutional groups) on social and professional networks, namely Facebook® and LinkedIn®.

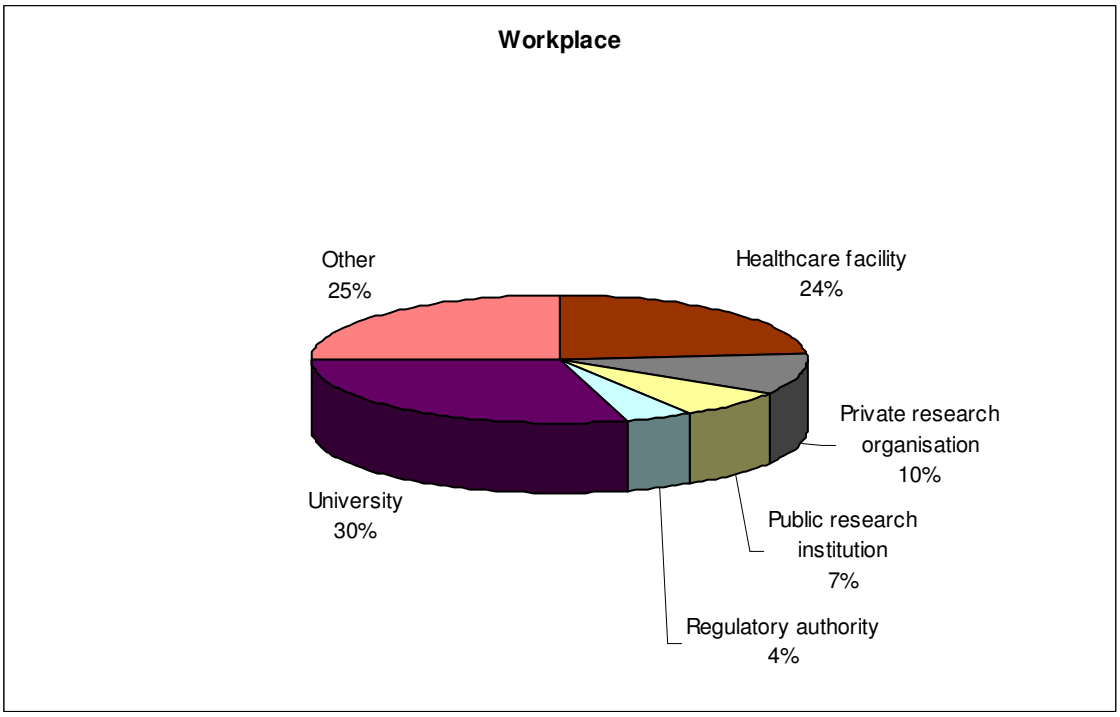
Considering only sent emails, the response rate was 15,2%. It is, however, reasonable to consider the real response rate lower, as the form was also present in social/ professional networks; this is also reinforced by the fact that some replies were from countries other than the United Kingdom or the United States, home to the universities of where the emails were sent.

Total number of replies was 76, of which 68 were considered valid, corresponding to a percentage of 89,5. For a form to be considered valid, it had to meet two criteria: adequate response to all mandatory questions; and the respondent was related with scientific research.

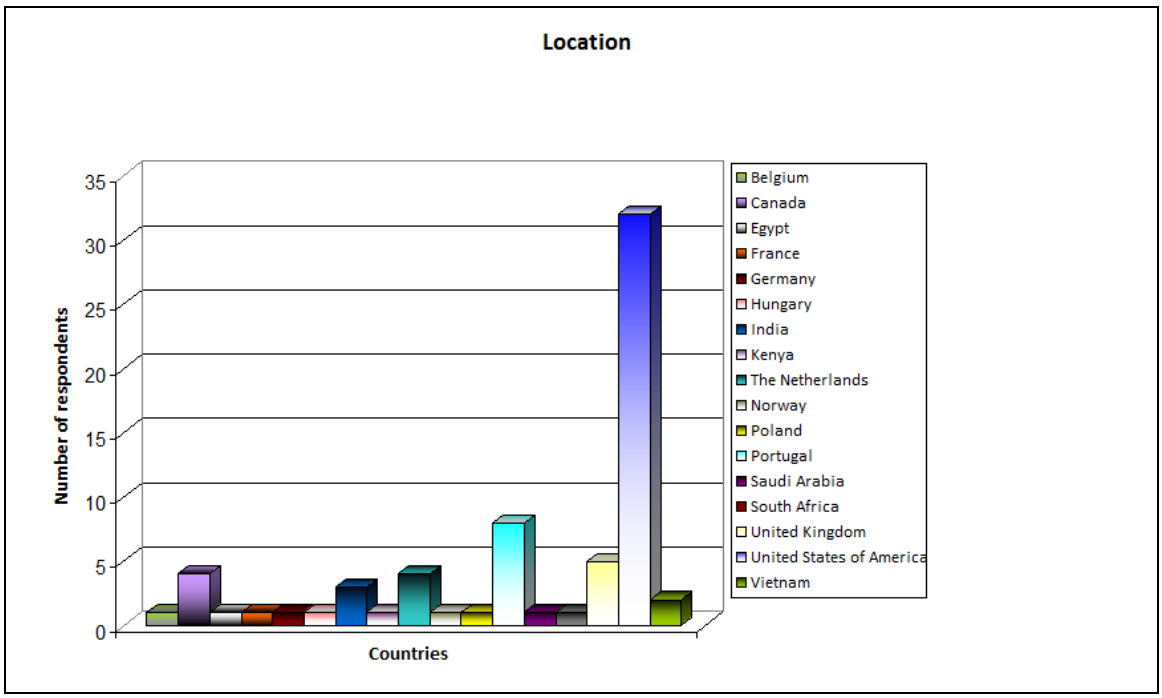
Graphs 15, **16** and **17** represent, respectively, basic training, workplace and location of the respondents.



Graph 15 – Basic training of the respondents



Graph 16 – Workplace of the respondents



Graph 17 – Location of the respondents

Core data regarding the critical factors when engaging in scientific research acquired from the questionnaires was used to define a Risk Severity Index as described in the literature; where Risk Severity Index is described as Impact X Probability (104).

Impact was calculated the following way:

1. Crescent numbers were assigned from the fifth to the most critical factor (from 1 to 5);
2. The frequency of each factor was then multiplied by its corresponding number and the values obtained *per* factor were added;
3. Based on the sum of each factor, an arbitrary value was specified for each one – to the highest sum it was given the number 12, the second highest the number 11 and so on.

Likelihood was calculated considering the relative frequency of each factor. Based on it, an arbitrary value was specified for each one.

Having calculated both the Likelihood and the Impact, is possible to assess the Risk Severity Index, according to the above mentioned formula.

After Risk Severity Index was calculated for each factor identified, one, two and three thirds percentile were calculated, so risk categories could be assigned to each factor, taking into account its Risk Severity Index.

Results are shown on **table 10**.

Table 10 – Risk Severity Index/ Risk Category

Factor	Risk Severity Index	Risk Category
Location	4	Noncritical
Qualified human resources	9	
Scientific area	15	
Economical outcome	16	
Professional prospectives	35	Critical
Publishing potential	36	
Scientific method	56	
Logistics	56	
Coordination with colleagues/ superiors	81	Very critical
Timespan/ planning of the project	100	
Funding	121	
Objectives/ potential applications	144	

The method chosen for data collection – an online questionnaire – had some advantages:

- It allowed a worldwide participation, with replies from people based in five continents, namely Africa, America, Asia and Europe; comprising different work cultures and standards;
- Access to investigators in top universities, that otherwise would not be accessible to this project;
- Could be easily shared;
- Simplified data treatment.

The questionnaire was designed so its structure could provide data to determine: 1) which risk factors are present on scientific research; 2) what is the likelihood of their occurrence and; 3) should they occur, at what level the organisation would be affected.

The likelihood of their occurrence *versus* their impact is called Risk Severity Index. This index **(Table 10)** is semi-quantitative, dividing risk into three categories: very critical, critical and noncritical.

Taking into account that the Risk Severity Index was calculated based on the opinion of a pool of experts, we consider it to be reliable for use as a "guideline" within the International Standard. Therefore, science-oriented organisations implementing a risk management plan, may find this information useful to be applied in the scope of and within ISO 31000:2009, namely when describing some of its points: definition of risk criteria, risk analysis and risk evaluation. This means that the input generated by this data can, ultimately, define how, when and if the organisation should address a given risk; with a more robust basis of support.

Despite its greatest usefulness is achieved when integrated in a risk management plan, data obtained can also be used as a standalone tool.

In this case, as no risk management plan was in place – nor deemed necessary – Risk Severity Index was used as an individual tool to follow the project progress. It allowed to direct efforts and manage time and resources, while conducting

Part A of this work. This way, decision-making on whether a risk should be dealt with primarily or its treatment could be postponed, was based taking into account not only the individual experience, but – most importantly – the collective one.

Despite other risks that always exist, the one that was mostly perceived from the beginning and during the work, was the time frame for its conclusion. So, faced with this risk, three major paths could be followed: 1) address the problem as soon as possible; 2) postpone treatment of the risk or; 3) disregard the risk.

Based on individual experience and context, it was decided this matter should be addressed as soon as possible. This assessment was also supported by what is laid down in **Table 10**.

Being the only identified risk, the approach was straightforward: address it as soon as possible. However, should there be a multiplicity of risks with limited resources to address them all simultaneously, along with no experience of the situation and/ or context, the calculated Risk Severity Index is a valuable tool to define how to act. Information provided by the Risk Severity Index, helps to identify – together with a risk management plan, if it exists – how the organisation should primarily direct its resources, to address which risks:

- Very critical risks – should be addressed firstly and concentrate the organisation resources. These risks are those more likely to occur and more damaging to the organisation.

- Critical risks – should be addressed after very critical risks. Medium likelihood and impact to the organisation.

- Noncritical risks – last to be addressed. Present minimal chance of occurring with minimal effects upon the organisation.

CONCLUSION

The method applied allowed to obtain data that could be treated to be used as a standalone tool or as a part of a risk management plan.

The Risk Severity Index determined for scientific research is a useful tool for organisations that work within this scope of activity. It allows them to better appreciate how to address risks, especially when the organisation has little experience with them. It provides a hierarchy, so that when multiple risks are encountered, a good resources management can be put in place, minimising hazardous consequences for the project and/or organisation.

This index, used as an individual tool also shows valuable for individuals or smaller organisations, that do not have a risk management plan. It points on where to act first and more actively, even if not within the frame of an integrated plan.

CONCLUSION AND FUTURE OUTLOOK

Despite using a simple and inexpensive process, it was possible to obtain an inclusion complex of drug-cyclodextrin. This may reveal useful for the pharmaceutical industry as a simple process might reduce the needed amount of active pharmaceutical ingredient to produce the desired therapeutic effects. Further studies could improve the kneading process, making it more efficient.

A directed risk management approach to scientific research is an added value for every project. Data acquired during this work shows to be promising as it includes the views of top scientists and is focused in this specific area. However, it is desirable that the sample would be larger to include the maximum number of expert opinions, providing a more balanced scoring of the Risk Severity Index.

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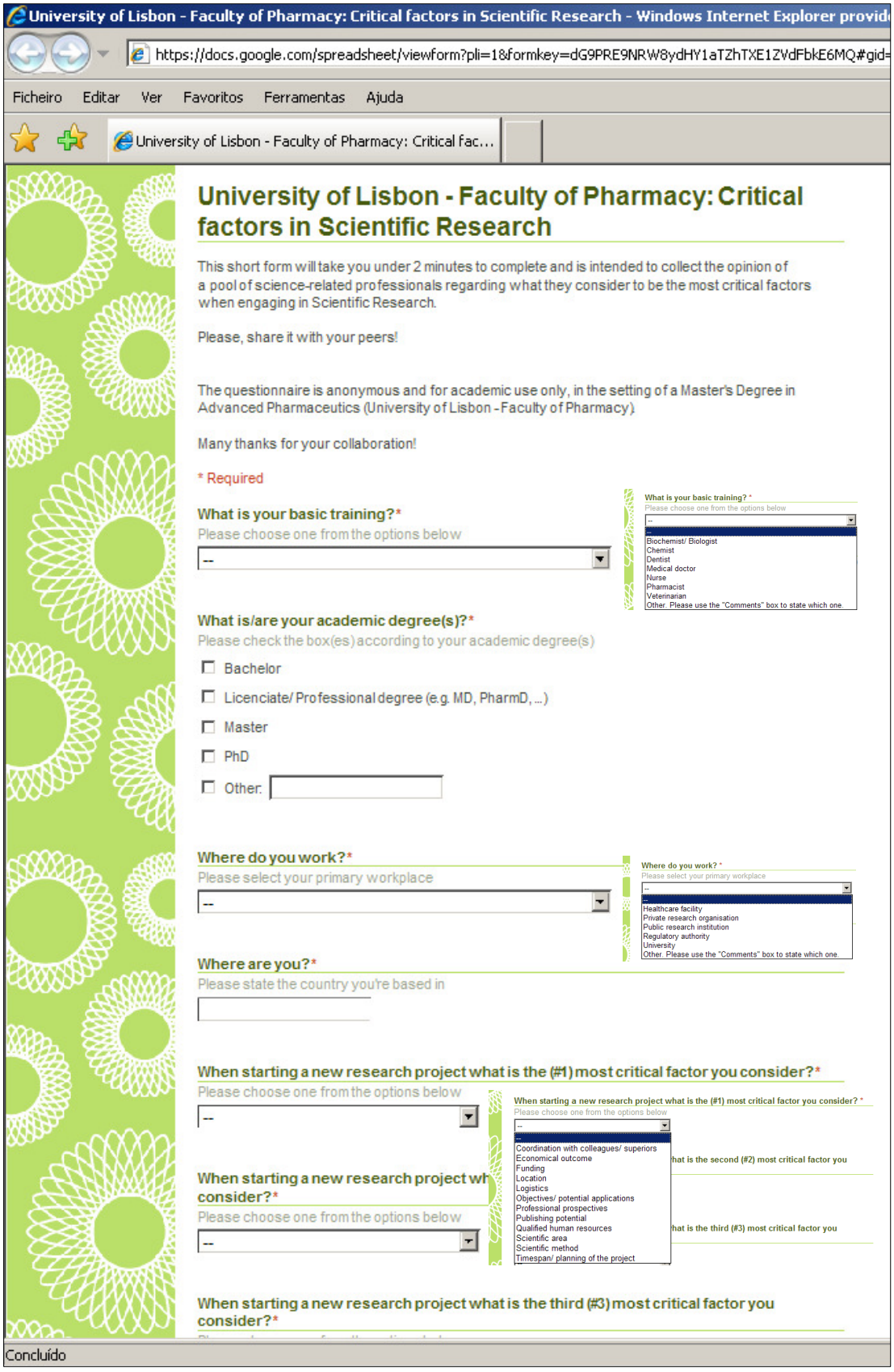
ANNEXES

ANNEX I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University of Lisbon - Faculty of Pharmacy: Critical factors in Scientific Research
This short form will take you under 2 minutes to complete and is intended to collect the opinion of a pool of science-related professionals regarding what they consider to be the most critical factors when engaging in Scientific Research.
Please, share it with your peers!
The questionnaire is anonymous and for academic use only, in the setting of a Master's Degree in Advanced Pharmaceutics (University of Lisbon - Faculty of Pharmacy).
Many thanks for your collaboration!
* Required
What is your basic training?*
Please choose one from the options below
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What is your basic training? *
Please choose one from the options below
Biochemist/ Biologist
Chemist
Dentist
Medical doctor
Nurse
Pharmacist
Veterinarian
Other. Please use the "Comments" box to state which one.
What is/are your academic degree(s)?*
Please check the box(es) according to your academic degree(s)
Bachelor
Licenciate/ Professional degree (e.g. MD, PharmD, ...)
Master
PhD
Other:
Where do you work?*
Please select your primary workplace
--
Where do you work? *
Please select your primary workplace
Healthcare facility
Private research organisation
Public research institution
Regulatory authority
University
Other. Please use the "Comments" box to state which one.
Where are you?*
Please state the country you're based in
When starting a new research project what is the (#1) most critical factor you consider?*
Please choose one from the options below
--
When starting a new research project what is the (#1) most critical factor you consider? *
Please choose one from the options below
Coordination with colleagues/ superiors
Economical outcome
Funding
Location
Logistics
Objectives/ potential applications
Professional prospectives
Publishing potential
Qualified human resources
Scientific area
Scientific method
Timespan/ planning of the project
When starting a new research project what is the second (#2) most critical factor you consider?*
Please choose one from the options below
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When starting a new research project what is the third (#3) most critical factor you consider?*
When starting a new research project what is the third (#3) most critical factor you consider?*
Concluído


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What other factors do you take into account when starting a new research project?
Comments
Feel free to send comments, make questions, leave your contact, et cetera
Thank you!
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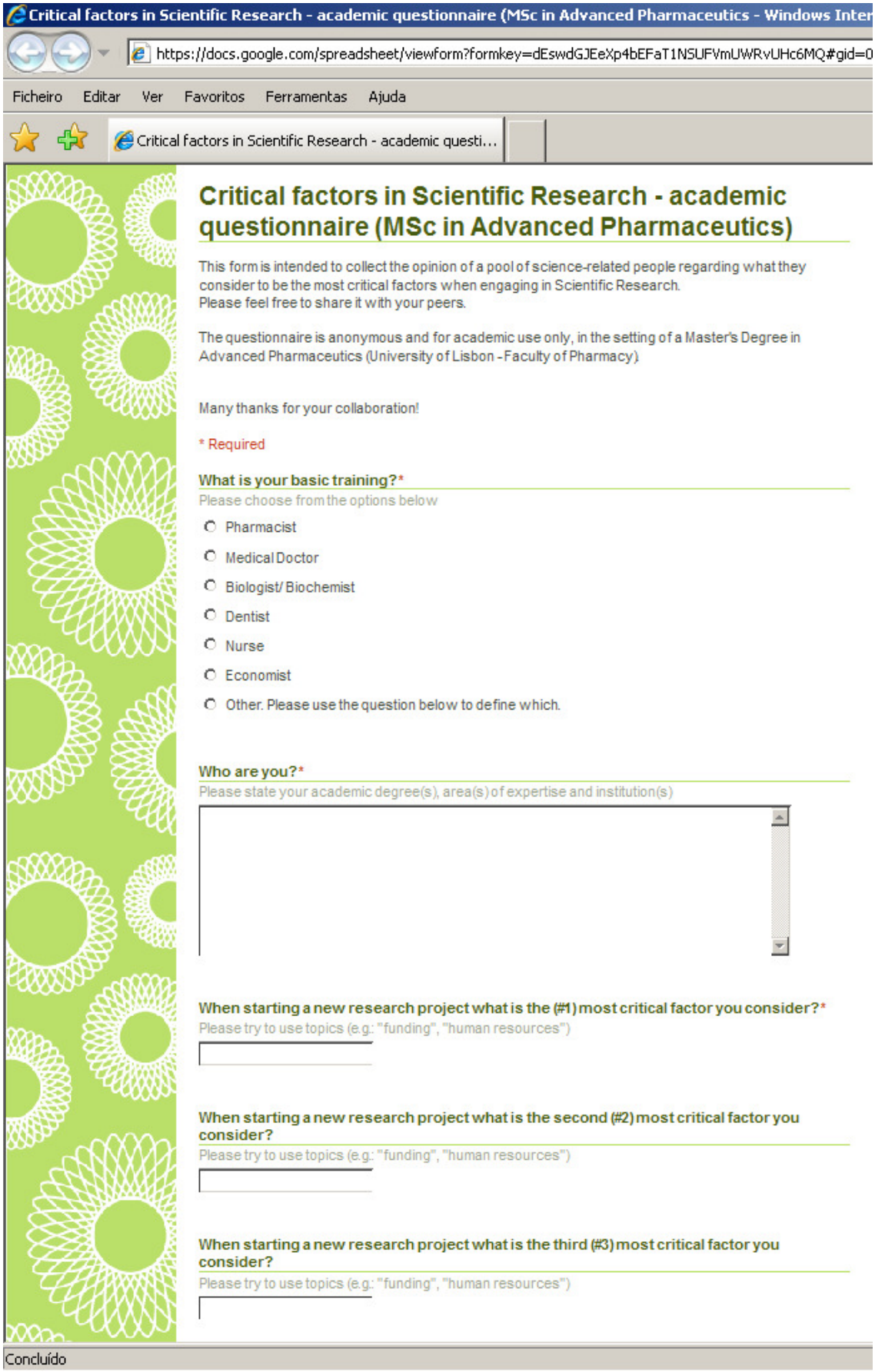
ANNEX II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Critical factors in Scientific Research - academic questi...
Critical factors in Scientific Research - academic questionnaire (MSc in Advanced Pharmaceutics)
This form is intended to collect the opinion of a pool of science-related people regarding what they consider to be the most critical factors when engaging in Scientific Research.
Please feel free to share it with your peers.
The questionnaire is anonymous and for academic use only, in the setting of a Master's Degree in Advanced Pharmaceutics (University of Lisbon - Faculty of Pharmacy).
Many thanks for your collaboration!
* Required
What is your basic training?*
Please choose from the options below
Pharmacist
Medical Doctor
Biologist/ Biochemist
Dentist
Nurse
Economist
Other. Please use the question below to define which.
Who are you?*
Please state your academic degree(s), area(s) of expertise and institution(s)
When starting a new research project what is the (#1) most critical factor you consider?*
Please try to use topics (e.g.: "funding", "human resources")
When starting a new research project what is the second (#2) most critical factor you consider?
Please try to use topics (e.g.: "funding", "human resources")
When starting a new research project what is the third (#3) most critical factor you consider?
Please try to use topics (e.g.: "funding", "human resources")
Concluído


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When starting a new research project what is the third (#3) most critical factor you consider?
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Please try to use topics (e.g: "funding", "human resources")
When starting a new research project what is the fifth (#5) most critical factor you consider?
Please try to use topics (e.g: "funding", "human resources")
What other factors do you take into account when starting a new research project?
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