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SYSTEMATIC DEVOLOPMENT OF FORMULATIONS

SUITABLE FOR PULMONARY APPLICATIONS

BY NEBULISATION

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1 SUMMARY (ENGLISH/GERMAN VERSION)

The main objective of this work was the elaboration of a systematic pathway for the development of formulations suitable for nebulisation. Furthermore, it was also an objective of this work to investigate the taste masking ability of the cyclodextrins not only for water insoluble drugs but also for water soluble drugs.
In this work, we investigated the solubility enhancement of 3 water insoluble drugs by using cyclodextrins in two pharmaceutical delivery forms: Solutions and Suspensions.
A preliminary conclusion was that through the use of cyclodextrins it was possible to increase the solubility of the 3 water insoluble drugs, Cyclosporin A, Azithromycin and Budesonide. In addition, all the formulations were stable and suitable to be nebulised using the intended eflow device.

- Cyclosporin A (CSA): it was possible not only to increase its water solubility but also to obtain droplets sizes suitable for nebulisation. For example, the nebulisation of the CSA lyophilizate resulted in an average droplet size of 3.58 µm with a TOR of 415 mg/ml. In addition, the lyophilizate of the cyclosporin A/cyclodextrin (CSA/CD) formulation kept at 25°C and 60% relative humidity was stable for at least 5 months.
- Azithromycin: in order to improve the solubility and also the taste of the azithromycin formulation, a new approach was developed. It involved increasing the intrinsic solubility of azithromycin with the help of pH adjustments in order to maximize the solubilisation capacity of the cyclodextrins without the formation of non-inclusion complexes. Consequently, the resulting formulation showed an improvement in masking the taste although the bitterness was not entirely removed. However, the taste of the formulation was tolerable for inhalation.
- Alternatively, the solubility of water insoluble drugs, such as cyclosporin A and azithromycin can also be increased by the preparation of nanosuspensions of Drug:CD nanospheres. For example, in the case of cyclosporin A nanosuspensions, the particle size of the nanospheres were 249 nm (P.I. =0.33).
- Budesonide: the results showed that is possible to have a general method that can be applied to several water insoluble drugs. For example, trough the use of cyclodextrins we were able to obtain a sterile and stable aqueous solution of budesonide that can be efficiently nebulised through the eflow® (MMD of 3.00 µm with a TOR of 330 mg/ml).

The taste masking ability of the cyclodextrins was also investigated. As a model drug, a water soluble substance, Pentoxifylline was used. The results obtained show that it is possible to prepare a pentoxifylline formulation with a taste suitable for pulmonary administration. In addition, the formulations can also be efficiently nebulised through the use of eflow®. As an example, a formulation containing 50 mg/ml pentoxifylline and 50 mg/ml 2-HP-β-CD was nebulised using the eflow®. The droplet size was 3.25 µm with a TOR of 485 mg/ml.

It was also an objective of this work the elaboration of a systematic pathway for the development of formulations suitable for nebulisation. Based on the knowledge collect during this work, a database system was built as an aid tool for the pharmaceutical formulation development process.
This system is able not only to reduce the time in the development formulation process but also decrease costs by reducing not only the development time but also by a more effective use of materials.

In conclusion, it was able to increase the solubility/stability and also to taste mask formulations containing both water insoluble and water soluble drugs. This opens new possibilities to the pulmonary delivery as an alternative non-invasive method of delivering drugs that currently can only be delivered orally or intravenously.

Das Hauptziel dieser Arbeit war die Weiterentwicklung eines systematischen Pfads für die Entwicklung von für Zerstäubung passenden Formulierungen. Außerdem war das auch ein Ziel dieser Arbeit zu untersuchen, der Fähigkeit des Cyclodextrins, nicht nur für unlösliche Wasserrauschgifte sondern auch für auflösbare Wasserrauschgifte, den Geschmack zu maskieren.
In dieser Arbeit untersuchten wir die Löslichkeitserhöhung von drei unlöslichen Wasserrauschgiften, indem wir Cyclodextrins in zwei pharmazeutischen Lieferformen verwendeten: Lösungen und Suspendierungen. Ein einleitender Beschluss war, dass durch den Gebrauch von Cyclodextrins es möglich war, die Löslichkeit der drei unlöslichen Wasserrauschgifte, Cyclosporin A, des Azithromycin und des Budesonide zu vergrößern. Außerdem waren alle Formulierungen stabil und passend, um zerstaübt durch das Verwenden des beabsichtigten eflow® Geräts zu sein.

- Cyclosporin (CSA): es war nicht nur möglich, seine Wasserlöslichkeit zu vergrößern sondern auch für Zerstäubung passende Tröpfchen-Größen zu erhalten. Zum Beispiel, lief der Zerstäubung des CSA lyophilizate auf eine durchschnittliche Tröpfchen-Größe 3.58 µ hinaus M mit einem TOR von 415 mg/ml. Außerdem war der lyophilizate des Cyclosporin A/Cyclodextrin (CSA/CD) Formulierung an 25°C behalten und relative Feuchtigkeit von 60 % seit mindestens 5 Monaten stabil.
- Azithromycin: um die Löslichkeit und auch der Geschmack der Azithromycin Formulierung zu verbessern, wurde eine neue Annäherung entwickelt. Es schloß die Erhöhung der inneren Löslichkeit von Azithromycin mit der Hilfe von pH Anpassungen ein, um die Solubilisierungs Kapazität des Cyclodextrins ohne die Bildung von Nichteinschließungskomplexen zu maximieren. Folglich zeigte die resultierende Formulierung eine Verbesserung in der Maskierung des Geschmacks, obwohl die Bitterkeit nicht völlig entfernt wurde. Jedoch war der Geschmack der Formulierung für die Einatmung erträglich.
- Wechselweise kann die Löslichkeit von unlöslichen Wasserrauschgiften, wie Cyclosporin A und Azithromycin auch durch die Vorbereitung von nanosuspensions von Drug:CD nanospheres vergrößert werden. Zum Beispiel, im Fall von Cyclosporin A nanosuspensions, war die Partikel-Größe des nanospheres 249 nm (P.I. =0.33).
- Budesonide: die Ergebnisse zeigten, dass das möglich ist, eine allgemeine Methode zu haben, die auf mehrere unlösliche Wasserrauschgifte angewandt werden kann. Zum Beispiel, durch den Gebrauch von Cyclodextrins wir waren im Stande, eine sterile und stabile wässrige Lösung von Budesonide zu erhalten, der effizient zerstäubt durch den eflow® sein kann (MMD 3.00 µ M mit einem TOR von 330 mg/ml).

Der Geschmack, der Fähigkeit des Cyclodextrins maskiert, wurde auch untersucht. Als ein Musterrauschgift, eine auflösbare Wassersubstanz, wurde Pentoxifylline verwendet. Die Ergebnisse erhielten zeigen dass es möglich ist, eine Pentoxifylline Formulierung mit einem für die Lungenverwaltung passenden Geschmack vorzubereiten. Außerdem können die Formulierungen auch effizient zerstäubt durch den Gebrauch von eflow® sein. Als ein Beispiel, eine Formulierung, die 50 mg/ml Pentoxifylline und 50 mg/ml 2-HP-ß-CD enthält; - CD war zerstäubtet durch das Verwenden des eflow®. Die Tröpfchen-Größe war 3.25 µ M mit einem TOR von 485 mg/ml.
Es war auch ein Ziel dieser Arbeit die Weiterentwicklung eines systematischen Pfads für die Entwicklung von für Zersträubung passenden Formulierungen. Gestützt auf die Kenntnisse versammeln während dieser Arbeit, ein Datenbanksystem wurde als ein Hilfswerkzeug für den pharmazeutischen Formulierungsentwicklungsprozess gebaut. Dieses System ist nicht nur im Stande, die Zeit mit dem Entwicklungsformulierungsprozess zu sparen sondern auch Abnahme-Kosten zu reduzieren. Anderseits, es führt nicht nur zu einer Reduzierung der Entwicklungsdauer sondern auch zu einem wirksameren Gebrauch von Materialien.
Schließlich es war im Stande, die Löslichkeit/Stabilität zu vergrößern und auch Maske-Formulierungen zu kosten, die sowohl auflösbaren als auch unlöslichen Wasserwasserrauschgifte enthalten. Das öffnet neue Möglichkeiten zur Lungenübergabe als eine alternative nichtangreifende Methode, Rauschgifte zu liefern, die nur zurzeit mündlich oder intravenös geliefert werden können.

2 INTRODUCTION

Pulmonary drug delivery has constantly being considered as a way to directly target disorders of the lung, as well as a non-invasive alternative for systemic conditions that can not be treated using, for example, with oral medications. While pulmonary delivery is routinely used for specific conditions such as asthma, recent advances in inhalation technology have provided the opportunity to increase the applications for pulmonary delivery to many therapeutic molecules.
The applications for pulmonary drug delivery are many, particulary in four key areas:

- *Targeted treatment of lung disorders;*
- *A non-invasive alternative to macromolecule drugs;*
- *Faster onset of action;*
- *Efficacious and patient-friendly therapies.*

2.1 The Inhalative Therapy

Growing attention has been given to the potential of the pulmonary route as a means for non-invasive administration for the local delivery of therapeutic agents and also for the systemic delivery (mainly peptides and proteins). This is because the lungs could provide a large absorptive surface area (up to 100 m2) but extremely thin (0.1 μm–0.2 μm) absorptive mucosal membrane and good blood supply. Consequently, the interest of using drug delivery systems for the treatment of pulmonary diseases is steadily increasing.
Furthermore, this route has the ability to deposit large concentrations at disease sites, to reduce the amount of drugs administered to patients (for example, only 20–10% of the amount administered by the oral route), to increase the local activity of drugs released at such sites, and to avoid the metabolization of drugs due to a hepatic first-pass effect.

No other non-invasive route of drug delivery provides the bioavailability potential of the pulmonary epithelium. The lungs are more permeable to macromolecules than any other portal of entry into the body (Lit. [1]). Some of the most promising therapeutic agents are peptides and proteins, which could be inhaled instead of being injected, thereby improving compliance. The lungs are even more permeable to small molecules than the gastrointestinal (GI) tract (Lit. [2]).
In contrast to oral delivery, where a drug can be heavily metabolized and altered by the enzymes of stomach and liver, the lungs exhibit only a small drug-metabolizing and efflux transporter activity (Lit. [3]–Lit. [5]). Consequently, small molecules can be efficiently delivered into the body through the lungs without the production of a complex array of metabolites.

No non-invasive route of delivery provides the speed of action that an inhaled drug can provide. Pulmonary drug targeting has long been used to treat lung diseases, particularly asthma and chronic obstructive pulmonary disease (COPD), non-cystic fibrosis (nCF) and cystic fibrosis (CF), bronchiectasis, bronchiolitis and recently influenza. Potentially, other pulmonary diseases such as parenchymal fibrosis, acute bronchitis, pneumonia and even carcinoma of the lung in situ can be treated by inhalation1.
By facilitating the systemic delivery of large and small molecule drugs through deep inhalation into the lungs, a unique and innovative delivery alternative is provided for therapies that must currently be administered by injection (i.v., i.m., s.c.) or oral delivery, which causes adverse effects or poor absorption.
In fact, the main advantage of a therapy via the lungs is the potentially improved therapeutic index, i.e. the ratio of therapeutic benefit to adverse effects. This applies mainly to the therapy of pulmonary diseases, but may also be applicable to systemic diseases due to reduced first-pass metabolism that may be associated with hepatocellular injury. Pulmonary delivery also offers the potential for better and possibly more economical treatment or prophylaxis of respiratory and systemic diseases (e.g. viral vaccines).

Pulmonary delivery provides a non-invasive method of delivering drugs into the bloodstream for those molecules that currently can only be delivered by injection. These include peptides and proteins, such as insulin for diabetes or interferon-beta for multiple sclerosis and most of the drugs developed in recent years by biotechnology companies. This direct delivery often results in a better treatment outcome while potentially requiring lower doses than given systemically - either orally or by injection.
Another advantage lies on the very rapid onset of action similar to the i.v. route and quicker than either oral delivery or subcutaneous injections2. More rapid delivery could benefit treatments for pain, seizures, panic/anxiety attacks, hypertensive crises, anaphylaxis (severe allergies food, insect bites), nausea, cardiovascular conditions (arrhythmia, strokes), and Parkinson's “lock-up”, where speed is important.
Inhalative therapy, in comparison to the oral route, can help avoid gastrointestinal tract problems such as poor solubility at low pH, low bioavailability, gut irritability, unwanted metabolites, food effects and dosing variability.

Natural mammalian peptides, (less than 30 amino acids), are broken down in the lung by ubiquitous peptidases and have very poor bioavailabilities. In general, proteins with molecular weights between 6,000 and 50,000 D are relatively resistant to most peptidases and have good bioavailabilities following inhalation. For larger proteins, the bioavailability picture is not clear.

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1 With regard to pulmonary infections, much higher concentrations of antibiotics can be achieved in the lungs by inhalation, which should accomplish greater and more rapid bacterial killing with less likelihood of developing bacterial resistance. Furthermore, topical delivery achieves reduced systemic side effects for equivalent therapeutic benefit.

2 One of the advantages, of inhaled insulin is, for example, that it is more rapidly absorbed than subcutaneously injected insulin and provides a more physiological response to a meal (Lit. [6]). Small molecules, particularly hydrophobic molecules, are absorbed within seconds after inhalation and can, this way, be used to treat a wide variety of symptoms that arise suddenly or require a quick response. Pain, panic, anxiety, nausea, cardiovascular crises, bronchoconstriction, sleep induction, spasms, Parkinson's lock-up, and hot flashes are some of the rapid-onset conditions that are addressable with inhaled medicines (Lit. [7]–Lit. [9]).

Although the lung is rich in antiproteases, aggregation of inhaled proteins will stimulate opsonization (coating) by special proteins in the lung lining fluids, which will then mark the aggregated proteins for phagocytosis and intracellular enzymatic destruction. Small peptides and proteins are absorbed more rapidly after inhalation than after subcutaneous injection. For other small molecules, inhalation is also a fast way to get into the body because drug efflux transporters and metabolizing enzymes are present in the lung at much lower levels than in the gastrointestinal tract:

- Lipophilic small molecules are absorbed extremely fast: t1/2 (abs) approximately 1 to 2 minutes;
- Water-soluble small molecules are absorbed rapidly: t1/2 (abs) approximately 65 minutes. Small molecules can exhibit prolonged absorption if they are highly insoluble or carry extremely negative charges.

For several years, inhaled drugs have been used for the treatment of many lung diseases but there are currently no inhaled drugs on the market for systemic disease. However, in several companies large and small molecules are under investigation as inhaled formulations for systemic applications[3].

Due to the potential of localized topical therapy in the lungs, the interest of using drug delivery systems for the treatment of pulmonary diseases is steadily increasing. Furthermore, this route offers the ability to deposit large concentrations at disease sites, to reduce the amount of drugs administered to patients (for example, only 20–10% of the amount administered by the oral route), to increase the local activity of drugs released at such sites, and to avoid the metabolization of drugs due to a hepatic first-pass effect (Lit. [10]).
As an example, it is known that asthma (in particular chronic persistent asthma) is related to the terminal bronchioles. For this reason and, aimed at improving the pulmonary targeting of a potentially useful therapy, several scientific articles have focused on the design of suitable dosage forms to specifically target the small airways and to increase the local bioavailability of drugs combined with carrier systems.
In addition, the design of a carrier system has a very important influence because of the limitations of chronic oral administration with respect to systemic side effects, including hepatic dysfunction, skeletal malformations, hyperlipidemia, and hypercalcemia (Lit. [11]).

At the present state, there are clinical results obtained with particular carrier systems which suggest that some of these may offer a practical alternative to systemic oral administration for chemoprevention trials or the treatment of lung diseases. This method can reduce the systemic complications associated with long-term administration and, consequently, increase the therapeutic value of targeted compounds.
Although the lungs are rich in enzymes, they also contain several protease inhibitors. For this reason, exogenous proteins may be protected from proteolytic degradation by these inhibitors. These characteristics allow the airways to be a useful route of drug administration in the inhaled or aerosol form.

[3] Recent advances in the development of particle technologies and devices make it possible to formulate, stabilize, and accurately deliver almost any drug to the lungs. More than 25 inhalation drugs on the market for treatment of lung diseases are all absorbed to some extent into the body, most of them quickly, and with very high systemic bioavailabilities (Lit. [56]).

However, the mechanisms of drug delivery to the lungs are more complex than for other routes. For the drug to reach the lungs, it depends on numerous factors, such as:

- The amount and rate of inhaled air;
- The respiratory pause;
- The particle size and characteristics (homogeneity, shape, electric charges, density, and hydrophobicity).

Nevertheless, the pulmonary delivery of a variety of drugs such as bronchodilators and steroids has been very successful (Lit. [12]).
In order to improve bioavailability and also to optimize the release of drugs targeted to specific sites into the lungs, several approaches have been investigated. These include advances in the fields of aerosol therapy, such as aerosol generators and drug delivery systems (for example, liposomes or nano- and microparticles).

2.2 Respiratory System

The human lung is an attractive route for systemic drug administration (Lit. [12]) due to its previously mentioned enormous adsorptive surface area (140 m^2) and thin (0.1–0.2μm) absorption mucosal membrane in the distal lung (Lit. [13]).
Approximately 90% of the absorptive area of the lung is attributed to the alveolar epithelium, which primarily consists of type I pneumocytes.[4] Because pulmonary drug administration is directly related to the respiratory structure and function, a small description of the basics of the lung and also of the drug entrance mechanisms is given below.

The human respiratory system is a complicated organ system of very close structure–function relationships.
In functional terms, the respiratory system consists of three major regions: the *oropharynx*, the *nasopharynx*, and the *tracheobronchial pulmonary region*. The conducting airway is composed of the nasal cavity and associated sinuses and the nasopharynx, oropharynx, larynx, trachea, bronchi, and bronchioles, including the first 16 generations of the airways of Weibel's tracheobronchial tree (Lit. [55]).
The conducting airway is responsible for the filtration, humidification and warming of inspired air. The respiratory region is composed of bronchioles, alveolar ducts, and alveolar sacs, including generation 17–23 of Weibel's tracheobronchial tree (Figure 1).

[4] Type I pneumocytes are extremely thin epithelial cells lining the alveoli of the respiratory tree. Although there as many type II pneumocytes as type I within each alveolus, type I occupies up to 90% of the surface area due to its arrangement into a squalors layer. Type I cells, joined to each other by tight junctions, form specialised connections with underlying capillaries at the thin portions of the alveolar-capillary complexes. It is at the complexes that the type I cells are in close enough proximity to the capillaries - 0.5 micrometers - to permit gaseous diffusion which is their key function (Lit. [55]).

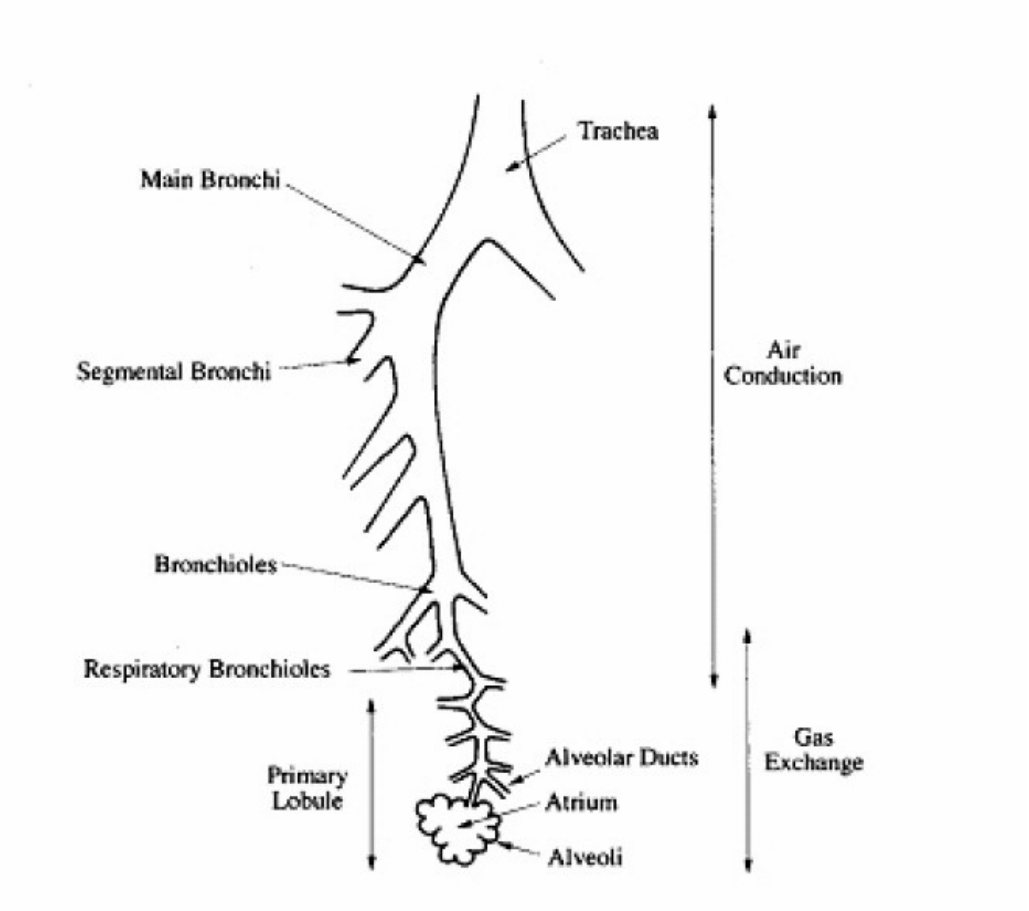

Figure 1: Tree structure of the lung (Lit. [14]).

	Generation		diameter (cm)	length (cm)	Number	total cross sectional area (cm^2)
conducting zone	Trachea	0	1.80	12.0	1	2.54
	Bronchi	1	1.22	4.8	2	2.33
	↓	2	0.83	1.9	4	2.13
	↓	3	0.56	0.8	8	2.00
	Bronchioles	4	0.45	1.3	16	2.48
	↓	5	0.35	1.07	32	3.11
	Terminal bronchioles	↓ 16	↓ 0.06	↓ 0.17	↓ $6{\cdot}10^4$	↓ 180.0
transitional and respiratory zones	Respiratory Bronchioles	17	↓	↓	↓	↓
		18				
		19	0.05	0.10	$5{\cdot}10^5$	10^3
	Alveolar ducts	20	↓	↓	↓	↓
		21				
		22				
	Alveolar sacs	23	0.04	0.05	$8{\cdot}10^6$	10^4

Figure 2: Schematic representation of the lung according to the model described by Weibel (Lit. [55]).

The respiratory gases circulate from air to blood and vice versa through 140 m^2 of internal surface area of the tissue compartment. This gas-exchange tissue is called the pulmonary parenchyma.
It consists of 130,000 lobules, each with a diameter of about 3.5 mm and containing approximately 2200 alveoli. The terminal bronchioles branch into approximately 14 respiratory bronchioles, each of which then branches into the alveolar ducts (Figure 2). The ducts carry 3 or 4 spherical atria that lead to the alveolar sacs supplying 15–20 alveoli. Additional alveoli are located directly on the walls of the alveolar ducts and are responsible for approximately 35% of total gas exchange.
It has been estimated that there are 300 million alveoli in an adult human lung. The diameter of an alveolus ranges from 250 to 290 μm; its volume is estimated to be 1.05×10^{-5} ml, and its air–tissue interface to be 27×10^{-4} cm^2. For these calculations, it is assumed that the lung has a total volume of 4.8 liters and a respiratory volume of 3.15 liters and that the air–tissue alveolar interface is 81 m^2.

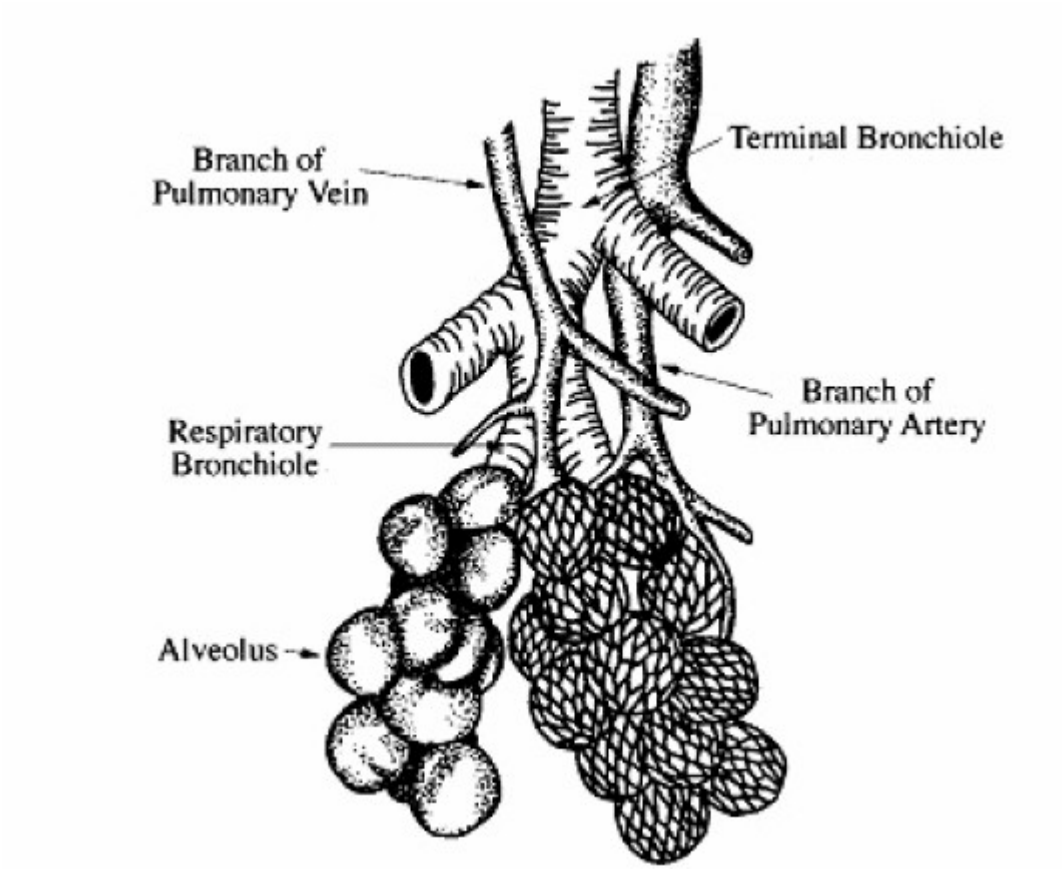

Figure 3: Structure and perfusion of the alveoli (Lit. [14]).

2.3 Barriers present in the lungs

The lungs also serve as biological barriers and defence mechanisms that protect the respiratory system. These barriers and mechanisms are listed below.

2.3.1 Pulmonary Surfactant

The elastic fibers of the lung and the wall tension of the alveoli could cause the lungs to collapse if these were not counterbalanced by the presence of the pulmonary surfactant system. This covers the alveolar surface to a thickness of 10–20 nm and is constantly renewed from the cell layer below. The surfactant is composed of 90% in weight of phospholipids, including 40–80% in weight of dipalmitoyl phosphatidylcholine (DPPC). Other main ingredients are phosphatidylcholines, phophatidylglycerols, other anionic lipids, and cholesterol (Lit. [15]).
The remaining fraction (10% in weight) is composed of 4 specific proteins: the hydrophiles SP-A and SP-C and the hydrophobes SP-B and SP-D. Enzymes, lipids, or detergents can destroy this surfactant.
If the pulmonary surfactant is removed quickly by pulmonary irrigation, no damage occurs because it is quickly replaced (half-life: 30 hours). The surfactant is only produced form the time of birth onwards, which is why premature babies (including premature caesarean babies) suffer from respiratory distress syndrome (RDS). In this case, replacement surfactants are administered to substitute for the missing natural surfactant (Lit. [16]-Lit. [18]).

2.3.2 Epithelial Surface Fluid

A thin fluid layer called the mucus blanket, 5 μm in depth, covers the walls of the respiratory tract. This barrier serves to trap foreign particles for subsequent removal and prevents dehydration of the surface epithelium by unsaturated air during inspiration. Hypersecretion of mucus is a result of cholinergic or α-adrenergic antagonists, which act directly on the secreting cells of the submucosal glands. Peripheral granules, in which mucus is stored, release a constant discharge and form a reservoir that will be secreted after exposure to an irritating stimulus. A state of disease can modify the distribution of the cell goblets and the composition of the fluids of the respiratory tracts.

2.3.3 Epithelium

The upper respiratory tract is made up of pseudostratified, ciliated, columnar epithelium cells (Lit. [19]). The bronchi, but not the bronchioles, have mucous and serous glands present. However, the bronchioles possess goblet cells and smooth muscle cells capable of narrowing the airway. The epithelium of the terminal bronchioles consists mainly of ciliated, cuboidal cells and a small number of secretory Clara cells (Figure 3). Each ciliated epithelial cell has

around 20 cilia with an average length of 6 μm and a diameter of 0.3 μm. Clara cells become prevalent in respiratory bronchioles.
In the alveolar ducts and alveoli, the epithelium is 0.1–0.5 μm thick. The alveoli are packed narrowly and do not have partitioning walls; the adjacent alveoli are separated by an alveolar septum with communication between alveoli via alveolar pores. The alveolar surface is covered with a lipoprotein film, which is the pulmonary surfactant. The alveolar surface is mainly composed of a single layer of epithelial cells—Type I alveolar cells—approximately 5 μm thick. Type II cells, cuboidal in shape, 10–15 μm thick, and situated at the junction of septa, are responsible for the production of alveolar lining fluid and the regeneration of type I cells during repair following cell damage from viruses or chemical agents.
The alveolar-capillary membrane, which separates blood from alveolar gases, is composed of a continuous epithelium, 0.1–0.5 μm thick (Figure 4). The maximum absorption occurs in the area where the interstitium is the finest (80 nm) because the pulmonary surfactant is also thin in this area (15 nm). The thickness of the air–blood barrier ranges from 0.2 to 10 μm. The most efficient gas exchange takes place if this air–blood barrier is less than 0.4μm in thickness.

2.3.4 Interstitium

The interstitium is the extracellular and extravascular space between cells in tissue. In order for a molecule to be absorbed from the airspaces to the blood, it must pass through the lung's interstitium. Within the interstitium there are fibroblasts, tough connective fibers (i.e., collagen fibers and basement membrane), and interstitial fluid, which slowly diffuses and percolates through the tissue.

2.3.5 Vascular endothelium

The endothelium is the final barrier to a molecule being absorbed from the airspace into the blood. Endothelial cells form capillaries that lie under Type I cells in the alveoli (Figure 4). The basic alveolar structure is the septum, which is composed of capillaries sandwiched between two epithelial monolayers (Lit. [20]).

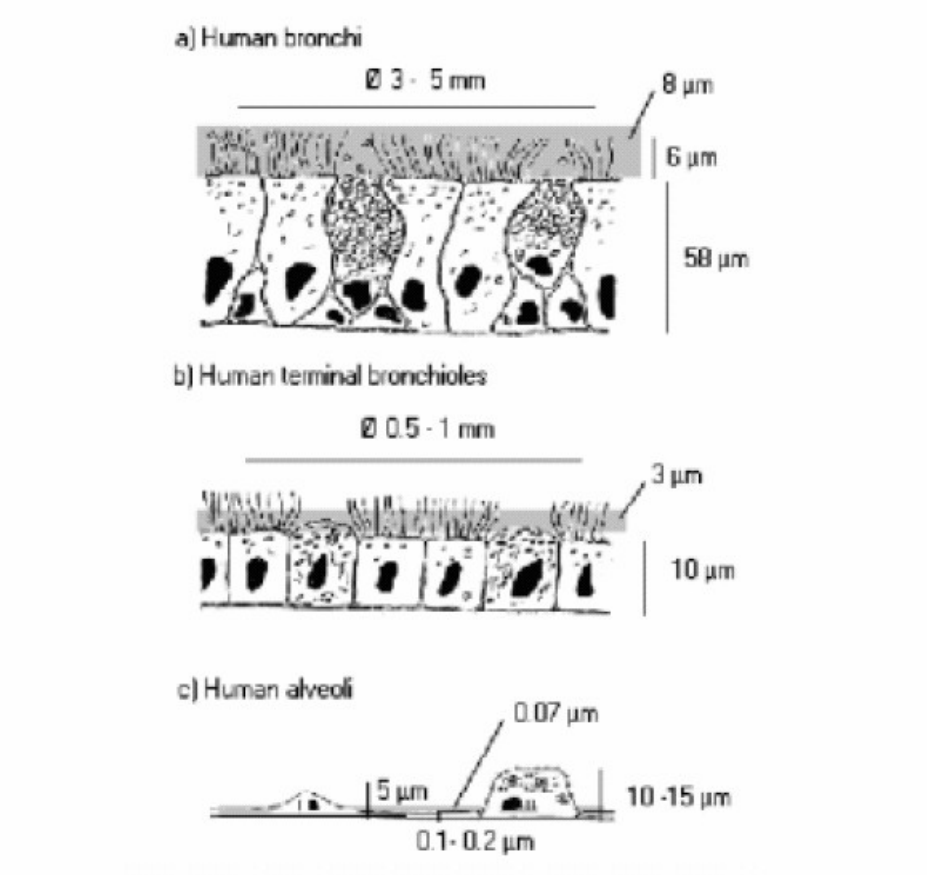

Figure 4: Typical lung epithelia in the different pulmonary regions and thickness of the surface fluid. (a) The bronchial epithelium (Ø 3–5 mm) showing the pseudostratified nature of the columnar epithelium, principally comprising ciliated cells 6 µm (c), interspersed with goblet cells (g) and basal cells (b). (b) The bronchiolar epithelium (Ø 0.5–1 mm) showing the cuboidal nature of the epithelium, principally comprising ciliated cells (c), and interspersed with Clara cells (cl). (c) The alveolar epithelium is comprise of extremely thin (Ø 5 µm) type I cell (I), which accounts for approximately 95% of the epithelial surface, and the cuboidal (Ø 10–15 µm) type II cell (II) (Lit. [20]).

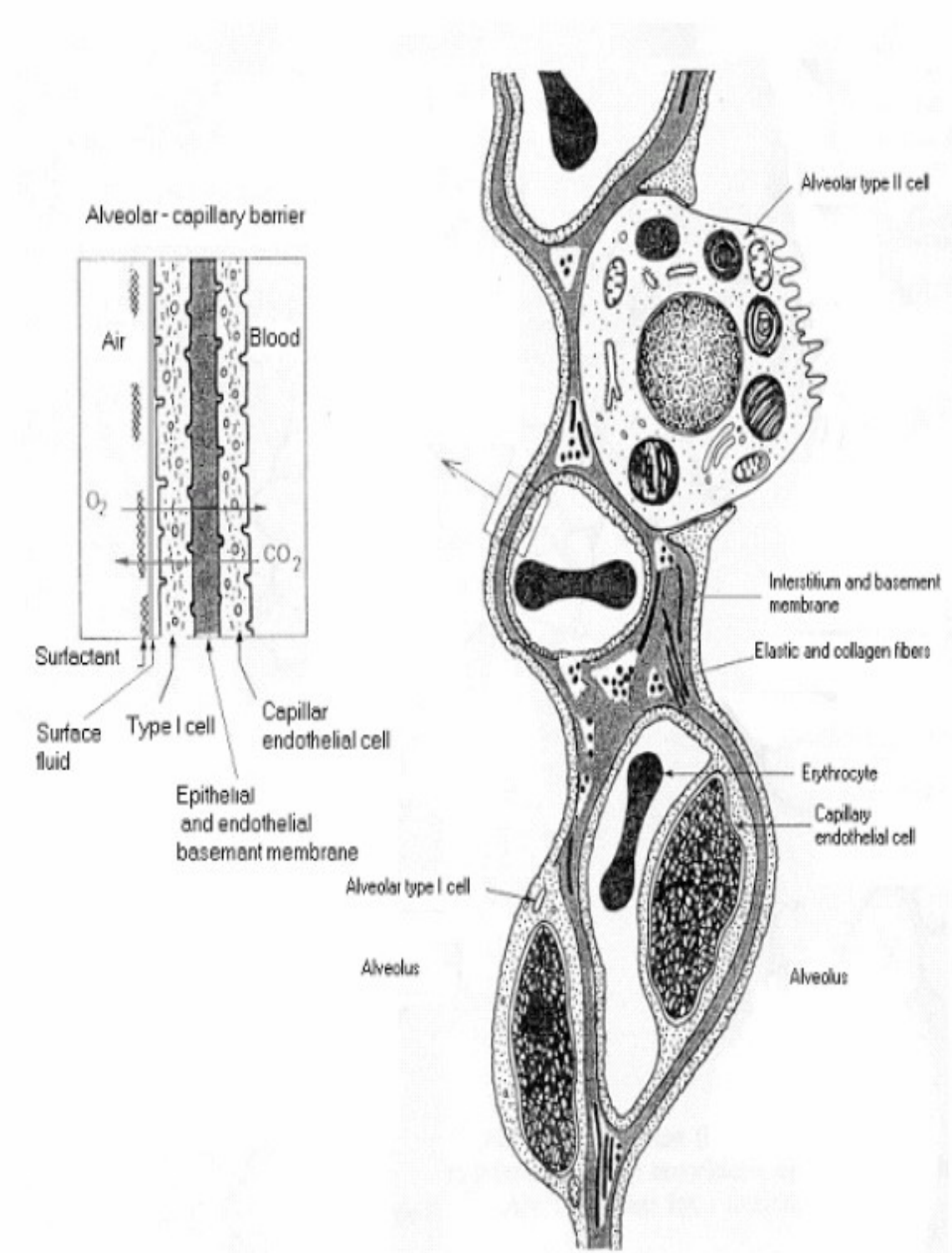

Figure 5: Alveolar–capillary membrane (Lit. [20]).

2.4 Factors Affecting Deposition of Particles

Deposition of aerosol particles in the bronchial tree depends on the granulometry of the particles and the anatomy of the respiratory tract. Aerosols used in therapy are composed of droplets or particles with different sizes and geometries. Generally, four parameters can be used to characterize the granulometry of an aerosol:

Mass median diameter (MMD) corresponding to the diameter of the particles for which 50% w/w of particles have a lower diameter and 50% w/w have a higher diameter.

Percentage in weight of particles with a geometrical diameter of less than 5 μm.

Geometric standard deviation (GSD) corresponding to the ratio of the diameters of particles from aerosols corresponding to 84% and 50% on the cumulative distribution curve of the weights of particles. The use of a geometric standard deviation to describe the particle size distribution requires that particle sizes are log-normally distributed. *If, as is frequently the case, particles are not log-normally distributed, the geometrical standard deviation is meaningless and a misleading representation of the distribution*. Heterogeneous aerosols have, by definition, a GSD of greater than or equal to 1.22 (Lit. [20]).

Mass median aerodynamic diameter (MMAD), which makes it possible to define the granulometry of aerosol particles by taking into account their geometrical diameter, shape, and density: MMAD = MMD × Density.

2.5 Mechanisms of Particle Deposition in the Airways

There are three main particle deposition mechanisms in the lung: inertial impaction, sedimentation and Brownian diffusion.
The deposition of particles administered by an aerosol in specific areas of the respiratory tract is related to the deposition mechanism along with the particle diameter (Lit [21]).

Inertial impaction is the most significant mechanism for the deposition of aerosol particles with an MMAD of more than 5 μm. It occurs in the upper respiratory tracts when the velocity and mass of the particles involve an impact on the airway. It is supported by changes in direction of inspired air and when the respiratory tracts are partially blocked. Hyperventilation can influence impaction, too.

Sedimentation occurs in the peripheral airways and concerns small particles from an aerosol with an MMAD ranging from 1 to 5 μm. Sedimentation is a phenomenon resulting from the action of gravitational forces on the particles. It is proportional to the square of the particle size (Stokes law) and is thus less significant for small particles. This kind of deposition is independent of particle motion. Sedimentation is mainly influenced by breath holding, which can improve deposition.

Brownian diffusion is a significant mechanism for particles with an MMAD of less than or equal to approximately 0.5 μm. The particles move by random bombardments of gas molecules and run up against the respiratory walls. Generally, 80% of particles with an MMAD of less than or equal to 0.5 μm are eliminated during exhalation.

The behaviour of the aerosolized particles in the body can be summarized in Figure 6.

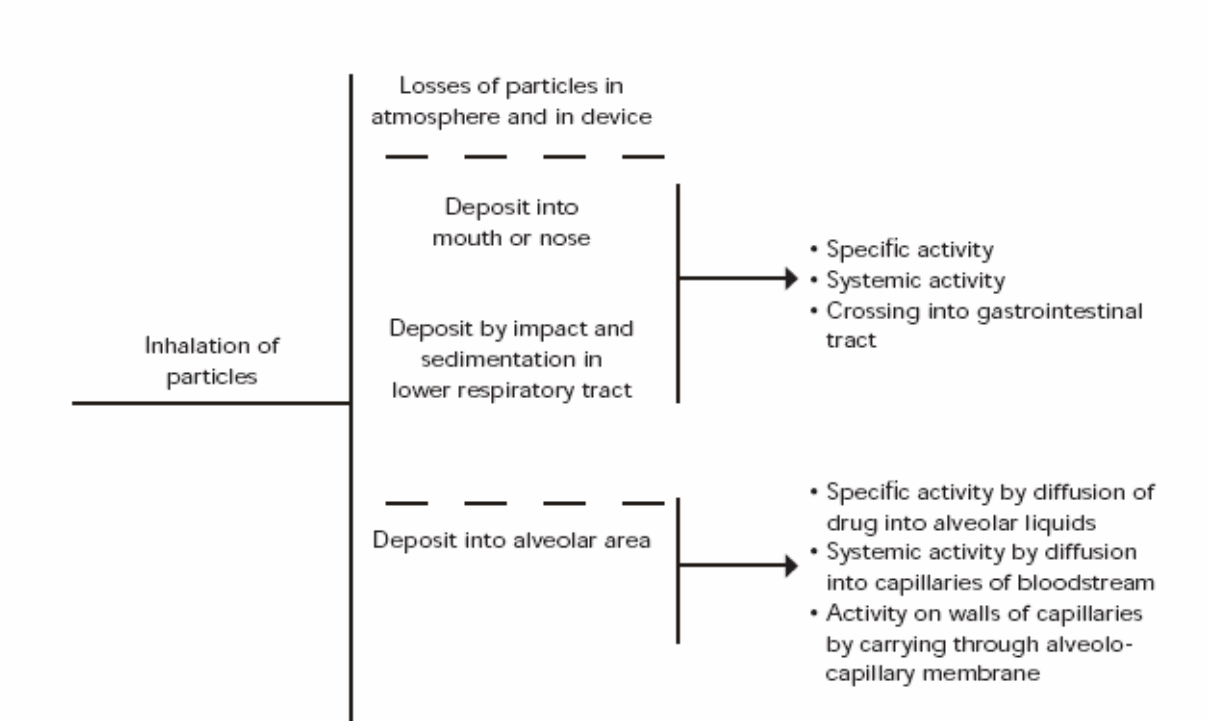

Figure 6: Behaviour of aerosolized particles into the body (Lit. [21]).

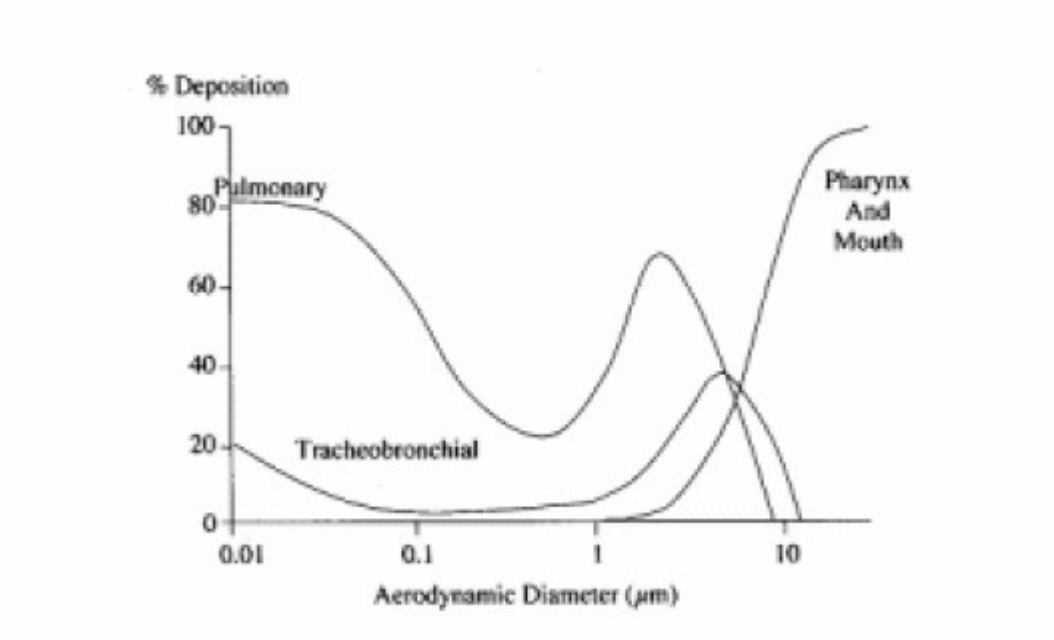

Figure 7: Dependence of deposition of particulates on particle size. (Lit. [21])

2.6 Influence of Particle Size

Big particles (>10 μm) come into contact with the upper respiratory tract and are quickly eliminated by mucociliary clearance. Particles with a diameter of 0.5–5 μm settle according to various mechanisms. The optimum diameter for pulmonary penetration was studied on monodispersed aerosols and is around 2–3 μm (Lit. [22]). Smaller particles can be exhaled before they are deposited; holding breath prevents this. Extremely small particles (<0.1 μm) appear to settle effectively by means of Brownian diffusion but are difficult to produce (Figure 6). Often the particle size does not remain constant once it reaches the respiratory tract:
Volatile aerosols become smaller with evaporation, and hygroscopic aerosols grow bigger with moisture from the respiratory tract. In addition, it has not yet been proven that the retention of inhaled particles depends on their geometric diameter (Lit. [23]).

2.7 Lung Permeability

The alveolar epithelium and the capillary endothelium have a very high permeability to water, to most gases and to lipophilic substances. However, there is an effective barrier for many hydrophilic substances of large molecular size and for ionic species. The alveolar Type I cells have tight junctions, limiting the penetration to molecules with a radius of less than 0.6 nm. Endothelial junctions are larger, with gaps of around 4–6 nm. Normal alveolar epithelium is almost completely impermeable to proteins and small solutes. Microvascular endothelium, with its larger intercellular gaps, is far more permeable to all molecular sizes, allowing proteins to flow into the systemic circulation. Pulmonary permeability increases in smokers and in states of pulmonary disease.
Soluble macromolecules can be absorbed from the lung by passing either through the cells (absorptive transcytosis) or between the cells (paracellular transport) (Lit. [24]). It has been postulated that molecules larger than ~40 kDa may be absorbed by transcytosis and then enter blood either via transcytosis in the capillary or post capillary venules; molecules smaller than ~40 kDa may directly enter the blood, primarily via the tight junctions of both the Type I cell and the capillary.

2.8 Clearance of Inhaled Particles from the Respiratory Tract

Particles deposited and not transported across the epithelium of the respiratory tract are cleared by either mucociliary clearance or a combination of mucociliary and alveolar clearance mechanisms.

2.8.1 Mucociliary clearance

The respiratory tract possesses series of defences against inhaled materials because of its constant exposure to the outside environment. The lung has an efficient self-cleaning mechanism known as the *mucociliary escalator*, in addition to other mechanisms such as coughing and alveolar clearance. The mucus gel layer (5 μm thick) floats above the sol layer, which is approximately 7 μm thick. The cilia extend through this layer so that the tip of the villus protrudes into the gel.
The coordinated movement of the cilia propels the mucus blanket and deposited foreign materials at a rate of 2–5 cm.min–1 outwards towards the pharynx, where they are swallowed. It has been estimated that 1 liter of mucus is cleared every 24 hours. Mucociliary clearance is influenced by various factors: physiological, environmental (S_2, CO_2, tobacco, etc.) and diseases (asthma, cystic fibrosis, etc.) (Lit. [25]).

2.8.2 Alveolar clearance

Particles deposited in the terminal airway units can be removed either by a nonabsorptive or an absorptive process (Lit. [26]). The nonabsorptive process involves the transport of particles from the alveoli to the ciliated region, where they are removed by the mucociliary clearance mechanism present in the conducting airway.
The absorptive process may involve either direct penetration into the epithelial cells or uptake and clearance by alveolar, interstitial, intravascular, or airway macrophages. In addition to their role in cleaning particles, macrophages also play an important part in inflammatory processes through the release of chemotactic factors to attract polymorphonuclear neutrophils from the pulmonary vascular bed to the area. Alveolar macrophages, 15–50 μm in diameter, lie in contact with the surfactant lining the alveoli. Foreign particles adhere to macrophages through either electrostatic interaction or interaction with receptors for some macromolecules, such as immunoglobulins. Following adhesion, macrophages ingest the particles by interiorization of vacuoles, surface cavitation, or pseudopod formation. The uptake of particles by macrophages is size dependent. Particles with a diameter of 6 μm are phagocytosed to a much smaller extent than those with a diameter of 3 μm. Moreover, particles with a diameter of less than 0.26 μm are minimally taken up by macrophages. The nature of the coating material also influences the rate of phagocytosis by alveolar macrophages (Lit. [27-28]).

2.9 Mechanism of absorption

The precise mechanisms of macromolecule absorption in the lungs are not well known. Most exogenous macromolecules are thought to be absorbed from the airspaces non-specifically through a combination of tight junctions and endocytic vesicles by processes that are diffusion-limited (Lit. [27]). Thus a large molecule takes longer to find its "opening" than a small molecule simply because the large molecule's random walk is at a slower pace than the small molecule. But the picture may be more complex. There is evidence that for certain endogenous molecules that normally occur in lung lining fluids, e.g. albumin (Lit.[28]),

immunoglobulins (Lit.[29]) and transferrin (Lit.[30]), there are specific receptor-mediated transport mechanisms on the alveolar epithelial cell that enable these proteins to be absorbed at higher rates than expected.

2.9.1 Small molecule drug absorption

Most small molecules that exhibit some water solubility are rapidly and efficiently absorbed from the lungs. Those that are more hydrophobic are absorbed even more rapidly—within seconds to a few minutes. Those that are more hydrophilic are absorbed within minutes to tens of minutes. Lewis Schanker and colleagues, in a series of papers between 1973 and 1986, explored many facets of pulmonary drug absorption in a variety of different animals (Lit. [31]-Lit. [47]). They quantitatively analyzed the amount of radiolabeled drug absorbed across the pulmonary epithelium following intratracheal and aerosol delivery and determined the comparative rates of absorption for different classes of compounds. They compared the characteristics between different species, sexes, and ages of animals and determined the effect of various noxious agents that damage epithelia on pulmonary absorption, as described in the following chapter.

2.9.1.1 Mechanism of small molecule absorption

Putting that data of Schanker and co-workers in the context of our current understanding of the molecular basis of transepithelial transport allows one to draw certain inferences about the potential mechanisms of absorption of the different classes of molecules. Lipid-soluble compounds are rapidly absorbed presumably because they can integrate into the lipid bilayer surrounding the cells. This constitutes the "transcellular pathway" in which compounds pass from the apical to basolateral side by travelling through the cellular membrane. Lipid-insoluble compounds, by contrast, most likely traverse the epithelium via a paracellular route, in which they pass through aqueous pores in the intercellular tight junctions. Although the nature of these junctions is not fully understood, it appears that a combination of molecular weight and degree of ionization determine the rate at which molecules can pass through. The less ionized a molecule, the faster its absorption rate, because it forms fewer interactions with the proteins and lipids that line the pore. The work of Schanker and associates suggests that for small hydrophilic compounds in the range of 100 to 1,000 D, the degree of ionization is likely to dominate, whereas for larger ones the molecular weight becomes an influential factor, too (Lit. [31]-Lit. [36]).

2.9.1.1.1 Active Transport

A number of compounds (e.g., disodium cromoglycate and cycloleucine) undergo active or "carrier-mediated" transport (Lit. [47]). Their absorption rates are saturable, such that the percent dose absorbed decreases with increasing concentration. In addition, their absorption is energy-dependent and can be inhibited by other compounds that share common structural features. These molecules enter cells by binding to specific carrier proteins on the cell surface, and use an energy-driven exchange to drive uptake against a concentration gradient.

Nonetheless, these molecules do not deviate significantly from other molecules of similar lipophilicity or molecular weight in terms of their absorption characteristics (Figures 1 and 2).

2.9.1.1.2 Slow Absorption of Inhaled Small Molecules

Although the rapid absorption of small molecules has many conceivable medical uses, there are situations when one might want to slow the absorption of an inhaled small molecule, either to keep it acting longer locally in the lung, or to regulate its absorption into the body. There appear to be at least two instances in which small molecules are much slower absorbed than the data of Schanker and co-workers might suggest. Very insoluble small molecules that slowly dissolve from the inhaled particle may stick in the lung for many hours or even days. Fluticasone propionate, amphotericin B, and all-trans retinoic acid are absorbed from the lungs over a period of hours due in part to their slow dissolution rate from relatively insoluble lipophilic particles (Lit. [48]- Lit. [49]). There may also be specific interactions (e.g., amphotericin B with endogenous sterols) that help to slow absorption.
Another instance in which soluble small molecules stay in the lungs longer than expected is the case with tobramycin and pentamidine, which are absorbed over a period of several hours following inhalation in humans (Lit.[50]- Lit. [51]). Here the mechanism of retention may be related to the multiple positive charges on the molecules that bind to the ubiquitous negative charges on the surface of cell membranes.
The absorption of small molecules can also be slowed via encapsulation in slow release particles such as liposomes (Lit. [52], - Lit. [53]).

2.9.2 Hydrophobic versus Hydrophilic small molecules

Table 1 provides a summary of a subset of the different molecules studied by Schanker and colleagues (Lit. [31]-Lit. [47]). The observed trend in half-life with lipophilicity (as estimated from the octanol-water partition coefficient, log P) is captured in Figure 7.
A natural break seems to occur for compounds that are hydrophilic (lipid insoluble, log $P < 0$), and lipophilic (lipid soluble, log $P > 0$). Hydrophilic materials cluster around a mean half-life of about 1 hour, whereas lipophilic drugs cluster around a half-life of about 1 minute. Interestingly, there does not seem to be a strong dependence of the half-life with log P, other than the clustering which occurs for the two classes of molecules. That is, decreasing log P from 0 to –5.0 results in little deviation in the measured half-life. Similarly, increasing log P from 0.0 to 4.0 results in no discernable difference. Absorption of lipophilic molecules appears to be nonsaturable over a wide concentration range. Certain molecules classified as lipid-soluble by Schanker and co-workers are absorbed more slowly than might be expected, based on their classification. These include tetracycline, sulphaguanidine and ethambutol. Each of these molecules had a log $P < 0$ indicating that they possessed significant hydrophilic character. Erythromycin was also absorbed more slowly than one might expect despite its log P value of 3.06. Erythromycin and other macrolides have been shown to interact with phospholipids, and such an interaction with lung lipids may slow down absorption of this class of antibiotics through the lung.

Table 1: The effect of molecular weight and lipophilicity on the rate of absorption of small molecules following intratracheal instillation in rats (Lit. [31]-Lit. [47]).

Compound	Class	Molecular Weight (D)	log P	$t_{1/2}$ (min)	k (min^{-1})
Guanidine	Lipid insoluble	59	–3.56	6.3	0.11
Urea	Lipid insoluble	60	–2.11	4.7	0.147
AIB (-aminobutyric acid)	Lipid insoluble	103	–2.54	57	0.012
Erythritol	Lipid insoluble	122	–2.29	35	0.02
N-methylnicotinamide	Lipid insoluble	136	0	50	0.014
Mannitol	Lipid insoluble	182	–3.1	60	0.012
p-Aminohippuric acid	Lipid insoluble	194	–0.89	41	0.017
3-o-methyl-D-glucose	Lipid insoluble	194	–1.69	58.7	0.012
Decamethonium	Lipid insoluble	258	–4.78	90	0.008
Sucrose	Lipid insoluble	342	–3.7	84	0.008
Cyanocobalamin (Vitamin B_{12})	Lipid insoluble	1,355	3.57	190	0.0036
Heparin	Lipid insoluble	6.000–20.000	4.31	552	0.0013
Dextran	Lipid insoluble	20,000		668	0.001
Amitrole	Lipid soluble	84	–0.86	1.3	0.533
Isoniazid	Lipid soluble	137	–0.7	1.9	0.365
Salicylic acid	Lipid soluble	138	2.26	1.0	0.693
Barbital	Lipid soluble	184	0.65	0.93	0.745
Sulphaguanidine	Lipid soluble	218	–1.22	41	0.017
Pentobarbital	Lipid soluble	226	2.1	1.0	0.693
Phenobarbital	Lipid soluble	232	1.47	1.0	0.693
Ethambutol	Lipid soluble	232	–0.41	40	0.017
Procainamide	Lipid soluble	235	–0.55	2.3	0.301
Sulphamethoxypyridazine	Lipid soluble	280	0.32	1.0	0.693
Sulphadimethoxine	Lipid soluble	310	1.63	1.0	0.693
Hydrocortisone	Lipid soluble	362.5	1.61	1.0	0.693
Dexamethasone	Lipid soluble	392.5	1.83	1.7	0.408
Tetracycline	Lipid soluble	444	–1.3	14	0.05
Erythromycin	Lipid soluble	734	3.06	6.3	0.11
Digitoxin	Lipid soluble	765	1.85	0.3	2.31
Digoxin	Lipid soluble	781	1.26	1	0.693

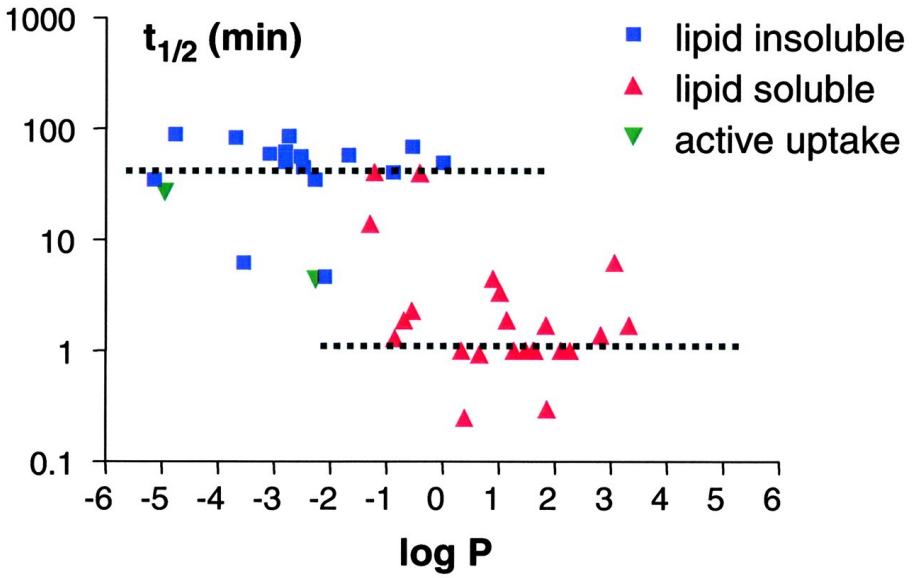

Figure 8: Pulmonary absorption data from Schanker and colleagues (Lit. [31]-Lit. [47]), demonstrating that the rate of drug absorption from the lungs is dependent on drug lipophilicity. Here, t1/2 represents the time taken for absorption of 50% of the initial dose through the lung following intratracheal administration in rats, and log P represents the octanol-water partition coefficient. Molecules characterized by Schanker and co-workers as lipid-insoluble or as lipid-soluble are shown as squares and triangles, respectively. Molecules with active uptake are denoted by inverted triangles.

It is interesting that within the molecular weight range from about 100 to 1,000 g/mol, there appears to be no dependence of half-life on molecular weight (Figure 8) (Lit. [31]-Lit. [36]). Again, the data seem to be classified simply on the basis of lipophilicity. More lipophilic drugs in this range pass through the lung rapidly, whereas more hydrophilic compounds pass much slower, but still fast compared with higher molecular weight compounds. Only when the molecular weight exceeds 1,000 D does the half-life increase significantly. Schanker and colleagues examined a series of high molecular weight carbohydrates which showed -similar to peptides and proteins much slower absorption at molecular weights in the tens of thousands (Lit. [31]-Lit. [36]).

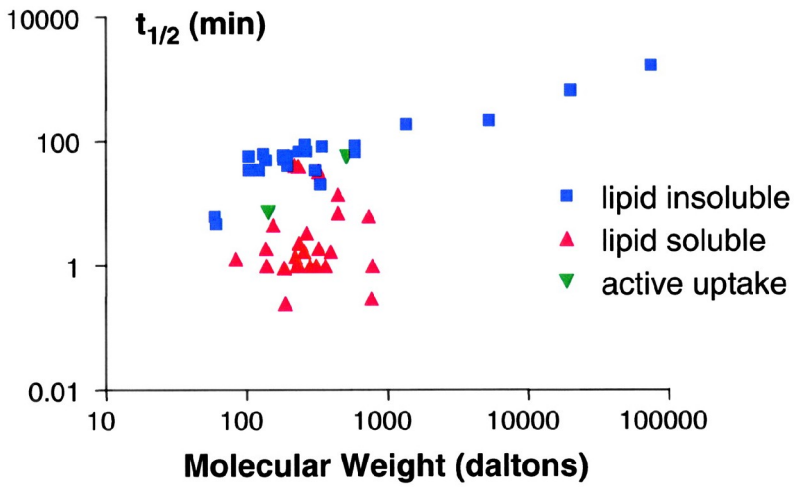

Figure 9: Relationship between molecular weight and rate of absorption through the lung (data from Schanker and co-workers (Lit. [31]-Lit. [47]). Molecules characterized by Schanker and colleagues as lipid-insoluble or as lipid-soluble are shown as squares and triangles, respectively. Molecules with active uptake are denoted by inverted triangles. (1Dalton=1 g/mol).

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2.10 Technological Problems

Pulmonary drug administration imposes strong requirements on the delivery device. This is due to the fact that the particle size of the powder or droplet greatly influences the delivery site, and thus the degree of drug absorption from the lungs.
The devices that are currently available for pulmonary drug administration were mostly developed to achieve local effects of the drug in the conducting airways, such as in asthma. These devices include:

a) Nebulisers,
b) Metered-dose inhalers (MDIs),
c) Dry-powder inhalers (DPIs).

The basic function of these three completely different devices is to generate a drug-containing aerosol cloud that contains the highest possible fraction of particles in the desired size range.

2.10.1 Nebulisers

Nebulisers are applied to aerosolize drug solutions or suspensions. There are two basic types: the *air jet* and *ultrasonic nebuliser*. The jet nebuliser is powered by compressed air while the ultrasonic nebuliser receives the energy required to aerosolise drugs from high frequency sound waves.

2.10.1.1 Jet nebulisers

Jet nebulisers have a two-fluid nozzle for atomizing the drug solution. Compressed air passes through a narrow hole and entrains the drug solution from one or more capillaries mainly by momentum transfer. The liquid break-up process depends on the design of the nozzle, the air pressure and the physical properties of the drug solution. Droplets in the required size range are entrained by the airflow from the nozzle.
Larger droplets impact on a baffle and are returned to the reservoir. Auxiliary airflows, generated by the patient, may pass through special vents to the nebulisation cup in order to improve droplet entrainment from the nozzle area.

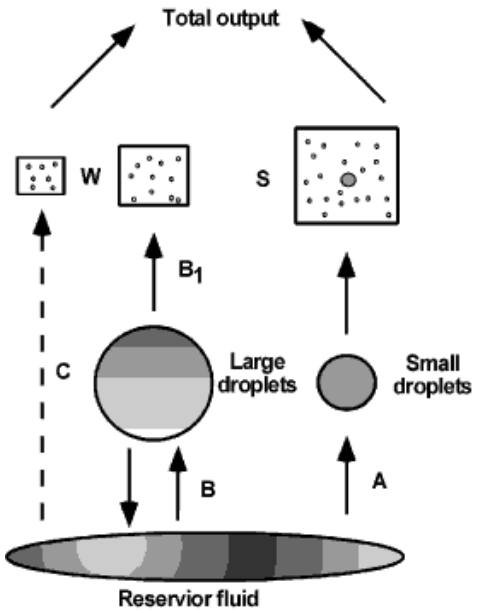

Figure 1: Schematic diagram of a jet nebuliser output. Droplets small enough will be carried out by air stream (route A), and the large droplets will be either recycled to the nebuliser reservoir (route B) or carried out by the outgoing air stream (route B1). Some of the solvent will be evaporated (route C). Adapted from Lit. [9].

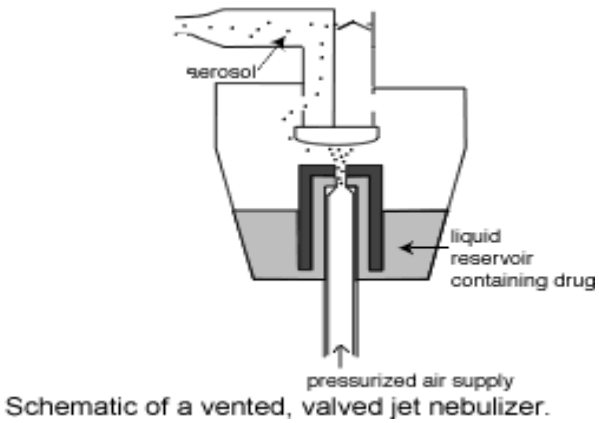

Figure 2: Schematic view of a jet nebuliser.

Figure 3: Image of PARI TurboBOY N jet nebuliser.

2.10.1.2 Ultrasonic nebulisers

Ultrasonic nebulisers use ultrasonic energy to convert liquid, usually an aqueous solution, into an aerosol for inhalation. The energy required to atomize a liquid is produced by a piezoelectric crystal transducer, usually a synthetic ceramic material, vibrating at a high frequency (1–3MHz). The frequency and the properties of the drug solution determine the droplet size distribution of the mist.
When an alternating electric current is applied, the crystal shrinks and expands and the resultant vibrations are transmitted to the nebulized fluid, either directly or via a coupling liquid, usually water. A fountain of liquid is produce at the liquid surface, with large droplets being emitted from its apex and a mist of small droplets being produced from the lower part. Figure 4 illustrates the two mechanisms proposed for the processes of liquid disintegration and aerosol generation within ultrasonic nebulisers.

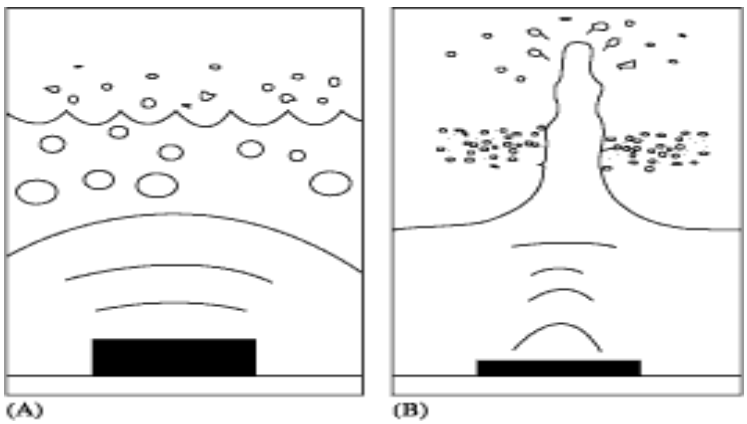

Figure 4: Proposed ultrasonic nebuliser aerosolization mechanisms. (A) Cavitation bubble formation at low frequency; (B) capillary-wave formation at high frequency (Lit. [10]).

Ultrasonic nebulisers exist in a number of basic designs that differ in the configuration of the piezoelectric crystal transducer, nebuliser chamber, baffles and auxiliary airflow systems (see Figure 5).

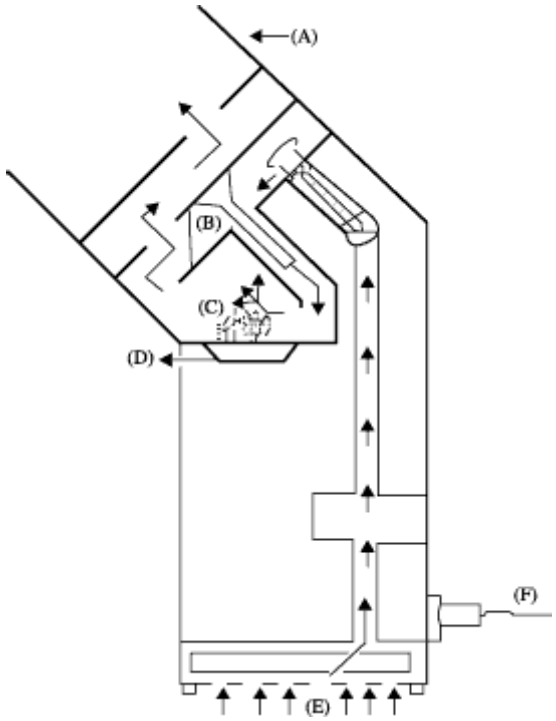

Figure 5: Schematic diagram of a typical ultrasonic nebuliser. (A) Face mask or mouthpiece; (B) Baffles; (C) Geyser of respiratory solution or suspension; (D) Piezoelectric crystal; (E)Internal fan; (F) Battery or electrical source. (Lit [10]).

Once the aerosol cloud is generated from the nebuliser fluid, it is transferred from the chamber and made available to the patient. Ultrasonic nebulisers produce a large number of droplets per unit volume, which tend to aggregate and settle in the absence of air circulating through the device. Larger droplets impact on the baffles or internal surfaces to return to the reservoir surface for recirculation under the influence of gravity.
Smaller droplets leave the device aided by an internal fan (e.g., Medix Electronic®, Easimist®) or by entrainment into the inspiratory flow of the patient (e.g., DeVilbiss Pulmosonic®).
Air velocity over the reservoir surface may be modified by fan speed (and flow constrictors), thereby influencing both droplet size and aerosol output rate. For instance, changing the fan speed setting of the Sonix 2000® (Medix) ultrasonic nebuliser can vary flow output between 1ml per 1min and 1ml per 6 min. So flow output is strongly dependent on device construction. The important design features influencing mass output and the particle size of the generated aerosols are summarized in Table 1.

Table 1: Design features of ultrasonic nebulisers determining particle size distribution and mass output (Lit. [11]).

Design features	Nebuliser characteristics
Piezoelectric crystal	Frequency of vibration
	Amplitude of vibration
	Surface morphology (flat or curved)
	Coupling between crystal and fluid
Fluid reservoir	Size
	Shape
	Baffles
Auxiliary air flow	Velocity

Whereas air-jet nebulisers are usually disposable or sterilizable, ultrasonic nebulisers are too expensive to be produced as disposable units and are thus used repeatedly, running the risk of bacterial contamination.
Cleaning nebulisers and connecting tubing is difficult, and the transfer of Gram-negative bacteria between nebulisation equipment and patients has been reported (Lit. [12] - Lit. [14]).

Although most ultrasonic devices share a basic design, some novel devices have been developed. For example:

a) The Standard Ultrasonic Nebuliser (Shimed Inc.):

Figure 6: Image of the Standard Ultrasonic nebuliser from Shimed Inc, Taiwan.

b) Bespak Piezo Electric Actuator® (Bespak plc) is a novel aerosol delivery system based on a piezoelectric crystal combined with an electroformed mesh.

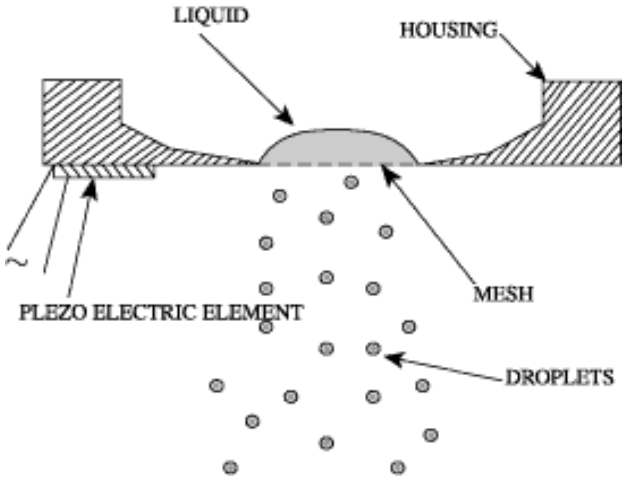

Figure 7: Schematic diagram of the spray head of the Bespak Piezo Electric Actuator. (Reproduced courtesy of Bespak plc, UK.)

Nebulisers represent ideal delivery systems for drugs that cannot be conveniently formulated into metered-dose inhalers or dry powder inhalers or when the therapeutic dose is too large for delivery with these systems.
Nebulized drugs may be inhaled during normal tidal breathing through a mouthpiece or face mask, permitting their use for patients, such as the hospitalized, the elderly, children and patients with arthritis, who experience difficulties with other devices.
However, the use of nebulisers has important limitations due to drug sensitivity: For example, drugs are often very unstable in aqueous solutions, and easily hydrolyzed. In addition, the process of nebulisation exerts high shear stress on the compounds, which can lead (in the case of proteins) to protein denaturation. Also, the droplets produced by nebulisers are rather heterogeneous, which results in very poor drug delivery to the lower respiratory tract.

2.10.2 Metered Dose Inhalers

Metered dose inhalers are pharmaceutical delivery systems designed for oral or nasal use, which can deliver aerosolised drugs to the respiratory tract. Typically, the MDI contains the active substance, dissolved or suspended in a liquefied propellant system held in a pressurized container that is sealed with a metering valve. Actuation of the valve discharges a metered dose of medicament as an aerosol spray through an actuator during oral or nasal inhalation[5].
The release of the liquid causes a fast evaporation of the propellant that produces a finely dispersed aerosol spray. The deposition, and hence the clinical efficacy, are therefore dependent on the mass of inhaled particles, which must have an appropriate aerodynamic size, usually below 5 µm, to be deposited in the lungs.

[5] MDIs are able supply to several hundred actuations, containing normally from about 10 to 500 µg of drug dispersed in a 25 to 100 µl metered volume of liquid.

Despite their apparent simplicity in use, MDIs are complex devices involving the integration of formulation, container, metering valve and actuator (see Figure 9). Changes to any one of these components will affect the overall performance of the MDI, which is designed to ensure that the delivered dose and the particle size distribution of the drug in the aerosol spray are consistent over both the labelled number of actuations in the MDI and for the duration of the shelf-life.

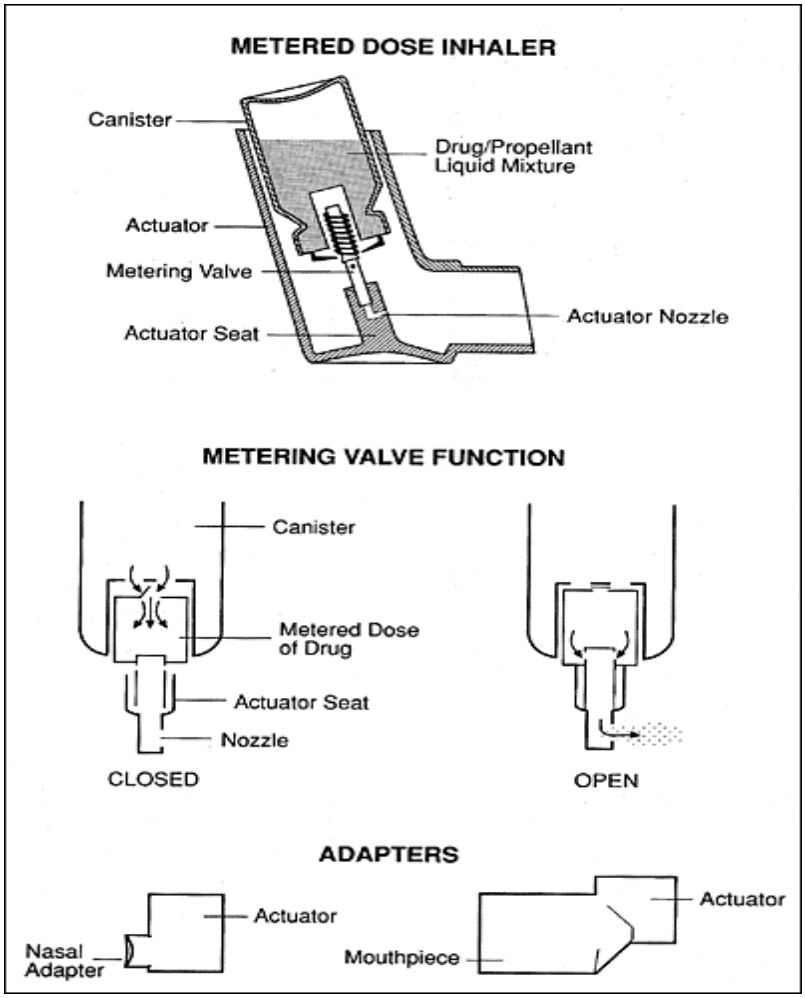

Figure 9: Sectional view of a metered dose inhaler (Lit. [6]).

The fine particle mass of a finely aerosolized drug delivered from an MDI is highly dependent on the atomization of the formulation and the subsequent spray dynamics. The aerosol characteristics depend heavily on interactions between:

- The propellant;
- The micronized drug particles;
- The formulation excipients;
- The design and dimensions of the metering valve;
- The key actuator variables (stem block, atomization orifice, actuator airflow paths, and mouthpiece design).

Figure 10: Image of the Autohaler® Inhaler from 3M Pharmaceuticals (Lit. [16]).

As discussed above, aerosol formation from MDI is a complex process influenced not only by the propellant but also by properties of the formulation and device design (e.g., valve volume and orifice size).
After aerosol droplets form at the exit of the spray nozzle, an aerosol plume begins to expand, and large particles travel along the axis of the actuator. Figure 11 shows photographs of aerosol formation using a commercially available MDI.

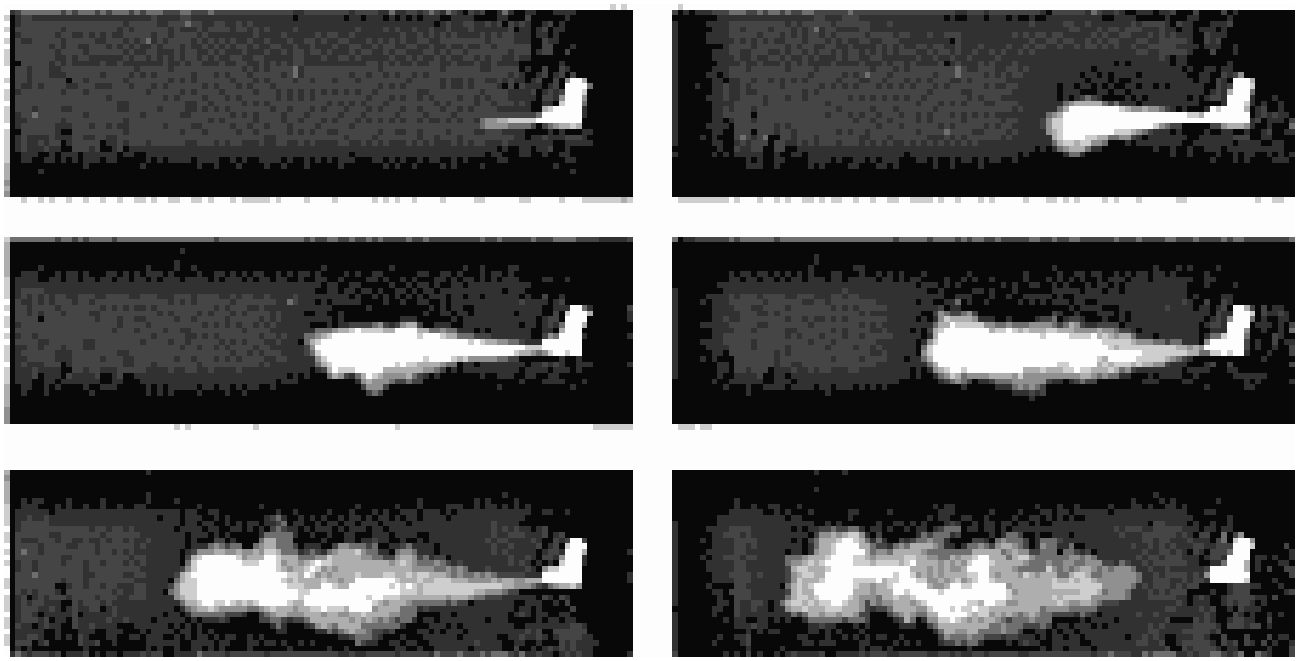

Figure 11: Formation of the aerosol plume as a function of time. Each frame has a time interval of 30 milliseconds(Lit. [17]).

The propellant or propellant mixtures used in the MDIs provide the energy necessary to generate a fine aerosol of drug particles suitable for delivery to the lungs or nasal cavity. Liquefied compressed gases are preferred over nonliquefied compressed gases such as nitrogen or carbon dioxide due to the following advantages in the inhalation therapy:

The discharge of defined aliquots of propellant from the MDIs will undergo flash evaporation to give an aerosol of very small particles.

The pressure inside the MDIs should remain consistent throughout the use of the entire contents, thus ensuring that the aerosol characteristics remain uniform during repeated discharges. At constant temperature, the vapour pressure remains constant while liquefied propellant remains. In contrast, aerosols generated using nonliquefied compressed gases coarsen during emptying of the MDI due to the decrease in gas pressure.

The ideal propellant for use in an MDI will exhibit the following properties (Lit. [7]):

- Non-toxic;
- Inert and non-reactive in the formulation;
- Chemically stable under a range of conditions;
- High purity;
- Acceptable taste and odour;
- Compatible with the packaging components (can, valve, actuator);
- Suitable vapour pressure;
- Suitable density to facilitate suspension stability;
- Suitable solvency properties;
- Preferably non-flammable;
- Acceptable cost.

The search for propellants of low or zero ozone depletion potential has led to the identification of a number of potential compounds.
Of the alternatives identified, the Hydrofluoroalkanes (HFCs) were targeted for development as replacements for the Chlorofluorocarbons (CFCs) in MDIs. Within this class, 134a and 227ea were adopted for inhalation after toxicity testing by two consortia of pharmaceutical companies: IPACT 1 for 134a and IPACT 2 for 227ea (IPACT: International Pharmaceutical Aerosol Consortium for Toxicity Testing)[6].

Although the MDI is simple to use for most people, there are certain sectors of the patient population that may have difficulties with the use of this device type. For example, very young children and older people have significant difficulty in coordinating their actuation of the device with breathing in.
Patients who have this coordination difficulty can, for example, be supplied with a dry powder inhaler, where the inspiration through the device is also responsible for the release and aerosolization of the powdered drug.

In addition, MDIs utilize propellants (CFCs and, increasingly, HFCs) to atomize the drug solution, which results in a more uniform spray than that achieved with nebulisers. However, proteins and peptides are susceptible to denaturation when they come into contact with these

[6] See Appendix 1.for a more detailed description.

propellants or with the large air–liquid interfaces that are constantly being generated during aerosolization (Lit. [1]).

The MDI technology is also limited by the drug dose that can be administered. The normal dose range for a MDI is between of 50 – 500 µg / actuation. Smaller doses may cause problems regarding dose uniformity. On the other hand, doses above 500 µg cannot be delivered with an acceptable percentage of respirable particles. Generally, increasing the dose/actuation above 500 µg is usually related with a decline of the fine particle fraction. This is especially clear with hygroscopic drugs such as disodium-cromoglycate.

2.10.3 Dry powders inhalers

A promising alternative to MDIs and nebulisers are the DPIs. Like the MDIs, most DPIs that are currently approved are made for pulmonary drug administration of locally acting drugs used for the management of asthma and chronic obstructive pulmonary diseases (COPDs), such as anti-asthmatic agents[7].

These devices differ not only in their forms of particle generation and delivery, but also with regard to design differences such as discrete or reservoir drug containment, the number of doses and the presence of a dose counter.

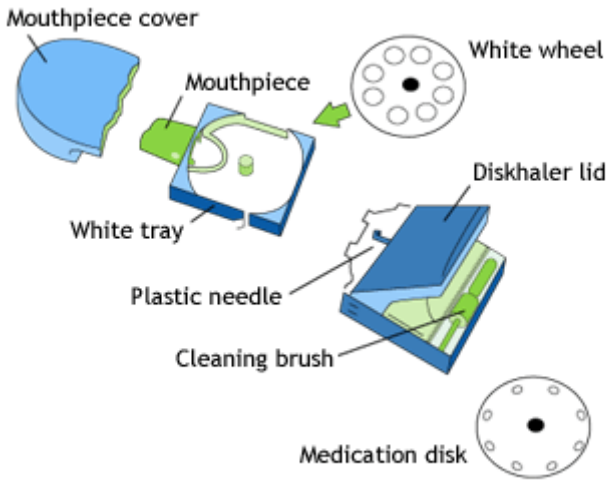

Figure 11: Image of the Autohaler® Inhaler from GlaxoSmithKline Pharmaceuticals (Lit. [18]).

[7] Examples of such devices include the Turbohaler (AstraZeneca, Wilmington, DE, USA), Diskhaler (GlaxoSmithKline, Research Triangle Park, NC, USA), Diskus (known as the Accuhaler in some countries, for example the UK, GlaxoSmithKline), Rotahaler (GlaxoSmithKline) and Aerolizer (Novartis Pharma, Basel, Switzerland).

Table 2 presents some of the most recent devices used in the field of DPI, most of them still focusing on local delivery of small molecule drugs to the airway for the treatment of asthma or COPDs. Nevertheless, some are also being used in clinical trials for systemic delivery of macromolecules such as insulin via the deep lung.

Table 2: More recent dry powder inhalation systems.

Name	Manufacturer	Packaging/metering	Energy source(s)
Pulvinal	Chiesi	Reservoir	Mechanical, patient inspiration
Easyhaler	Orion	Reservoir	Mechanical, patient inspiration
Clickhaler	ML Labs	Reservoir	Mechanical, patient inspiration
Discus	Glaxo	Multidose blister	Mechanical, patient inspiration
Monohaler	Astra	Unit dose	Mechanical, patient inspiration
AIR[b]	Alkermes	Unit dose	Mechanical, patient inspiration
Spiros[b]	Dura	Multidose blisters	Mechanical, not driven by patient inspiration
Inhance™	Inhale	Unit dose	Mechanical, not driven by patient inspiration

[b]In clinical trials; not yet available on the market.

Dry powder inhalers are generally described as ‘breath actuated’ devices, because the inspiratory air stream releases the dose from the dose system and supplies the energy for the generation of fine drug particles from the powder formulation. Because the efficiency of dose release and powder disintegration increases with increasing inspiratory flow rate for most DPIs, these devices would be better described as ‘breath controlled’ devices.

Basically, devices used as dry powder inhalers contain four basic functional elements:

- **Powder container**: Dry powder inhalers may contain the dry powder formulation in many different forms. The first DPI, the Spinhaler™ contained single doses in capsules. Other systems, like the Diskus™ or Diskhaler™ may contain the metered dose in blisters, whereas systems like the Turbohaler™, or Novolizer™, have multi-dose containers.
- **Dosing system**: It can be a Unit-dose, Multidose or Reservoir system.
- **Disintegration principle**: In general, the powders in the inhaler are not formulated as single particles, but as adhesive mixtures or spherical pellets. These mixtures or pellets are suitable for processing and metering. However, the particle size of these mixtures or pellets is far too large for lung deposition. Therefore, the pellet or mixture has to be disintegrated to make an aerosol cloud with the desired particle size (< 5 µm). Many different disintegration principles exist. They may vary from a simple screen

(Rotahaler™) to twisted powder channels (Turbuhaler™) or a cyclone chamber as used in the Novolizer™.

- **A mouthpiece**: The mouthpiece may be used to control the direction of the aerosol cloud in the mouth and throat, in order to reduce drug deposition in the oropharyngeal cavities.

One of the current DPIs in the market is the HandiHaler® in combination with the Spiriva® capsules (tiotropium bromide inhalation powder).[8]

The HandiHaler® inhalation device has the following components (Figure 12):

1) Dust cap;
2) Mouthpiece;
3) Base;
4) Piercing button;
5) Center chamber.

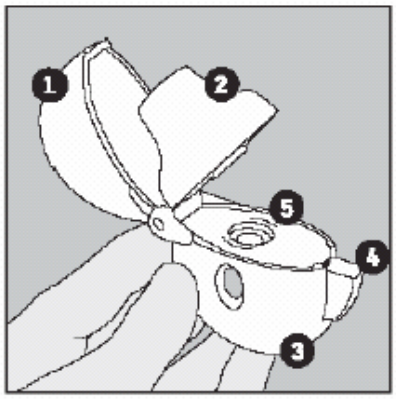

Figure 12: Schematic view of from HandiHaler® Boehringer Ingelheim International GmbH (Lit. [15]).

The normal operation procedure of a DPI is described below, using the HandiHaler® and SPIRIVA® capsules as an example. It requires the following steps:

a) Open the blister (Figure A and B).
b) Open the HandiHaler® device (Figure 1).
c) Insert the SPIRIVA® capsule and close the mouthpiece (Figure 2 and 3).
d) Press the HandiHaler® button (Figure 4).
e) Breathe out completely (Figure 5).
f) Inhale the medication (Figure 6).

[8] SPIRIVA®/ HandiHaler® are indicated for the long-term, once-daily, maintenance and treatment of bronchospasm associated with chronic obstructive pulmonary disease.

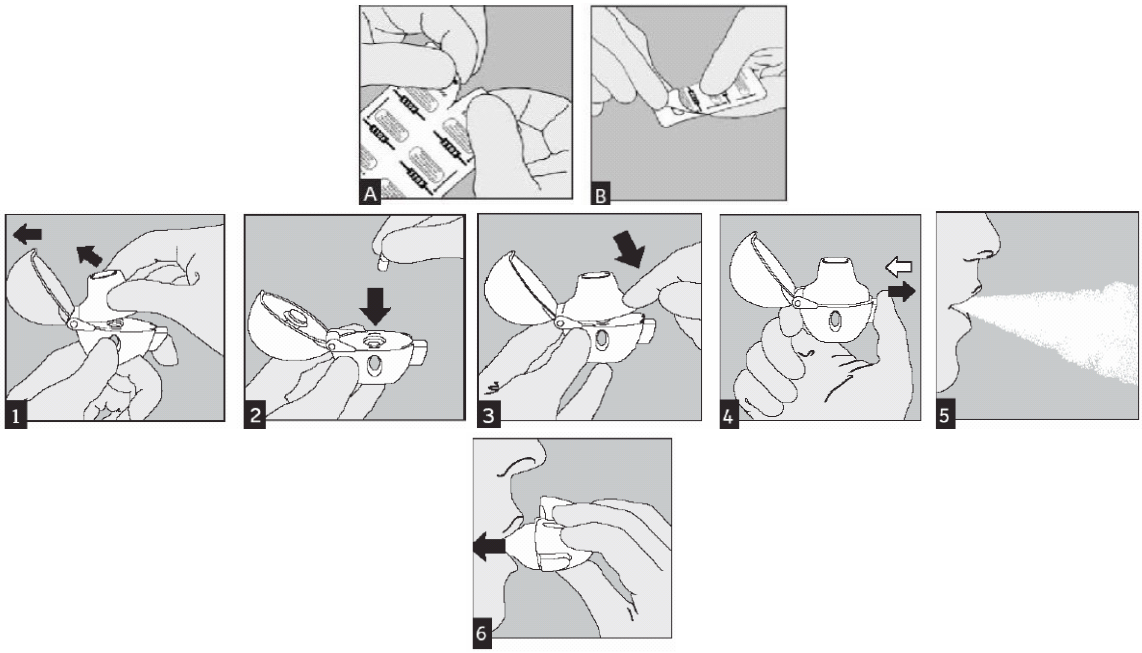

Figure 13: Operation steps in using the HandiHaler® and SPIRIVA® (Lit. [15]).

Drug delivery to the lower respiratory tract performed by these DPIs depends strongly on inspiratory air velocity. Consequently, delivery efficiency in children, elderly persons or adults with certain disease conditions will not always result in reproducible pharmacokinetics and pharmcodynamic responses with some devices. Additionally, dose-to-dose variations are greater. Furthermore, dose emission for some can be dependent on inhalation flow rates (Lit. [3], Lit. [4]).
The deposition pattern of the inhaled dry powder aerosol can be strongly influenced by the patient's inhalation dynamics and lung anatomy. At high inhalation flow rates, a given particle will have a greater tendency to impact the back of the throat or to deposit in the upper airways. For those delivery systems requiring high flow to deaggregate the powder particles, deep lung deposition is less accessible. The proliferation of device designs has been in part the result of attempts to minimize dosing variability regardless of source.

The target for lung deposition varies depending on the therapy under consideration. In the treatment of asthma by β-adrenergic agonists, the central airways are generally targeted. On the other hand, therapies intended to treat alveolar disease, chronic obstructive pulmonary disease or systemic conditions must reach the peripheral regions of the deep lung.

As a summary, table 3 presents the main advantages and disadvantages of dry powder inhalers:

Table 3: Advantages and disadvantages of dry powder inhalers.

Advantages of dry powder inhalers	Disadvantages of dry powder inhalers
Propellant free	Potential difficulties to obtain dose components uniformity
Less need for patient coordination inspiratory flow profile	More expensive
Less potential for formulation problems	Less protection from environmental effects and patient abuse
Less potential problems with drug stability parameters	-------

Subchapter conclusion

In conclusion, using the inhalation route to deliver therapeutic aerosols is a common practice in the treatment of patients with various airway diseases. Drug delivery via inhalation offers many advantages in the administration of pharmaceutical compounds, because the drugs are delivered directly to the site of action. Therefore, the required therapeutic dose for each treatment is lower than if the dose is administrated via oral or parenteral routes. Consequently, the inhalation route reduces adverse effects due to systemic absorption and intensifies the amount of drug deposited to the targeted tissue.
The delivery of drugs to the lung depends on administration by any one of three methods: nebulisers, metered-dose inhalers and dry powder inhalers.

The nature of the drug substance and its therapeutic target may influence which lung-delivery dosage form is more appropriate for the drug. For example, nebulisation is based on the fact that the drug can be well dissolved in an aqueous medium at a concentration suitable for convenient dosing.
Conversely, drugs developed for the metered-dose inhalers must be dissolved or suspended well in a nonaqueous propellant medium at a concentration appropriate for doses metered in volumes. On the other hand, for the dry powder inhalers, the physical properties of the drug substance determine the degree with which processing will give a stable powder. This powder can, in turn, be effectively aerosolized in milligram quantities by the inhaler device to deliver the proper drug dosage.

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2.11 eFlow Electronic Inhaler

Several parameters have to be taken in consideration in order to optimize drug delivery by inhalation products. The device design along with the formulation properties, play a very important role in the desired performance characteristics:

- Particle size distribution;
- Delivered dose;
- Delivery efficiency;
- Treatment time.

Nebuliser performance depends largely on their design and mode of operation (Lit. [1]-Lit. [2]). Advanced delivery efficiency was achieved by so called breath enhanced jet nebulisers using inspiratory and expiratory valves to control flow through the system and, more recently, by mechanical breath triggering (Lit. [3] -Lit. [4]).
However, new electronic aerosolisation techniques enable the use of miniaturized sensors as a mean of controlling the activation of the device (Lit. [5]-Lit. [6]).

The novel eFlow electronic inhaler (PARI GmbH, Germany) utilizes two different concepts (Lit.[7]):

- A patent protected valved aerosol chamber (Lit.[8])
- A sensor based breath controlled activation mechanism.

The eFlow® (PARI GmbH, Germany) is a novel electronic nebuliser platform, that creates the maximum benefit for the patient when the device is optimized together with the drug formulation to be administered. The ability of an inhalation device to be used in a wide range of applications, particularly with new inhaled medications, must deal with several challenges during development:

- variable viscosities of the distinct drug formulation;
- fragile molecules (like with proteins and peptides);
- suspensions;
- variable fill volumes and dosages;
- environmental issues;
- critical dose-response relations;
- need to avoid systemic and local side effects;
- patient compliance.

Nebulization of liquids is an effective means of aerosol generation for pulmonary drug delivery.
Atomisation theories predict that aerosol size and output characteristics are dependent not only upon the operating principles, conditions and mechanical construction of the nebuliser but also upon the physicochemical properties of the nebulised fluids (Lit. [14]).
Nebulisers have the ability of delivering drugs in large doses, especially in cases where, for technical reasons, formulation as metered dose or dry powders inhalers is not feasible. In addition, since the drug can be delivered during the relaxed tidal breathing, there are no atomisation or actuation synchronization requirements.
The therapeutic efficiency relies on the pulmonary penetration and deposition of the aerosol. Thus, several factors, in particular, the droplet size and mass output have a very important influence on the therapeutic efficacy.
In regard to that particle size, the respirable doses are designated as those droplets less than approximately 5 µm, which are assumed to penetrate into the peripheral regions of the lung (Lit. [6]). Several factors can influence the particle size and output characteristics of a nebuliser. These can include:

- Rate air flow (Lit.[13] -Lit. [14]);
- Intrinsic design (Lit. [15]);
- Volume of nebuliser fluid (Lit. [16]);
- Physicochemical properties of the formulation (Lit. [17]).

The aerosol quality, especially size and output characteristics, depends not only on the operating principles and conditions, mechanical construction and geometry of nebulisers, but also on the physicochemical properties, such as surface tension and viscosity of the nebulised

fluid. An aerosol can be defined in terms of the increase in the liquid surface area resulting from nebulisation. The surface area before break-up of the solution[9] is simply that of the liquid cylinder as it emerges or the free surface of the fluid mass in the chamber of the nebulisers. After atomisation, the area is the sum of the surface areas of all the individual droplets. Consequently, surface tension is important for nebulisation because it represents the force that resists the formation of new surface area. The minimum energy required for nebulisation is related to the surface area and also to the increase in liquid surface area. Therefore, surface tension forces tend to impair atomisation quality by opposing any distortion or irregularity on the liquid surface, thereby delaying formation.

Since most of the currently used pharmaceutical nebuliser solutions are aqueous (normally with low solute concentration), the surface tension and viscosity may not vary significantly.
However, surfactants are required in specific formulations to suspend the drug and these agents will evidently lower surface tension.
In accordance to Lit.[18] -Lit. [21], it was concluded that mean droplet size of liquid sprays was directly related to liquid viscosity and surface tension while the overall effect of liquid density on drop size was small. In Lit. [21] it is reported that the presence of a surface active agent (dodecylbenzene sulphonate 0.1-1.0%) influenced the spraying of aqueous solutions: In average, the drop size decreased by approximately 30% in comparison with water and the polydispersity of the system increased.

In addition to the effect of the surface tension on the aerosol droplet size, viscosity also plays an important part in the aerosol quality. In (Lit. [23]), the effect of viscosity was investigated using water, sucrose (10-60% w/v) and sodium citrate (7-36% w/v) as tests solutions. The results showed that, in the case of ultrasonic devices, the droplets size was proportional to the viscosity.
However, in this work a new electronic nebuliser (eflow®, Pari GmbH, Germany) is used to nebulize the drug formulations. It is based on a vibrating membrane principle (Lit. [24]) which differs from the commonly used jet nebulisers. For this reason, the effect of viscosity and surface tension on the eflow® nebulisation efficiency was investigated. This information will be very important as a guide to the future drug development formulations as a way of screening possible formulations.

2.12 Vibrating Membrane Principle

The working principle behind the eFlow® nebuliser is the Vibrating Membrane Principle.
The eFlow® has the ability to deliver a wide range of drug volumes (0.5–5 ml) and dosages (0.01–1000 mg), allowing the patient to take its treatment during consecutive spontaneous breathing.
The core component of the system is the aerosol head. It contains the perforated membrane and ring-shaped piezo-electric actuator which is driven by an electronic circuit to vibrate the membrane. The membrane is made of stainless steel with the holes being precisely shaped by a laser drilling process.

9 For a more detailed description of the break-up processes please refer to the chapter 2.10.

The process of generating an aerosol by the eFlow® nebuliser is explained below:

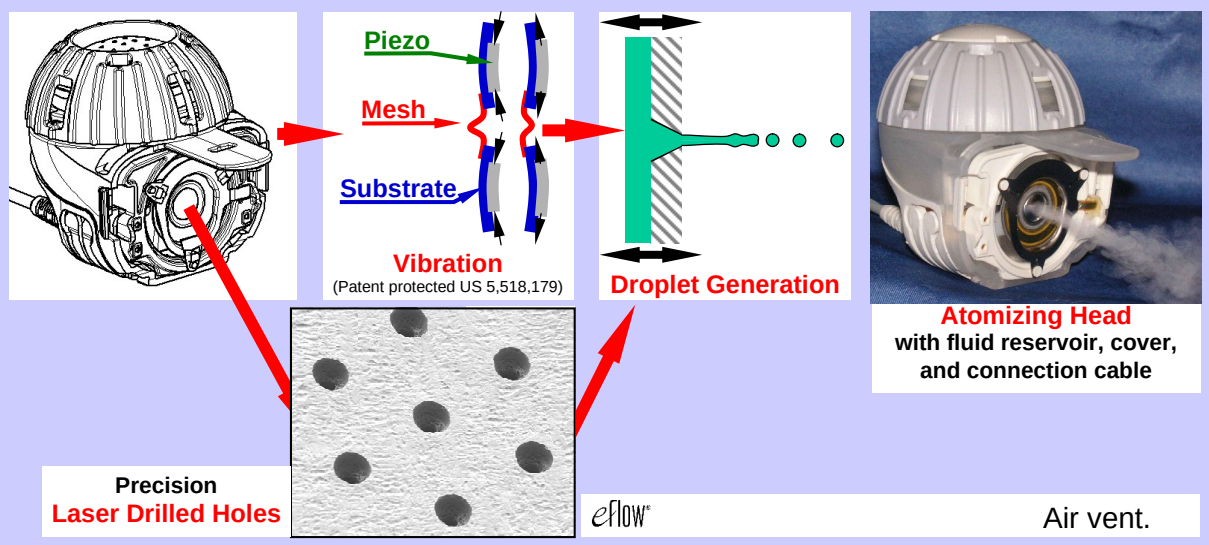


Figure 1: Aerosol generation principle.

Description:

- The atomizing head consists of an annular, metallic substrate, a thin membrane in the center and an annular piezo ceramic;
- Activation of the piezo with a high frequency current causes the membrane to vibrate;
- A patent protected “bending mode” design allows for highly efficient energy utilization;
- Several thousand holes are drilled in the centre of the membrane using a high precision laser drilling process;
- The pressure variations caused by the vibration force the fluid to be ejected from the holes on one side of the membrane thus creating well defined aerosol droplets;
- Many different parameters such as droplet size or the physical-chemical properties of the drugs, must be taken into account in order to optimize an inhalation device for a specific drug, therapy, or patient group;
- In addition, interdependencies between these factors require an increased effort in the fine-tuning;
- The optimization can be performed with respect to :
    - *A) Particle size distribution;*
    - *B) Aerosol generation rate;*
    - *C) Drug delivery rate;*
    - *D) Patient guidance;*
    - *E) Delivered dose;*
    - *F) Formulation aspects (e.g. surface tension, viscosity).*

A) Particle Size Distribution

- **MMD range:** 2.5 µm to > 5 µm
- **GSD** approx. 1.5
- **Primary parameter:** Hole exit size (Figure 2) with precise control of MMD and GSD by laser drilling process and inline quality assurance

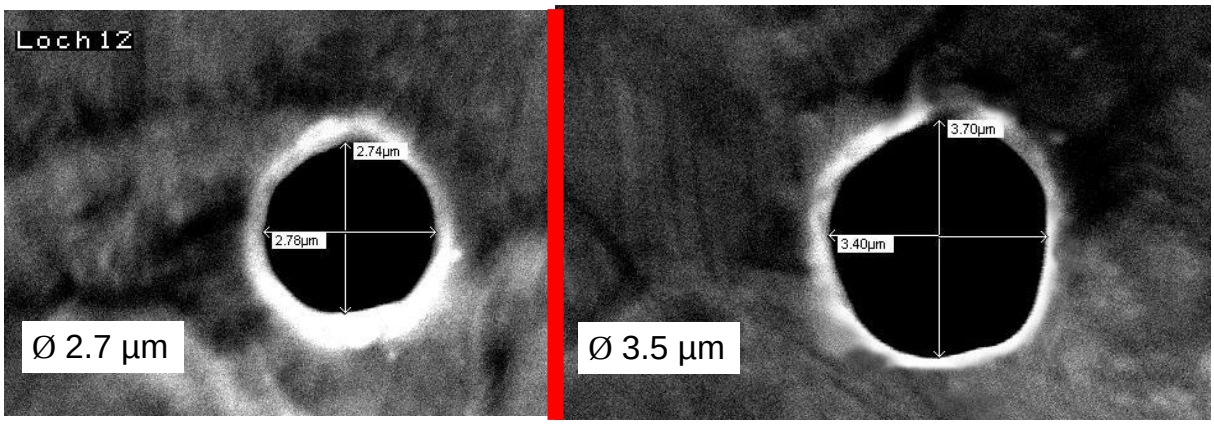


Figure 2: SEM pictures of different exit hole sizes.

B) Aerosol Generation Rate

Generation rate: 0.4 ml/min (@ MMD = 3.0 µm) to 1.2 ml/min (@ MMD = 4.5 µm)
Highly efficient aerosol generation due to "bending mode" activation

Parameters:

- Hole size;
- Number and distribution of holes (Figure 3);
- Power input to Atomizing Head;
- Design and dimensions of Atomizing Head;
- Manufacturing processes.

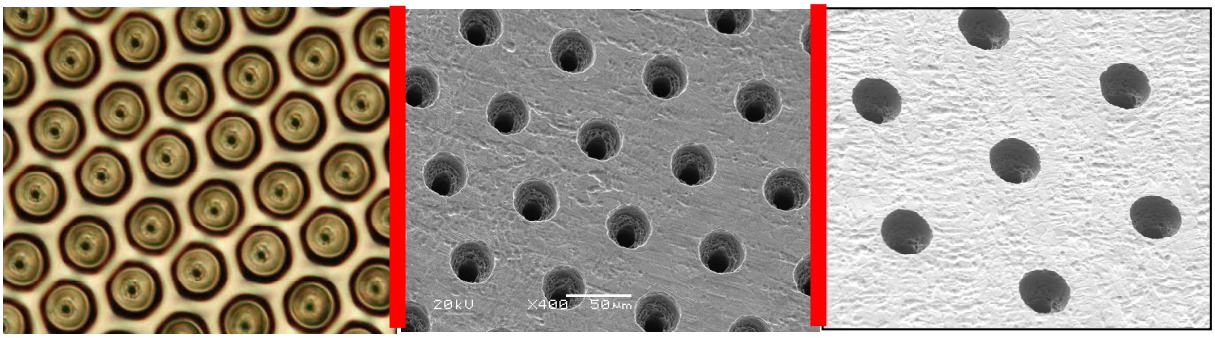


Figure 3: Pictures of different holes distributions (same magnification).

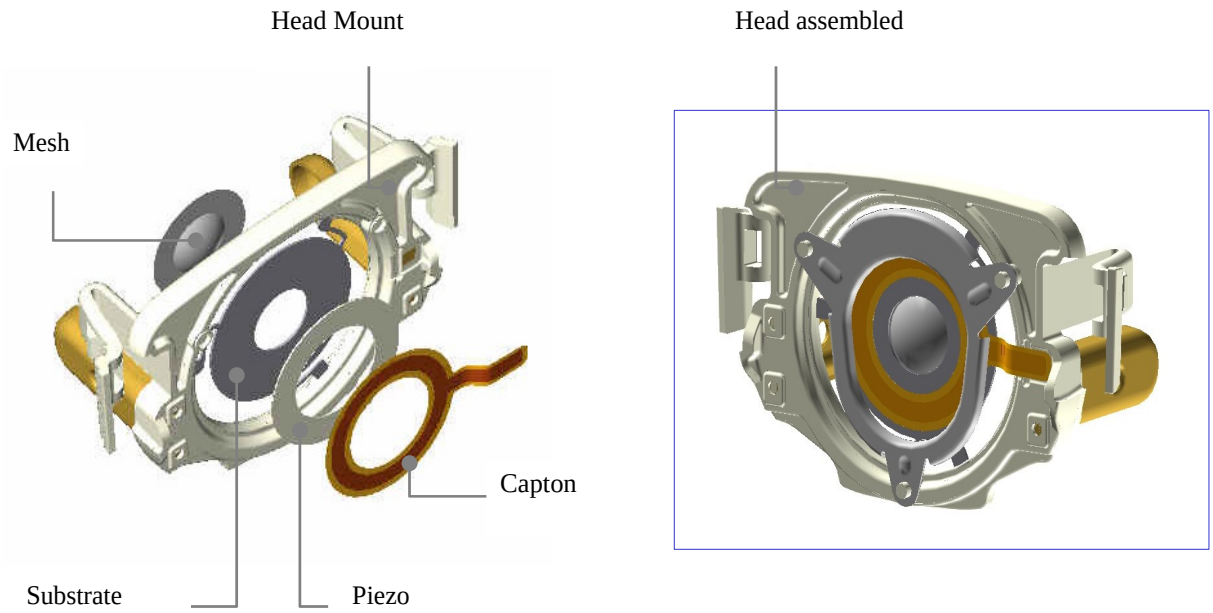


Figure 4: Different parts of the Atomizing Head.

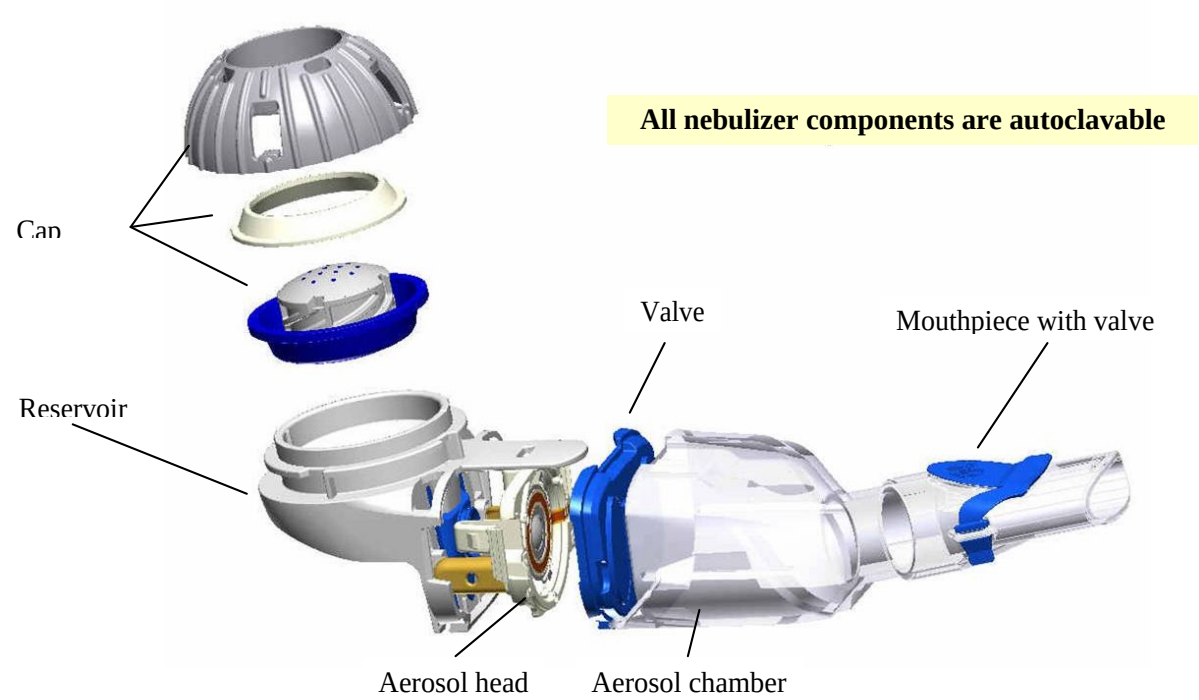


Figure 5: View of the different components of the eFlow.

C) Drug Delivery Rate (example):

32.1 mg/min (100 mg/ml Tobramycin) compared to 8.6 mg/min (60 mg/ml Tobramycin) with jet nebuliser Lit.[9]

Parameters:

- Aerosol generation rate;
- Activation mode (breath trigger vs. continuous);
- Nebuliser design (valves, aerodynamics).

D) Patient Guidance

Sensors and feedback mechanisms may increase patient compliance:

- **Passive approach:** Adaptation to breathing pattern;
- **Active approach:** Feedback to influence breathing pattern.

E) Delivered Dose

Adjustable over a wide range

Parameters (Figure 6):

- Nebuliser design (aerodynamics);
- Valve System;
- Activation mode (breath trigger vs. continuous).

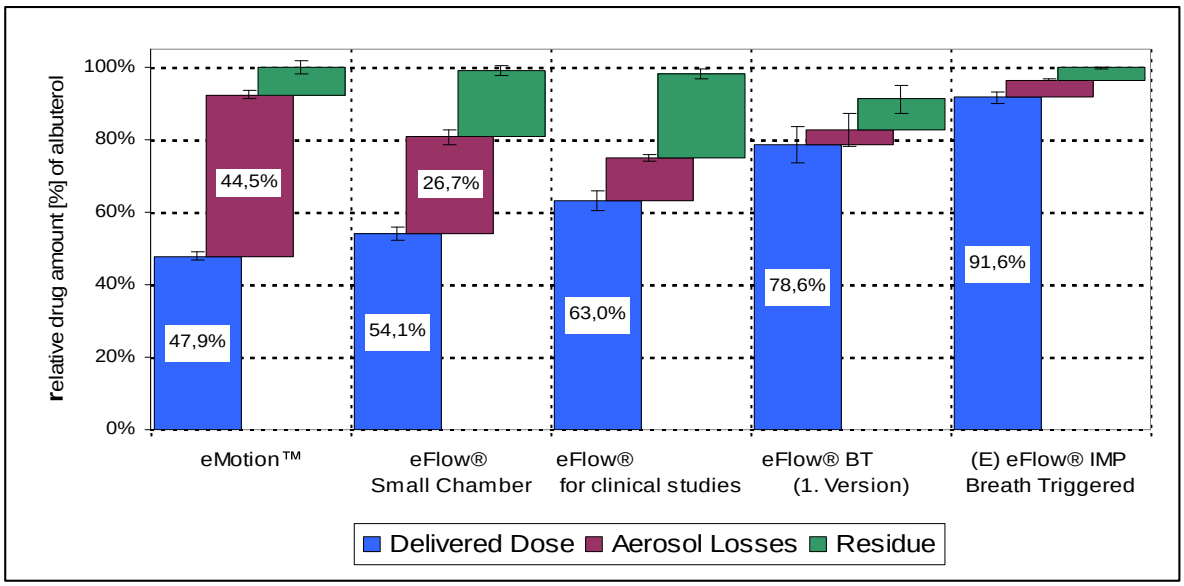


Figure 6: Drug distribution of salbutamol following nebulisation by different eFlow® devices (Lit.[10]).

Examples

eFlow® for Clinical Trials

- For customization with novel nebuliser medications (Lit.[11]).
- The device was used in several clinical studies, gamma scintigraphy deposition studies and patient acceptance tests.
- Treatment can be as fast as 2 – 3 minutes.

Fill volume: 0.5 – 4 ml
Delivered dose: > 60 %
Tailored droplet size

eFlow® Baby

Fill volume: 0.5 – 4 ml
Delivered dose: >60 %
Droplet size: 2.8 µm

- For novel paediatric nebuliser medications (Lit.[12]).
- Available for clinical studies.
- Treatment time < 5 min.

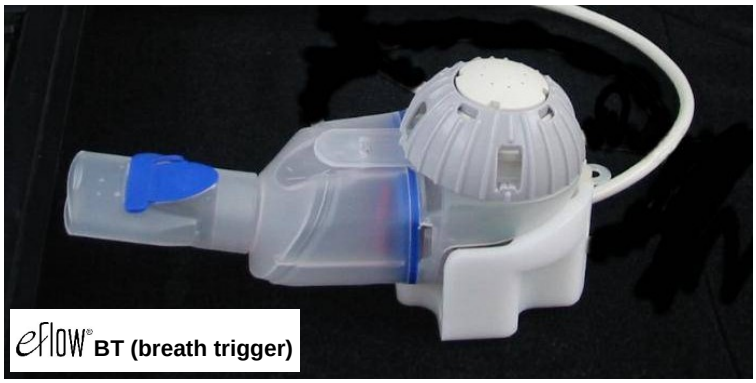
eFlow® Breath-Trigger

Fill volume: 0.5 – 4 ml

Delivered dose: >80 %

Tailored droplet size

(MMD 2.5 µm to > 5 µm)



- Highly efficient delivery of novel nebuliser medications (Lit.[10]).
- Available for clinical studies

eFlow® rapid

Fill volume 2 – 6 ml
Delivered dose >20 %
Droplet size 3.8– 4.2 µm

- Adapted for equivalent delivered dose with current nebuliser medications in CF.
- Treatment time cut by half (3 – 7 min) in comparison with the standard therapy.

eMotion™

- Adapted for current nebuliser medications used in Japan with a treatment time cut by half in comparison with the standard therapy.

Fill volume: 0.5 – 4 ml
Delivered dose: ~ 40 %
Droplet size: 4.2 µm

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3 WORK OBJECTIVES

The respiratory tract has become an increasingly attractive route of application for a wide range of active substances with either topic or systemic pharmacological activity. The major advantages are a rapid onset of the therapeutic effect, a lowering of the required dose (as compared to systemic administration) and a reduction in unwanted side-effects (increased therapeutic index).

However, the delivery of the drug substances via nebulisation constitutes an interesting challenge. This is not only due to the natural properties of the respiratory system but also to the physicochemical properties of the drugs, which combined have an important influence. In addition, the operation requirements of the eflow device also play a very important role.
As model drugs, the following actives substances were investigated:

- Cyclosporin A;
- Azithromycin;
- Pentoxifylline;
- Budesonide.

Although the drugs chosen share common physicochemical properties such as poor water solubility or strong bitter taste, the development of a nebulised formulation constituted an unique challenge for each of them.
One of the drugs investigated in this work was cyclosporin A. It is a hydrophobic cyclic peptide and a first line immunosuppressive drug used in the treatment and prevention of graft rejection.
However, its poor water solubility constituted an important hurdle to overcome, especially in the development of a formulation suitable for nebulisation[10].
In fact, its low value of water solubility in an aqueous solution (water solubility of cyclosporin A at 23°C: 10.41 µg/ml) and its solubility only in organic solvents such as acetone or chloroform reduced the field of possibilities that could be used in the formulation development.
Furthermore, the stability and taste of the formulation also represented an important objective to be reached.
In addition, in the development of the formulation, the parameters and requirements of the eflow® (for example, viscosity limits) also needed to be taken in consideration. For example, the use of oils as a way of solubilising the cyclosporin A has the disadvantage of greatly increasing the viscosity of the formulation. Consequently, the nebulisation of such formulations is not possible due to it's the high viscosity value.

[10] The use of CSA has been limited due to a broad toxicity profile, including nephrotoxicity and hepatotoxicity. As an example, it has also been reported that Cremophor EL®, a solubilizing agent, present in the commercially available intravenous dosage form of cyclosporin A, was nephrotoxic and haemolytic (Lit. [89]-[90]).

Given the above-mentioned reasons, it was decided to use cyclodextrins as a way of overtaking these difficulties. Cyclodextrins have the potential of being not only solubility/stability enhancers for water insoluble drugs but also for the pulmonary delivery of proteins.

The development of a formulation for azithromycin shares the same main objectives as the development of a cyclosporin A formulation but at a different level. For example, the taste and also the stability of the formulation are very important parameters to take in consideration in the formulation development.
In fact, the taste of the azithromycin formulation is essential in the formulation development process due to patient compliance. If patients, particularly a young patients, do not like the taste, it is more difficult to persuade them to continue with the therapy. Consequently, taste masking is an important factor in patient compliance. This represents a very difficult challenge in the development process because of the extremely bitter taste of the azithromycin molecules.
On the other hand, the stability of an azithromycin formulation also constitutes a considerable challenge. Furthermore, azithromycin can degrade in aqueous solutions, especially in the presence of acids.
Equally important, azithromycin is a water insoluble drug, which means that the increase of it's the water solubility is also one of the objectives to be achieved.
Similar to cyclosporin A, it was also decided to investigate the use of cyclodextrins as means of improving the solubility/stability and also the taste of the azithromycin formulations.

Pentoxifylline is a water soluble drug that possesses, however, a bitter taste. Therefore, the main objective in the development process was the improvement of the taste of the formulation.
To that end, it was considered the use of cyclodextrins due to their taste masking ability.

Budesonide is a water insoluble drug and it was also studied in this work. The development process shared the same problems and objectives as the development processes for cyclosporin A and azithromycin. Consequently, the development of the budesonide formulation was based on the work previously developed for cyclosporin A and azithromycin.
It had as main objectives the improvement of the water solubility and the stability of budesonide formulations.

As a conclusion, for the development of formulations suitable for nebulisation one has to take in consideration not only the physicochemical properties of the drugs and the respiratory system but as operation requirements of the eflow device. All this variables need to weighted and considered in the development process in order to achieve the proposed objectives for each of the drug formulations.

4 CYCLODEXTRINS

Cyclodextrins have gained most attention in the pharmaceutical area and drug delivery by improving solubility and/or stability of various drug molecules.
Cyclodextrins (CDs) were first isolated by Villiers in 1891, and characterized by Schardinger in 1904.

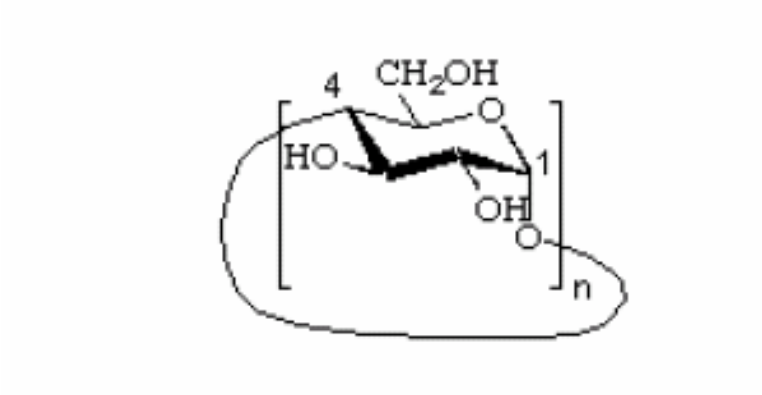


Figure 1: Cyclodextrin with n glucose units. According to Lit.[1].

Cyclodextrins are capable of forming inclusion complexes with various molecules, altering this way their physical/chemical properties. There are three well-known naturally produced cyclodextrins, α-, β-, and γ-cyclodextrin (α -, β -, and γ -CD), consisting of 6, 7, or 8 glucose units, respectively.

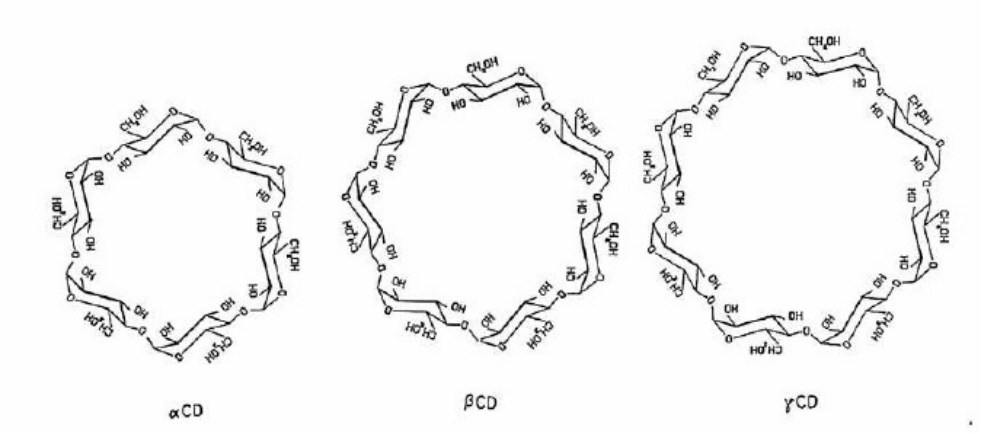


Figure 2: Chemical structure of α-, β-, γ-CDs. According to (Lit. [1]).

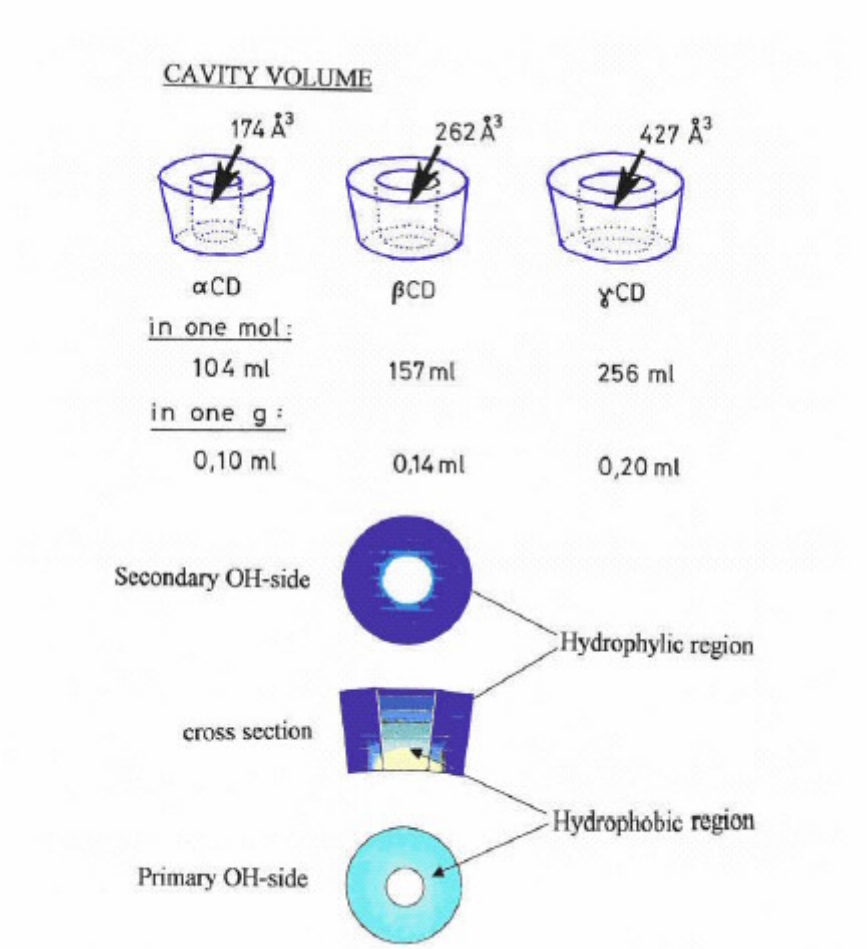


Figure 3: Dimensions and hydrophilic/hydrophobic regions of the CD molecules. According to (Lit. [1]).

Cyclodextrins containing more than eight glucose units have been reported, but due to low yield and complicated purification, these cyclodextrins are less characterized and have presently no value in commercial applications (Lit. [1]- Lit. [2]).
Cyclodextrins with less than six glucose units in the ring cannot be formed enzymatically due to steric reasons, but can be obtained by chemical synthesis (Lit. [1]- Lit. [3]). Like the large cyclodextrins, those are also currently of minor commercial interest.

4.1 Physicochemical Properties of Cyclodextrins

Because of their specific properties, cyclodextrins have gained attention, which is partly explained by their unique structures. As a consequence of the α(1,4)-linked of the glucose units, all the secondary hydroxyl groups are situated at the one side of the two edges, whereas all the primary hydroxyl groups are situated on the other edge (see Figure 9).
The cyclodextrin structure provides a molecule shaped like a segment of a hollow cone with an exterior hydrophilic surface and interior electron rich hydrophobic cavity (for characteristics of α-, β- and γ-CD see Table 1).

The hydrophilic surface generates a relative good water solubility for the cyclodextrins and the hydrophobic cavity provides a favourable environment for the hydrophobic parts of a guest molecule to be held in. This association isolates the guest molecules from the aqueous solvent and may increase the guest's water solubility and/or stability.

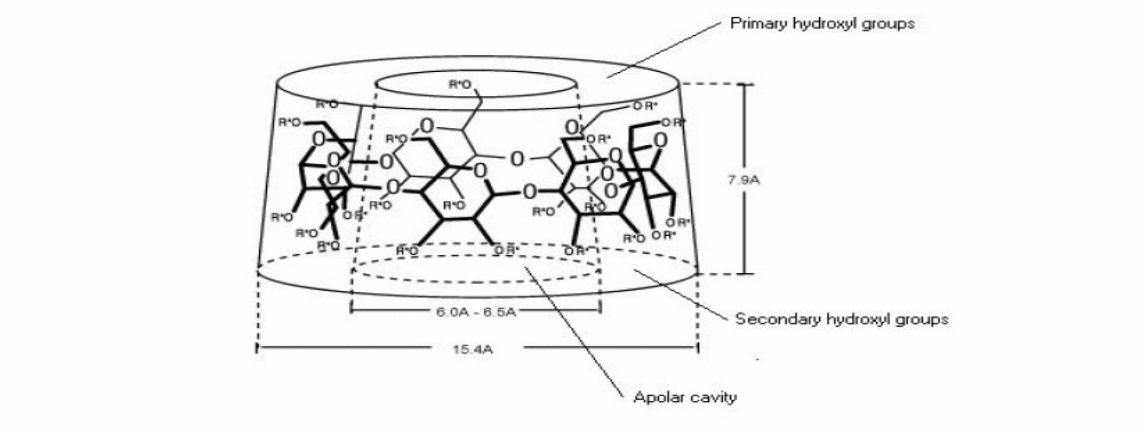


Figure 4: Shape of β -CD molecule (Lit.[92]).

Table 1: Some characteristics of α-, β - and γ -CD (Lit. [1]) and (Lit. [4]).

Parameters	**α-CD**	**β-CD**	**γ-CD**
Number of glucose units	6	7	8
MW [g/mol]	972	1135	1297
Solubility in water at room temperature [g/100 ml]	14.5	1.85	23.2
[a]D at 25°C [°C]	150±0.5	162±0.5	177.4±0.5
Cavity diameter [pm]	470-530	600-680	750-830
Height of torus [pm]	790±10	790±10	790±10
Diameter of outer periphery [pm]	1460±40	1540±40	1750±40
Approximate volume of cavity (106 pm3)	174	262	427
Approximate cavity volume in mol of cyclodextrin	104	1257	256
Approximate cavity volume in 1 g of cyclodextrin	0.1	0.14	0.2
pKa (by potentiometry) at 25°C	12.332	12.202	12.081
Hydrolysis by A. oryzea α-amylase	Negligible	Low	Rapid
Enthalpy of solution, DH° [kJ mol-1]	32.1	34.8	32.4
Entropy of solution, DS° [J K-1 mol-1]	57.8	49	61.5

4.2 Cyclodextrin-Guest Complexation

The various cyclodextrins can be considered as an empty cavity of molecular size. When the cavity is filled with a molecule of another substrate, it is called an inclusion complex[11].
Inclusion complexes are systems that consist of two or more molecules, in which one of the molecules, the host, includes entirely or partially the guest molecule. The molecules in the inclusion complex are only in contact by physical forces and without covalent binding (Lit. [1]- Lit. [5]).
Moreover, the dissociation-association equilibrium is probably the most characteristic feature of the host-guest association (Lit. [1]).

4.2.1 Overall Inclusion Complex Stability Constant

The equilibrium between the guest and cyclodextrin is fundamental in the measurement of the inclusion complex stability constant. The general equation can be expressed as:

$$\mathbf{n[Guest] + m[CD] \leftrightarrow [Guest_nCD_m]}$$

where *n* and *m* are the number of mole for the guest molecule and cyclodextrin, respectively. The equilibrium constant *K* or inclusion complex stability constant for the process can be defined as:

$$\mathbf{K= [Guest_nCD_m]/(Guest]^n[CD]^m)}$$

where *n* and *m* are the number of mole for the guest molecule and cyclodextrin, respectively.

The simplest formation of a complex between the cyclodextrin and a guest molecule is a stoichiometric proportions of 1:1. Cyclodextrin complexes with two guest molecules per cyclodextrin or vice-versa are also quite common [Lit.1].

4.2.2 Mechanism of Inclusion Constant

Since the cavity of cyclodextrins is hydrophobic, the inclusion of a molecule in the cyclodextrin cavity is basically a substitution of the water inside the cavity with a less polar substance. The substitution of water from the cavity with a more non-polar guest is energetically favourable for both the cyclodextrin and the guest, which is illustrated in figure 10.

[11] Since no covalent bond is established between the host and guest, the expression inclusion complex has been used to describe the process.

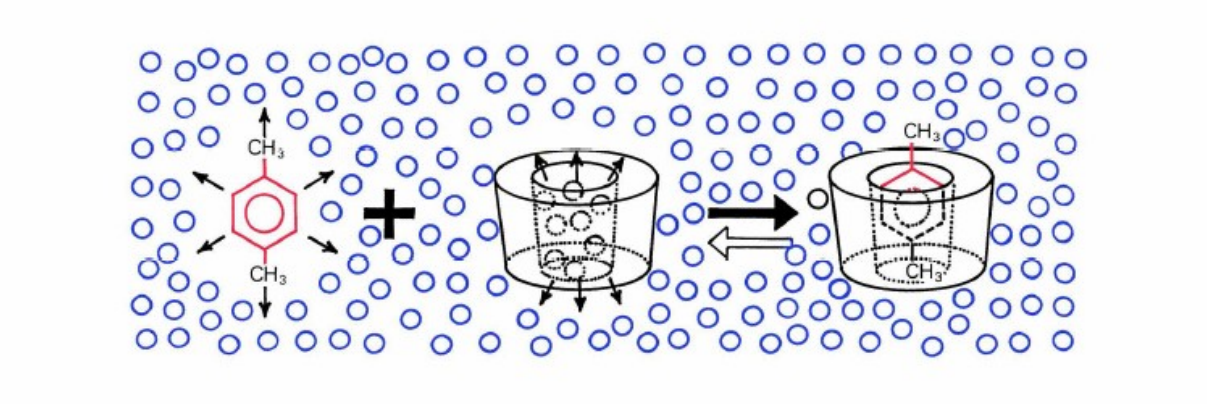


Figure 10: Schematic illustration of inclusion complexation of p-xylene by a cyclodextrin. The small circles represent the water molecules (Lit. [1]).

The 3D structure of the parent CD provides a cavity (Figure 11) that is hydrophobic relative to an aqueous environment. The sequestration of hydrophobic drugs inside the cavity of the CD can improve the drug's solubility and stability in water, the rate and extent of dissolution of the drug:CD complex, and the bioavailability of the drug when dissolution and solubility are limiting the delivery. These properties of the CDs enable the creation of formulations for insoluble drugs typically difficult to formulate and deliver with more traditional excipients.

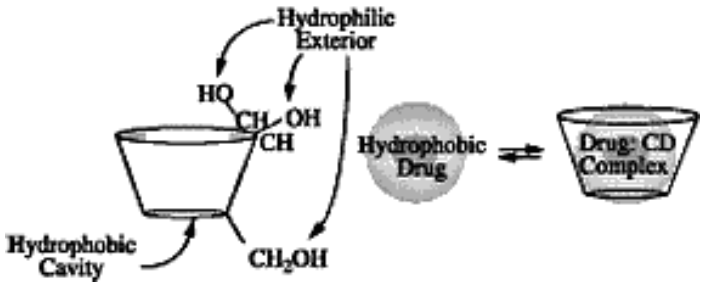


Figure 11: Complexation of drugs inside the hydrophobic cavity of CDs (Lit. [6]).

CDs form inclusion complexes with hydrophobic drugs through an equilibrium process (Figure 11), quantitatively described in Equation 1 by an association or stability constant (K a:b),

$$K_{a:b} = [Drug_a\ CD_b]/(\mathrm{Drug}t]^a[CD]^b)$$

where a and b represent the molar ratio of the sequestered drug molecule to the CD. The magnitude of this associate constant can be used to compare the binding effectiveness of different CDs. Various complexes with different ratios of drug-to-CD molecules can be formed, depending on the type of CD used and the size and physicochemical characteristics of the drug molecule. In dilute solutions and/or if the drug fits entirely into the CD cavity, a 1:1 complex results.

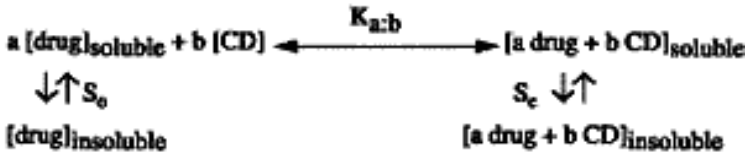


Figure 12: Equilibrium process describing the interaction between a CD and an insoluble drug molecule to form a soluble or insoluble complex. (Modified from (Lit. [6]).

However, if the cavity is large enough, two drug molecules may be accommodated, resulting in the formation of a 2:1 complex. Conversely, if the drug is very large, then several CD molecules might enclose the drug for the formation of 1:2 or higher order complexes. Although each complex has a finite stoichiometry, more than one complex may be formed in a given system. Depending on the method used to determine the association constant, it is possible to obtain a description of the stoichiometry of the complex (a:b).

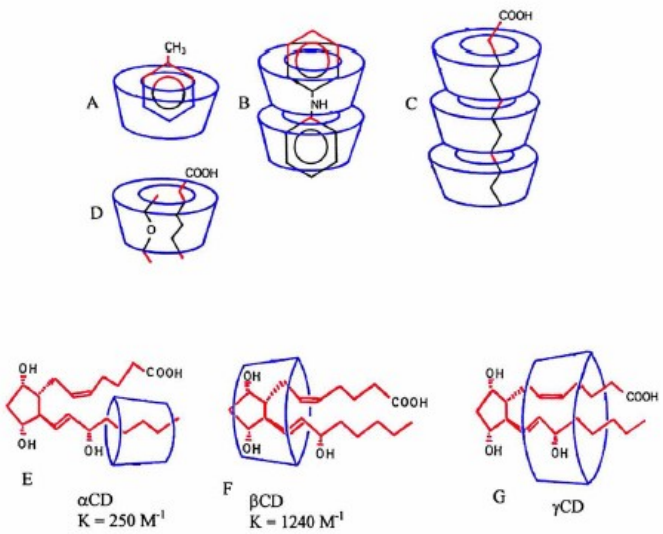


Figure 13: Examples for CD-complexes: the same CD with different guests, and the same guest with different CDs. Toluene/βCD (A), diphenylamine/βCD (B), long-chain fatty acid/CD (C), short chain fatty acid + diethyl ether ternary βCD complex (D), prostaglandin E2/αCD (E), prostaglandin E2/βCD complex (F), prostaglandin E2/γCD (G) (Lit. [1]).

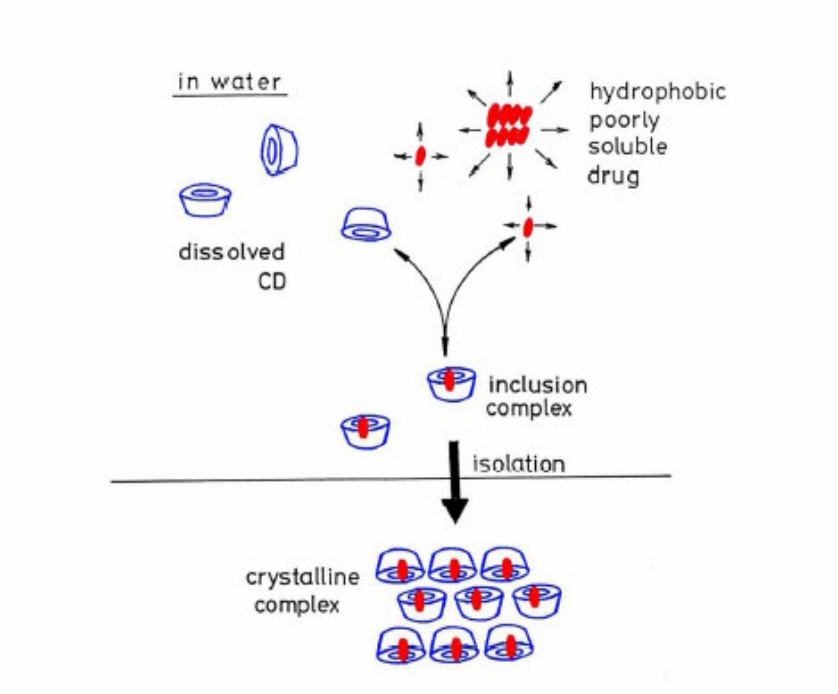


Figure 14: Schematic representation of the production of a CD complex (Lit. [1]).

The “driving force” in the complexation is not fully understood, but it seems to be a combination of different effects depending on the specific guest and cyclodextrin.
These effects can be hydrophobic interaction, van der Waals interaction, hydrogen bonding, dipoledipole interaction, and release of “enthalpy-rich” water (Lit. [1]-Lit. [5]).

a) Hydrophobic Interaction

Hydrophobic interaction occurs when non-polar molecules tend to cluster together in an aqueous environment due to removal of apolar surfaces from contact with water. The structure of the surrounding water is a critical factor in “classical” hydrophobic interaction. The interaction results in a slightly positive $\Delta H°$ and a large positive $\Delta S°$ at low temperature, and its thereby said to be “entropy driven” (Lit. [7], Lit. [8] and Lit. [9]). The fact that the entropy change is positive, even though the molecules are clustering together, shows that there must be a contribution to the entropy from the solvent and that solvent molecules must be more free to move once the solute molecules have herded into small aggregates (Lit. [10]).
Since the majority of the cyclodextrin complexation is “enthalpy driven”, it seems obvious that hydrophobic interactions are of minor contribution compared to the other driving forces and therefore several authors have reported that hydrophobic interactions do not need to be taken into consideration (Lit. [7], Lit. [8] and Lit. [9]). However, cyclodextrin is a “semi-polar” molecule where “semi-polar” means a cavity more hydrophobic than water but less hydrophobic than n-octanol base on the dielectric constant of toluidinyl groups after inclusion, which provides an environment suitable for interaction with hydrophobic guests (Lit. [1]). If hydrophobic interactions occur, there should be no expectation that the “classical” system is applicable in the cyclodextrin system (Lit. [9]).

A model describing a “non-classical” hydrophobic interaction between semi-polar molecules seems to be relevant to the cyclodextrin complexation where molecules may have either a negative DH° or DS°. In this model, the “driving forces” for complexation can be either a favourable enthalpy or entropy change that relies on the magnitude and sign of DH° and DS° (Lit. [7- Lit. [9]). However, this model cannot solely be applied to predict the complexation between cyclodextrin and guest, since the complexation is a combination of different effects as mentioned above.

b) Van der Waals Interaction

When two molecules are brought close together, they both attract and repel each other depending on the distance that separates them. The attraction force of the molecules is caused by instantaneous and short-lived imbalance in the electron distribution of an atom that generates a temporary dipole. These short-living induced dipoles result in an induction electron distribution of the neighbouring atom that generates a temporary polarization. This polarisation minimizes the electron-electron repulsion between the atoms also known as induced dipole-induced dipole interaction or London dispersions forces. Other forces involved are dipole-induced dipole and permanent dipole. Common for all these repulsive and attractive forces, known as van der Waals forces, are that they neither are non covalent nor non ionic (Lit. [11]- Lit. [12]).
These forces are usually weak for all kind of interactions, but are likely to be numerous in the cyclodextrin cavity and thereby have to be taken into consideration (Lit. [1]- Lit. [7]).

c) Hydrogen Bonding Interaction

If hydrogen is close to an atom that is very good at attracting electrons (like N, O, or F) the hydrogen end of the bond becomes very positively charged and the other atom becomes negatively charged (*i.e.*, polar). Hydrogen is the smallest atom in the periodic table, which makes it possible for hydrogen atom and the other atom to get very close together. The combination of high polarity and close approach result in the interaction being particularly strong due to the force of attraction between two opposite charges. This is proportional to the magnitude of their charges divided by the square of the distance between them. In fact, the interaction is so strong that it dwarfs all other dipole-dipole attractions (Lit. [13]).
The hydrogen bonding is considered to play an important role in the stability of the cyclodextrin complexes in aqueous solution. It may, furthermore, contribute to a conformational change either in the cyclodextrin, the guest, or both, which results in a more stable complex (Lit. [9]).

d) Release of “Enthalpy-rich” Water

When water is substituted from the cavity of the cyclodextrin a decrease in energy occurs. This is caused due to an increase in solvent-solvent interaction, since the surface contact between solvent and cyclodextrin cavity, as well as between solvent and guest molecule, are reduced. Furthermore, water inside the cyclodextrin cavity cannot possess its tetrahedral hydrogen bonding capacity compared to those in the surrounding solvent, and it is therefore often reported as “high energy” water or “enthalpy rich” water. One of the main driving forces for complexation could, therefore, be the release of this “high energy” water from the cyclodextrin cavity, which, allowing them to form their full compliment of hydrogen, bonds with the surrounding water (Lit. [1], Lit. [7] and Lit. [8]).

4.3 Evaluating Complex Formation

One of the most common methods of determining association constants and stoichiometry is the *phase solubility technique* (Lit. [27]). The technique involves adding an equal weight (in considerable excess of its normal solubility) of the compound to be complexed into each of several vials or ampoules. A constant volume of solvent is added to each container.
Successively increasing portions of the complexing agent are then added to the vessels. The vessels are then closed and the contents brought to solubility equilibrium by prolonged agitation at constant temperature. The solution phases are then analyzed for total solute content. A phase diagram is constructed by plotting the molar concentration of dissolved solute, found on the vertical axis, against the concentration of complexing agent added on the horizontal axis.
Two general types of phase solubility profiles are generated: *Type A* where soluble complexes are formed and *Type B* where complexes of limited solubility are formed.
The A-type diagrams are defined by the absence of any form of precipitation of the inclusion complexes after equilibrium has been attained and by an increase in solubility of the drug as a result of complexation with cyclodextrin. The B-type solubility diagrams on the other hand indicate precipitation of a drug-cyclodextrin complex at a certain concentration of the cyclodextrin.

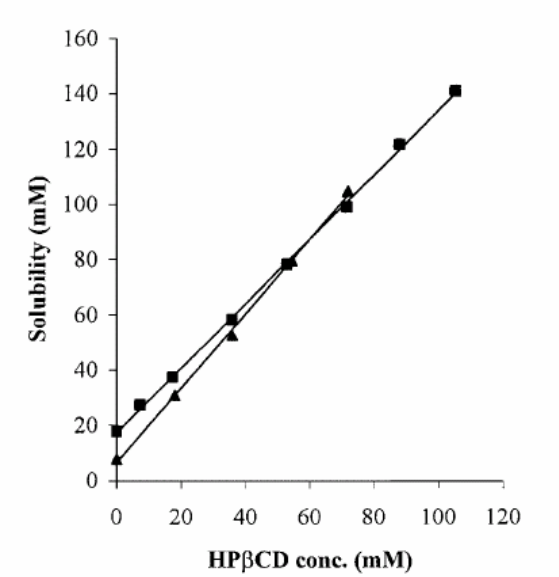


Figure 15: The A-type phase-solubility diagrams of the sodium salts of ibuprofen (■) and diflunisal (▲) in 2-HP-β-CD containing pH 6.0 aqueous 0.1 M phosphate buffer solution at ambient temperature (Lit. [87]).

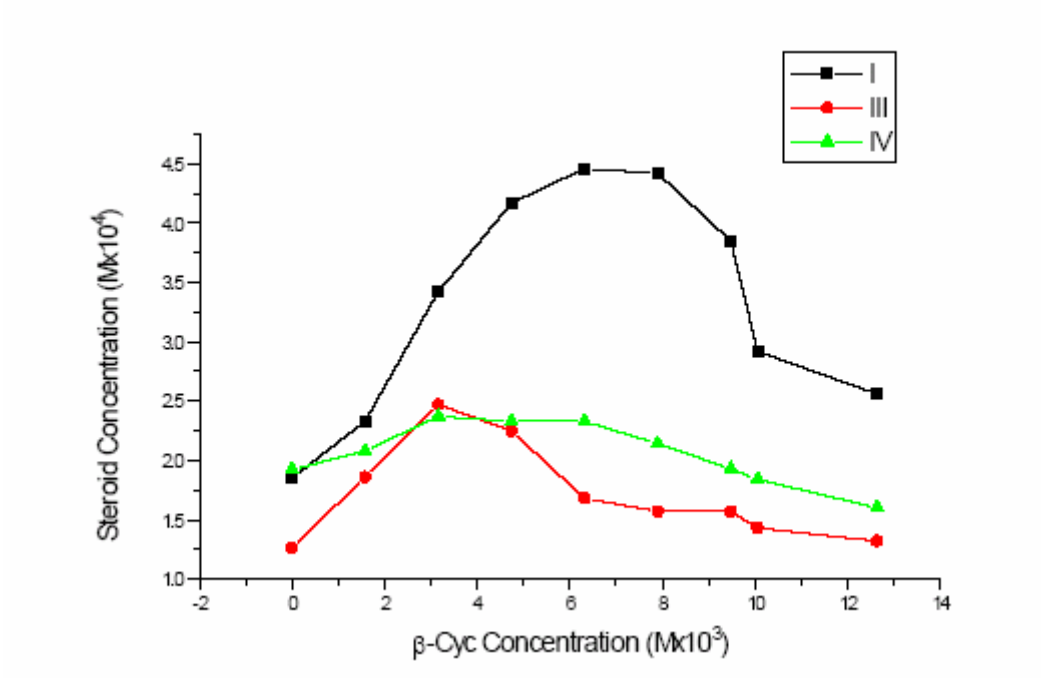


Figure 16: The B-type phase solubility diagrams of 3 heterocyclic steroids compounds 1, 3 and 4 in water – ethanol system, in the presence of β-CD (Lit. [88]).[12]

In Type A diagrams, an increase in solubility of the compound occurs as the amount of complexing agent increases. Soluble complexes are formed between the compound and the complexing agent, thereby increasing the total amount of compound in solution. Depending

[12] **I**-stigmasteryl-5'-nitrobenzo[b]-thiophen-2'-carboxylate; **III-** citosteryl-5'-nitrobenzo[b]-thiophensulphone-2'-carboxilate (**3**); **IV**-cholesteryl-6-bromobenzothiazolyl carbamate.

on the nature of the complexes formed, the diagram can be linear, AL, or show curvature in a positive, AP, or negative, AN, fashion (Figure 16). Linear diagrams are formed when each complex contains only one molecule of complexing agent. When more than one molecule of complexing agent is found in the complex, an AP-type diagram is formed. AN diagrams are uncommon but may result if self-association is present or high concentrations of complexing agent cause alterations in the nature of the solvent.

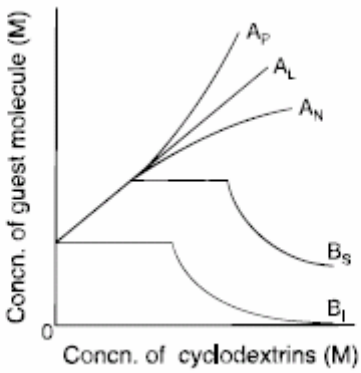


Figure 17: Possible types of phase solubility diagrams.

Type B diagrams are observed when complexes of limited solubility are formed. In Figure 16, the segment xy in curve BS shows the formation of a complex that increases the total solubility of the compound. This is similar to a Type A diagram. At a certain point, however, the solubility of the complex is reached and as additional compound goes into solution, some solid complex precipitates. Then, the entire excess solid compound added to the vials has been consumed by this process. Further addition of complexing agent beyond this point results in depletion of the compound from solution by complex formation. Curve BI is interpreted in a similar manner except that the complex formed is so insoluble that no increase in solubility is observed.

The stoichiometry of the complexes can often be determined from the ascending and descending portions of these diagrams if certain assumptions can be made (Lit. [27]). If a 1:1 complex is formed, the association constant $K_{a:b}$ can be determined from the slope of the initial linear portion of the phase solubility curve, and the intrinsic solubility of the compound, S_0, using the following equation:

$$\mathbf{K_{1:1} = [Slope]/[S_o\ (1\text{-}Slope)]}$$

Additional methods are available to determine these association or stability constants including spectroscopy (UV, fluorescence, NMR, and ORD-CD), potentiometry, microcalorimetry, surface tension, membrane permeation, electrophoresis and freezing point depression. Chromatographic methods include HPLC and TLC techniques.
When cyclodextrins are used to solubilise water insoluble drugs, it is generally assumed that the solubilization proceeds through inclusion complex formation, e.g. that the lipophilic water-insoluble drug molecules, or some lipophilic moieties of the drug molecule, are taken into the hydrophobic central cavity of the water-soluble cyclodextrin molecules.

Linear phase-solubility diagrams (i.e. Figure 16, plots drug solubility versus cyclodextrin concentration) are usually assumed to indicate formation of 1:1 drug/cyclodextrin inclusion complexes or at least complexes that are first order with respect to cyclodextrin. Positive deviation from linearity is thought to indicate higher-order inclusion complexes such as formation of 1:2 drug/cyclodextrin complexes at higher cyclodextrin concentrations (Lit. [77]–Lit. [79]). Such higher order systems are characterized by stepwise binding constants, e.g. that 1:2 complex is formed by association of the 1:1 complex with one additional cyclodextrin molecule. Frequently, the stoichiometry is obtained by simply fitting the phase solubility diagrams to the appropriate equation without any further verification. In some cases, proposed stoichiometry and structure of a given drug/cyclodextrin complex is verified through nuclear magnetic resonance (NMR) investigations (Lit. [79]).

However, these are usually performed in dilute solutions whereas the phase-solubility studies are based on investigations of saturated drug solutions. Other methods for stoichiometry verifications are based on theoretical gas-phase computer modelling of complexes that do not describe well the actual conditions in saturated aqueous solutions (Lit. [80]). Thus, the conventional descriptions of 1:1 drug/cyclodextrin complexes, as well as of those containing different stoichiometry, are not as unambiguous as one might think.

Cyclodextrins are all known to self-associate and to form complexes with various types of compounds (Lit. [81]-Lit.[84]). It is also well known that many drugs are surface active and that these drugs form micelles, which are stabilized by water-soluble polymers in a similar way as micelles (Lit. [85]-Lit. [86]). Cyclodextrin complexes are also known to self-associate to form water-soluble aggregates or micelles. These cyclodextrin aggregates and micelles, and their effects on drug solubilization, are not accounted for in the current complexation models (Lit. [87]-Lit. [89]).

The stoichiometry of drug/cyclodextrin complexes cannot be derived exclusively from simple phase solubility studies. Self-association of surface active drugs, lipophilic drug molecules, and drug/cyclodextrin complexes, as well as drug solubilization through non-inclusion interactions with drug/cyclodextrin complexes, will influence both the shapes and mathematical interpretation of the phase-solubility diagrams.

In summary, drug/cyclodextrin complexes can self-associate to form water-soluble aggregates of several drug/cyclodextrin complexes.

4.4 Self-association of cyclodextrin complexes

Aqueous cyclodextrin solutions have been regarded as true solutions where individual cyclodextrin molecules as well as cyclodextrin complexes form a homogeneous molecular dispersion in a continuous system of water molecules.
In recent years it has been shown that cyclodextrins and cyclodextrin complexes self-associate to form aggregates or micelle-like structures (Lit). Furthermore, it has been shown that water-soluble polymers do interact with such systems and that the aggregates can solubilise drugs through non-inclusion complex formation (Lit). Various techniques can be applied to enhance the water-solubility of drug/cyclodextrin complex aggregates. For example, addition of small amount of water-soluble polymers to aqueous complexation media increases the solubility of the aggregates. Likewise, addition of various salts, such as sodium acetate, can increase the solubility of drug/cyclodextrin complexes.
Formation of drug/cyclodextrin complexes depends on the chemical structure and the physicochemical properties of both the drug and the cyclodextrin. For an inclusion complex to be formed some lipophilic moiety of the drug molecule has to fit into the hydrophobic cyclodextrin cavity. Also, low aqueous solubility is not always due to lipophilicity of the drug molecules.
The effects of the molecular structure and physicochemical properties of both the drug and the cyclodextrin on the formation of drug/cyclodextrin complexes are:

- **Size of the cyclodextrin cavity:** the size of the cyclodextrin cavity will influence the complex formation. For instance, the α-cyclodextrin cavity is too small for naphthalene and only the γ-cyclodextrin cavity can accommodate anthracene. α-Cyclodextrin can be used for small molecules or side-chains of larger molecules (e.g., prostaglandins), β-cyclodextrin is very useful for complexing molecules containing a phenyl group, a group encompassing many drugs, and γ-cyclodextrin can be used for complexation of larger molecules such as macrolide antibiotics;

- **Molar substitution (or degree of substitution) of the cyclodextrin molecule**: chemically modified cyclodextrins of lower degree substitution are frequently better complexing agents than the same derivatives with higher molar substitution;

- **The intrinsic solubility of the drug:** the lower the intrinsic drug solubility, the greater is the relative solubility enhancement obtained via cyclodextrin complexation. Drugs that possess aqueous solubility in the µg/ml range generally demonstrate much greater relative enhancement than drugs possessing solubility in the mg/ml range;

- **Hydrophilic drugs possessing low intrinsic solubility in water:** zwitter ionic drugs, and other polar drugs of limited aqueous solubility, generally demonstrate low complexing abilities. The basis for such poor enhanced solubility is that the low aqueous solubility of the drug is frequently due to high crystal energy rather than their lipophilicity. Once in solution, the hydrated drug

molecules often have little tendency to be included into the hydrophobic cyclodextrin cavity. However, many ionisable drugs are capable of forming cyclodextrin complexes and although the ionized form tends to form complexes less avidly than the unionized form, it is frequently possible to enhance aqueous solubilization of ionisable drugs by appropriate pH adjustments;

- **Ion pairing:** compared to neutral cyclodextrins, enhanced complexation is frequently observed when the drug and cyclodextrin molecules have opposite charge while decreased complexation is observed when the drug and cyclodextrin carry same type of charge.

It might also be mentioned that various vehicle additives commonly used in pharmaceutical preparations, such as sodium chloride, buffer salts, surfactants, preservatives and organic solvents, often attenuate the ability of cyclodextrins to solubilise drugs. Thus, solubility studies should be completed a) using the intended formulation and b) under normal production conditions. In general, the complexation efficiency of cyclodextrins is rather low, and thus relatively large amounts of cyclodextrins are needed to complex small amounts of a drug.
Due to toxicological considerations, formulation and production costs, it is important to use as little cyclodextrin as possible in pharmaceutical preparations. The complexation efficiency is equal to the intrinsic solubility of the drug times the stability constant of the drug cyclodextrin complex. Enhanced complexation efficiency can be obtained by increasing the apparent intrinsic solubility of the drug, by increasing the apparent stability constant of the drug/cyclodextrin complex or by increasing both simultaneously. Some methods that can be applied to enhance the complexation efficiency are:

- **Drug ionization**: Unionized drugs do usually form more stable complexes than their ionic counterparts. However, ionization of a drug increases its apparent intrinsic solubility resulting in enhanced complexation;

- **Salt formation**: it is sometimes possible to enhance the apparent intrinsic solubility of a drug through salt formation;

- **Complex-in-complex**: in some cases the apparent intrinsic solubility of a drug can be increased through formation of metal complexes;

- **The acid/base ternary complexes:** It has been shown that certain organic hydroxy acids (such as citric acid) and certain organic bases are able to enhance the complexation efficiency by formation of ternary drug/cyclodextrin/acid or base complexes.

- **Polymer complexes**: water-soluble polymers form a ternary complex with drug/cyclodextrin complexes increasing the observed stability constant of the drug/cyclodextrin complex. This again increases the complexation efficiency;

- **Solubilization of cyclodextrin aggregates**: organic cations and anions are known to solubilise uncharged drug/cyclodextrin complexes that have limited aqueous solubility. This will enhance the complexation efficiency during preparation of, for example, solid drug/cyclodextrin complex powder;

- **Combination of two or more methods**: frequently, the complexation efficiency can be enhanced even further my combining two or more of the above mentioned methods. For example drug ionization and the polymer method, or solubilization of the cyclodextrin aggregates by adding both polymers and cations or anions to the aqueous complexation medium.

4.5 Pharmacokinetics of the Drug/Cyclodextrin Complexes

Complexation of molecules to cyclodextrins occurs, with some exceptions, through a non-covalent interaction between the molecule and the cyclodextrin cavity. This is a dynamic process whereby be the guest molecule continuously associates and dissociates from the host cyclodextrin. Although most complexation schemes depict the complex as a single entity, the complex is more realistically composed of a family of species with the depicted complex representing some average. It probably represents the specie with the longest lifetime.

For example, in a 1:1 complexation, the association is usually described as in Scheme 1. Two important parameters can be defined for the inclusion process. First, is the complexation strength or constant (*K*) defined by Scheme 1 and Eq. (1), where [CD] and [D] are the concentrations of free cyclodextrin and free drug molecule, respectively.
Second, is the lifetime (t) of the complex, also defined in Scheme 1 and Eq. (2), measured when the equilibrium is disturbed. The constants k_f and k_r are the forward and reverse rate constants, respectively, and k_{obs} is the observed rate constant for the reestablishment of the equilibrium after it is perturbed.
If the complex is diluted such that dissociation of the complex is complete, the half-life will be that associated with k_r.[13]

$$\mathbf{K=k_f/k_r=[DCyD]/[D_f][CyD_f]}$$
$$\mathbf{K_{obs}=l/\tau= k_f(CyD_f) + [D_f][+ K_\tau}$$

$$\mathbf{D_f + CyD_f <=> DCyD}$$

Scheme 1: The interaction of drug, D, with a cyclodextrin, CyD, to form an inclusion complex, DCyD, with a binding constant of K, where K=kf /kr. According to (Lit. [76]).

Several factors can have influence the dissociation of the drug from the cyclodextrins complexes. For example:

[13] Taking in consideration that k_{fis} a second order rate constant while k_r is a first order rate constant.

4.5.1 Competitive displacement

Competitive displacement of drugs from their cyclodextrin complexes can have a significant influence *in vivo*. As an example of the competitive displacement of a drug from its cyclodextrin complex is the work of Tokumura et al on the water insoluble drug cinnarizine (Lit. [73]-Lit. [74]). A β-cyclodextrin complex of the water insoluble drug, cinnarizine, was more soluble than cinnarizine alone. After oral administration of the complex, improvement in cinnarizine availability were less than expected based on *in vitro* dissolution experiments. It was suggested that cinnarizine was too strongly bound to the cyclodextrin such that complex dissociation was limiting oral availability.
Co-administration of phenylalanine, a displacing agent, improved the availability of cinnarizine from the complex but not from conventional tablets of cinnarizine.
When cinnarizine was administered to dogs as HP–β-CD or (SBE)–β-CD aqueous solutions of pH 4.5, or as a (SBE)–β-CD/ cinnarizine complex in capsules, oral availability was essentially complete without the need for a displacing agent (Lit. [75]). In other words, dissociation of cinnarizine from these two cyclodextrins was not limiting.

4.5.2 Dilution

The effect of the dilution on the drug release from the cyclodextrin complex depends on the route of administration. For example, dilution is minimal when a complex is administered ophthalmically or pulmonary. However, after oral drug administration, some dilution is likely to occur but again, dilution alone is probably insufficient to account for the relative good absorption of drugs administered as cyclodextrin complexes. Unlike, ophthalmic delivery, residence time in the GI tract is longer, allowing time for other factors to contribute to complex dissociation.

4.5.3 Protein binding

Scheme 2 shows a possible three equilibrium processes that can occur following the administration of a drug/cyclodextrin complex.

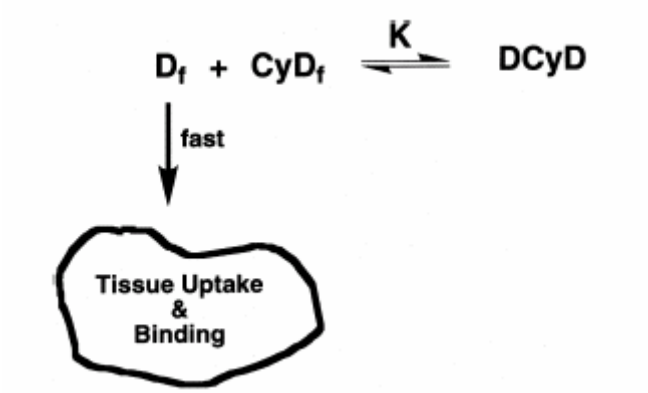
$$\begin{array}{ccccc} \mathbf{D_{1,f}} & + & \mathbf{CyD_f} & \leftrightarrow & \mathbf{D_1CyD} \\ + & & + & & \\ \mathbf{P} & & \mathbf{D_{2,f}} & & \\ \downarrow & & \downarrow & & \\ \mathbf{D_1P} & & \mathbf{D_2CyD} & & \end{array}$$

Scheme 2: Protein binding and displacement equilibrium competing with drug/ cyclodextrin inclusion complexation. According to ([76]).

The scheme represents the binding of drug (D) to a cyclodextrin (CyD), binding of drug to a protein (P) and the binding of a competing agent (D) to the cyclodextrin. K_1, K_2 and Kp are the binding constants for 1:1 drug/ cyclodextrin complexation, 1:1 competing agent / cyclodextrin complexation and drug/ protein binding, respectively.
As one can see, the protein binding does have an effect on facilitating drug dissociation from cyclodextrin complexes. However, the level of influence of the protein binding on the drug dissociation from the cyclodextrin complexes needs to be determined for each of the drug/cyclodextrin complexes.

4.5.4 Drug uptake into tissue

A potential contributing mechanism for drug release from cyclodextrin is preferential drug uptake by tissues. Scheme 3 illustrates this point: if the drug is lipophilic and has access to tissue not available to the cyclodextrin or the complex, the tissue then acts as a ‘sink’ causing dissociation of the complex based on simple mass action principles. This mechanism may become more relevant for highly bound drugs or when the complex is administered at a site where dilution is minimal, e.g., after ocular, nasal, sublingual, pulmonary, dermal or rectal administration.



Scheme 3: Selective tissue uptake facilitates complex dissociation. According to (Lit. [76]).

Upon administration, drugs appear to be rapidly and quantitatively released from cyclodextrin complexes. Dissociation due to dilution appears to be the major release mechanism although other factors such as competitive displacement of the drug from the complex, drug binding to plasma and tissue components, uptake of the drug by tissues not available to the complex or cyclodextrin and cyclodextrin elimination may also contribute for more strongly bound drugs.

In conclusion, the association and dissociation of molecules to and from cyclodextrins are dynamic processes. The reactions occur at very rapid rates for both strong and weak complexes with the average lifetime of the molecules in the host cavity being in the milli- to micro-second time range or shorter.
With these processes occurring at such rates, the kinetics of release of a drug molecule from cyclodextrin cavity should not be a limiting factor.

4.6 Benefits of Complexation

4.6.1 Improvement in solubility, dissolution and bioavailability

One of the most important applications of cyclodextrins in the pharmaceutical field is to enhance aqueous solubility of drugs through inclusion complexation.
The solubility of a guest compound can be changed upon complexation with a cyclodextrin either to increase or to decrease the solubility. When a guest compound is complexed by a cyclodextrin, the guest in the cavity of the cyclodextrin is essentially surrounded by the molecule of cyclodextrin. The hydrophobic groups of the guest that would be in contact with the solvent in the free state interact with the atoms of the cavity of the cyclodextrin instead. The outer surface of the cyclodextrin interacts with the solvent. As a result, this outer surface of the cyclodextrin contributes to the solubility of the complex and not the portion of the guest interacting with the cavity of the cyclodextrin.

4.6.2 Reduction of Unpleasant Side Effects and Bitter Taste

Improvements in the rate and extent of dissolution of a drug can improve the rate of absorption of the drug. Reducing the contact time between the drug and the tissue mucosa can help minimize tissue irritation produced by drugs. Nonsteroidal anti-inflammatory drugs cause a high incidence of gastrointestinal ulcerative lesions that are a result of both local irritation from the drug and systemic inhibition of prostaglandin synthesis by the drug. CD formulations of naproxen cause fewer gastric lesions associated with the acute local tissue irritation than produced by the drug alone (Lit. [15]).
Complexation with CDs can also have the effect of reducing the amount of contact with taste receptors. This can be of great benefit in the preparation of oral solutions. Not only are the drugs “masked” from the receptors by inclusion in the CD cavity, but the increased hydrophilicity enables the easier removal of the bitter substance from the receptor surface as well. The apparent concentration of the uncomplexed bitter drug is a function of the complexation constant, the amount of free CD, and the water solubility of the drug (Lit. [16]). Complexation has been used to mask the unpleasant bitter taste of a number of drugs such as acetaminophen (Lit. [16]).

4.6.3 Improvements in Drug Stability

CDs are normally thought of as stabilizing agents in pharmaceutical formulations (Lit. [17]-Lit. [18]). They have been shown to stabilize drugs to hydrolysis Lit. [19] and hydrolytic dehalogenation, oxidation, decarboxylation and isomerization, both in solution and in the solid state(Lit. [20-23]). The nature of the stabilization or destabilization depends on the CD used (parent and functional groups of any derivative) and on the position of the guest molecule inside the CD. If the molecule is positioned such that the area of instability is located outside the CD, no effect on stability may be observed. When the position allows interaction of the CD hydroxyls (or derivative functional groups) with a hydrolytically prone

site, decreased stability may be observed but if the site is located fully within the CD, enhanced stability usually results.
Stabilization is not limited to small compounds, since larger molecules such as peptides and proteins can also form complexes that result in enhanced chemical and physical stability (Lit.[23]). The CDs will typically interact with functional groups present on exposed surfaces of the macromolecules and often form multiple complexes (several CDs per molecule).

4.6.4 Reduction in Volatility

Inclusion complexes have been prepared with a number of volatile substances (Lit. [24]- Lit.[25]) including spices, flavours, essential oils, and several drugs. CD complexation has been shown to reduce the volatility and improve the stability of many compounds. Examples include lemon oil and other flavouring agents (Lit. [25]- Lit. [26]).

4.7 Use of Cyclodextrins as Drug Carriers

As mentioned previously, CDs are cyclic nonreducing oligosaccharides containing 6, 7, or 8 glucopyranose units (α-, β-, or γ-CD, respectively).
The CD exterior, containing hydroxyl groups, is hydrophilic, whereas the central cavity is relatively lipophilic (Lit. [28-30]). The selection of CDs is also based on structural modifications to reduce toxicity. Some of these modifications are discussed below.

- **Sustained Drug Release**

For pulmonary administration of the drug, CD makes it possible to protect the drug from enzymatic degradation, to release the drug in a sustained pattern, and, as a result, to reduce the number of administrations required and prevent the high peak concentrations frequently encountered following single-dose administration.

- **Bioavailability Enhancer**

CDs have the ability to increase drug bioavailability by enhancing drug permeation through biological membranes. The preferred explanation for this phenomenon is that CDs increase the aqueous solubility of water-insoluble drugs. But the situation is actually more complicated, because CDs are also known to decrease drug bioavailability. This is, therefore, not solely a question of increased aqueous drug solubility (Lit. [28]).

4.8 Toxicological Considerations

β-CD permeates lipophilic membranes with considerable difficulty and, thus, is virtually non-toxic when used in oral or topical formulations. The acute toxicity of β-CD, administered by the oral route, was studied in rats and dogs and did not reveal any toxicity. Therefore, even if they are swallowed during or after pulmonary administration, CD will not be toxic. Nevertheless, CD exerted a relatively mild and reversible effect on the ciliary beat frequency of both chicken embryo trachea and human nasal adenoid tissue in vitro in a concentration-dependent manner. (Lit. [31]-Lit. [32]). Consequently, CD appears to be non-toxic for both the upper and lower airways.

Even though some of the cyclodextrins are not suitable for parenteral use, because of its toxicity, they can still be used in other kinds of formulation.

Table 2 include the cyclodextrins with potential interest as drug carrier and how they can be used.

Table 2: Description of cyclodextrins with potential interest as drug carriers.

Derivatives	**Examples**	**Characteristic**	**Possible use (dosage form)**
Hydrophilic Methylated β -CD	Methylated-β-CD	Soluble in cold water and in organic solvents, surface active, haemolytic	Oral, dermal, mucosal[14]
	DIMEB		
	TRIMEB		
Hydroxyalkylated-β -CD	Hydroxyalkylated β -CD	Amorphous mixture with different degrees of substitution, highly water soluble, low toxicity	Oral, dermal, mucosal, parenteral(i.v.)
	2-HP-b-CD		
	Branched-β -CD	Highly water soluble, low toxicity	Oral, mucosal, parenteral (i.v.)
	G- β -CD		
	M- β -CD		
Ionisable Anionic β -CD	Anionic β -CD	Highly water soluble, low toxicity	Oral, mucosal, parenteral (i.v.)

[14] Mucosal: nasal, sublingual, ophthalmic, pulmonary, rectal, vaginal, etc.

4.9 Pulmonary Administration of Cyclodextrins

Insulin

The relative effectiveness of CD and derivatives as pulmonary insulin absorption enhancers was investigated in rats (Lit.[33]). There was an improved hypoglycaemic response when insulin was administered intratracheally in the presence of CD. The relative effectiveness of CD in enhancing pulmonary insulin absorption as measured by pharmacodynamic relative efficacy followed the rank order of DM-β-CD > α-CD > β-CD>γ-CD > HP- β-CD. Pharmacokinetic analysis also revealed near complete insulin uptake from the pulmonary sacs upon co administration with 5% DM-β-CD. However, an absolute bioavailability of only 22% was obtained in the presence of 5% HP-β-CD. Relatively low acute mucotoxicity was observed. The absolute bioavailabilities following pulmonary insulin administration with CD revealed that the thinner epithelial cell layer of the respiratory mucosa in comparison with the intestinal mucosa offered less resistance to CD-promoted insulin uptake (Lit. [33]).

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[92] http://www.cyclodex.com/indexc.html.

5 MATERIALS AND METHODS

5.1 Materials

The following materials were used in this work:

Table 1: List of materials used

Substance	Synonym	Charge/Lot Number	Supplier
Ascorbic acid	Vitamin C	99K29GN-703239 32043334	Bufa B.V. Pharmaceuticals products, Uitgeest, Holland Merck GmbH, Darmstad, Germany
Aspartame		433698/1	Fluka AG, Buchs, Switzerland
Azithromycin		410150	Hande Industry & Trade Holdings Limited, China
Calcium chloride-Dihydrate		TAG 43081012	Merck GmbH, Darmstad, Germany
Chitosan		4040701	Pfannenschmidt GmbH, Germany
Cyclosporin A		FPO 1472 C	Fährhaus Pharma GmbH, Hamburg, Germany
Citric acid monohydrate		00125HH-155795	Bufa B.V. Pharmaceuticals products, Uitgeest, Holland
Cyclodextrin	Cavamax W6 (α-CD)	60P301	Wacker Chemie GmbH, Munich, Germany
Cyclodextrin	Cavamax W7 (ß-CD)	70P225	Wacker Chemie GmbH, Munich, Germany
Cyclodextrin	Cavamax W8 (γ-CD)	80P200	Wacker Chemie GmbH, Munich, Germany
Cyclodextrin	Cavasol W8 HP (2-Hydroxypropyl-ß-cyclodextrin)	83B003	Wacker Chemie GmbH, Munich, Germany
Cyclodextrin	Heptakis (2,6-Di-O-Methyl)-ß-Cyclodextrin	043K0717	Sigma-Aldrich Chemie GmbH, Munich, Germany
Cyclodextrin	(2-Hydroxypropyl-ß-cyclodextrin	447841/1	Fluka AG, Buchs, Switzerland

Cyclodextrin	(2-Hydroxypropyl)-ß-cyclodextrin	454270/1	Fluka AG, Buchs, Switzerland
Cyclodextrin	Hydroxypropyl-ß-Cyclodextrin	04909TA-483	Fluka AG, Buchs, Switzerland
Cyclodextrin	Kleptose HP (Hydroxypropyl-ß-cyclodextrin)	E2004	Roquette Inc., Lestrem, France
Cyclodextrin	Kleptose HPB (2-Hydroxypropyl-ß-cyclodextrin)	E0018	Roquette Inc., Lestrem, France
Cyclodextrin	Kleptose HPB (2-Hydroxypropyl-ß-cyclodextrin)	E0020	Roquette Inc., Lestrem, France
Cyclodextrin	maltosyl-α-CD-Mixture	97135	Dexy Pearl / Ensuiko sugar refining CO.,LTD, Japan
Cyclodextrin	Trappsol(6-0-Maltosyl-β-CD)	804712	Cyclodextrin Technologies Development Inc., Florida, USA
Cyclodextrin	Trappsol(Hydroxy propyl-β-CD)	060474T005	Cyclodextrin Technologies Development Inc., Florida, USA
Cyclodextrin	Trappsol (Hydroxypropyl-β-CD)	090174B008	Cyclodextrin Technologies Development Inc., Florida, USA
Cyclodextrin	Heptakis(2,6-Di-O-Methyl)-ß-Cyclodextrin	043 K 0717	Sigma-Aldrich Chemie GmbH, Munich, Germany
Cyclodextrin	(2-Hydroxypropyl)-ß-cyclodextrin	447841/1	Fluka AG, Buchs, Switzerland
Cyclodextrin	Hydroxypropyl-ß-Cyclodextrin	04909TA-483	Sigma-Aldrich Chemie GmbH, Munich, Germany
Cyclodextrin	Trappsol (Hydroxypropyl β-CD)	1203109J2	Cyclodextrin Technologies Development Inc., Florida, USA
Cyclodextrin	Ethylated-ß-cyclodextrin	CYL-292	CycloLab, Budapest, Hungary
Cyclodextrin	Trappsol (Hydroxypropyl β-CD)	090174B008	Cyclodextrin Technologies Development Inc., Florida, USA
Cyclodextrin	Trappsol (Maltosyl Alpha CD Mixture)	91203	Cyclodextrin Technologies Development Inc., Florida, USA
Cyclodextrin	Trappsol (Maltosyl α-CD Mixture)	91203	Cyclodextrin Technologies Development Inc., Florida, USA

Glucose Monohydrate		K28721342 K31253942	Merck GmbH, Darmstad, Germany Merck GmbH, Darmstad, Germany
Hydroxypropyl-methylcellulose		390367/1 33301	Fluka AG, Buchs, Switzerland
Lipoid S 100	Soja lecithin	790260-1	Lipoid GmbH, Ludwigshafen, Germany
Lipoid S 100	Soja lecithin	790474-4/27	Lipoid GmbH, Ludwigshafen, Germany
Lipoid S 100	Soja lecithin	790501-4 / 23	Lipoid GmbH, Ludwigshafen, Germany
Lipoid S 100	Soja lecithin	790509-2/22	Lipoid GmbH, Ludwigshafen, Germany
LysineMonohydrat		K27386233	Merck GmbH, Darmstad, Germany
Magnesiumsulfat Hexahydrat		431328/1	Fluka AG, Buchs, Switzerland
Pentoxifylline		0010102-S 23924705	LKT Labs, Minnesota, USA LKT Labs, Minnesota, USA
Pluronic F 68		X02316P Y07018P	Bioconcept, Allschwil, Switzerland Bioconcept, Allschwil, Switzerland
Polyvinyl-pyrrolidon K25		368948/123198	Fluka AG, Buchs, Switzerland
Polyvinyl-pyrrolidon K30		375755/141798	Fluka AG, Buchs, Switzerland
Sodium acetate		032K0177	Sigma-Aldrich Chemie GmbH, Munich, Germany
Sodium ascorbate		43457025 32960244 43457025	Merck GmbH, Darmstad, Germany Merck GmbH, Darmstad, Germany Merck GmbH, Darmstad, Germany
Trehalose		S4112361	Merck GmbH, Darmstad, Germany
Tween 80		40762194 083K0588	Merck GmbH, Darmstad, Germany Sigma-Aldrich Chemie GmbH, Munich, Germany

Tyloxapol		083K0588	Sigma-Aldrich Chemie GmbH, Munich, Germany
Vitamine E acetate	alpha-Tocopherol-acetate	2450412	Merck GmbH, Darmstad, Germany

The other chemicals were of pharmaceutical or reagent grade and were used without further purification.

5.2 Physicochemical Properties of the Drugs Substances

5.2.1 Cyclosporin A

Cyclosporin is a cyclic oligopeptide produced by the fungus *Tolypocladium inflatum Gams*, or *Cylindrocapron lucidum Booth*.
It is a hydrophobic cyclic peptide built from nonmammalian amino acids with low oral bioavailability Lit [34]. Cyclosporin A (CSA) is one of several biologically active cyclosporins produced by these fungi (Lit. [35]).

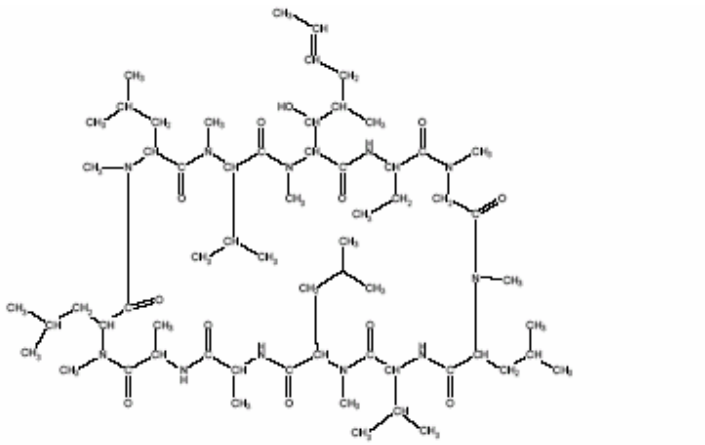


Figure 1: Structure of Cyclosporin A.

It is a first line immunosuppressive drug used to prevent transplant rejection and to threat autoimmune diseases. CSA causes selective suppression of cell mediated immunity by inhibition of T cells Lit.[35].
Cyclosporin A is a potent immunosuppressive agent extensively employed to avert graft rejection in kidney, liver and bone marrow transplant patients and to delay or prevent disease progression in patients with autoimmune disease and inflammation (Lit. [36-39]). The immunosuppressive effect of CSA is due to a selective and reversible inhibition of T-lymphocytes (Lit. [40-41]). CSA selectively inhibits interleukin-2 (IL-2) driven proliferation of activated T-lymphocytes.

In spite of the great clinical importance of the drug, their extended usage has often been limited by several disadvantages including low bioavailability, narrow therapeutic window, nephrotoxicity and hepatotoxicity (Lit. [42-43]).
Nephrotoxicity may appear acutely, associated with reversible hemodynamic changes, or as a result of interstitial fibrosis after chronic dosing (Lit. [44]- Lit. [45]) It has also, been reported that Cremophor EL®, a solubilizing agent, present in the commercially available intravenous dosage form of cyclosporin A, is nephrotoxic and haemolytic (Lit. [46-47]).

5.2.1.1 Physicochemical properties of cyclosporin A

Cyclosporin A occurs as white prismatic crystals from acetone. It is insoluble in water and *n*-hexane and very soluble in all other organic solvents such as methanol, ethanol, acetone, ether, and chloroform. Cyclosporin A has a melting point of 148-151 °C (natural) and 149-150 °C (synthetic). It is stable in solution at temperatures below 30 °C but is sensitive to light, cold and oxidization. When heated to decomposition, cyclosporin A emits toxic fumes of nitrogen oxides (NO_x).

5.2.2 Azithromycin

Azithromycin is a macrolide antibiotic belonging to the azalide group. It has *in vitro* activity against many gram-positive and gram-negative aerobic and anaerobic bacteria. It also has greater stability than erythromycin in the presence of an acid.
Azithromycin binds to the 50S ribosomal subunit of the 70S ribosome of susceptible organisms, thereby inhibiting RNA-dependent protein synthesis.

5.2.2.1 Physicochemical properties of azithromycin

Azithromycin, as the dihydrate, is a white crystalline powder with a molecular formula of $C_{38}H_{72}N_2O_{12}\cdot 2H_2O$. It is not water soluble but it is soluble in Acetone, Chloroform, Methyl Chrolide, Ethyl Acetate, Ethanol, Methanol and Dimethyl Formamide. It has a pH between 9-11 and a molecular weight of 785.0.

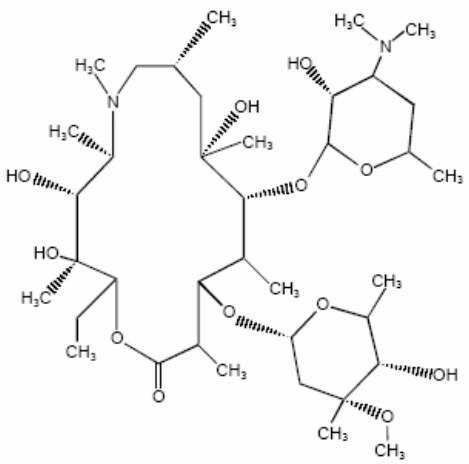


Figure 2: Structure of Azithromycin.

5.2.3 Pentoxifylline

Pentoxifylline (PTX) is a xanthine derivative that has generated widespread interest in the field of oncology based on its reported potential ability to ameliorate radiation- and chemotherapy-induced toxicity.
The radiosensitivity of the lung tissue limits the dose of radiation which can be delivered to tumours in the thoracic region. Radiation-induced lung damage may arise depending on the total dose of radiation, the fractionation schedule, the volume of lung tissue irradiated, the existence of prior lung disease and the use of chemotherapeutic drugs in the treatment of the disease (Lit. [48-50]). Damage to endothelial or epithelial cells is assumed to be the initial step leading to radiation pneumonitis and ultimately to pulmonary fibrosis.
However, the process of injury and repair initiated by irradiation is also a function of activation of cells to produce important biological mediators, such as cytokines, which modulate diverse aspects of the inflammatory and fibrogenic response.
Therefore, the pathophysiological tissue response after lung irradiation implies the induction of numerous cytokines which form the basis for the multicellular interactions of the inflammatory and fibrogenic process associated with radiation injury (Lit. [51-57]). The relative role of cytokine dysregulation versus direct tissue injury from irradiation for the pathogenesis of radiation pneumonitis/fibrosis remains elusive.
Tumor necrosis factor- α (TNF-α) is thought to be a key mediator for the pathogenesis of radiation pneumonitis, because it shows a varied spectrum of biological activities. TNF-α exerts in particular proinflammatory effects by inducing the expression of adhesion molecules that recruit leukocytes into the sites of tissue damage, by priming leukocytes for oxidant production, and by inducing production of prostaglandins and other mediators of inflammation. TNF-α inhibits anticoagulatory mechanisms and therefore promotes thrombotic processes (Lit. [58]). In addition, TNF-α exerts fibrogenic effects by stimulating the growth of fibroblasts and increasing the collagen deposition (Lit. [59]). Therefore, a pharmacological regulation of the TNF- α production at the initial stage could possibly halt the progression of radiation-induced injury. A drug which suppresses the production of TNF-α but lacks the many side effects of glucocorticoids might be useful as an anti-inflammatory agent.
In addition, PTX down-regulates the production of proinflammatory cytokines, particularly TNF-α, in response to noxious stimuli and inhibits granulocyte-mediated cytotoxicity after TNF- α exposure and may, therefore, provide protection against radiation-induced, cytokine-mediated cellular damage (Lit. [60-63]). PTX is able to inhibit the synthesis of messenger RNA (mRNA) for TNF-α in mouse peritoneal macrophages at the transcriptional level (Lit. [63-64]). Also in humans, PXT is able to reduce the release of TNF-α by peripheral blood monocytes (PBM) (Lit. [66]). Compared with other new agents, PTX is relatively inexpensive and has few side effects. A recent study suggests that patients with progressive sarcoidosis respond to PXT treatment (Lit. [67]). This beneficial clinical effect may be due to the interference of the drug with TNF-α production from alveolar macrophages (AM), because these cells frequently release increased amounts of TNF-α in patients with sarcoidosis (Lit.[68-70]).
In Lit. [71] it is referred the use of PTX for the treatment of bronchopulmonary dysplasia (BPD). The treatment of bronchopulmonary dysplasia with steroids still remains controversial. For this reason, a new approach was investigated using nebulised pentoxifylline

in the successful treatment of five premature neonates with bronchopulmonary dysplasia. PTX has a strong anti-inflammatory effect, mainly through its inhibitory influence on neutrophils, macrophages and monocytes. Cytokines produced by those cells may prolong inflammation and impair healing of the immature lung. PTX also inhibits synthesis of ICAM-1 and plasma soluble intercellular adhesion molecule, the concentration of the latter correlates with the risk of BPD development (Lit. [72]). Moreover, PTX as a methylxanthine derivative has a bronchodilator, diuretic and respiratory muscles stimulant effect.
The route of PTX administration was established by a pilot trial which showed that in the nebulised form, the drug exerted a significantly greater effect than when given by intravenous infusion. One possible explanation for this may be the diminished penetration of PTX from blood to the foci of lung tissue inflammation and destruction.

5.2.3.1 Physicochemical properties of pentoxifylline

Pentoxifylline is a white or almost white crystalline powder with a molecular weight of 278.3. It is soluble in water and sparingly soluble in alcohol. It is also freely soluble in chloroform, methyl alcohol and slightly soluble in ether.

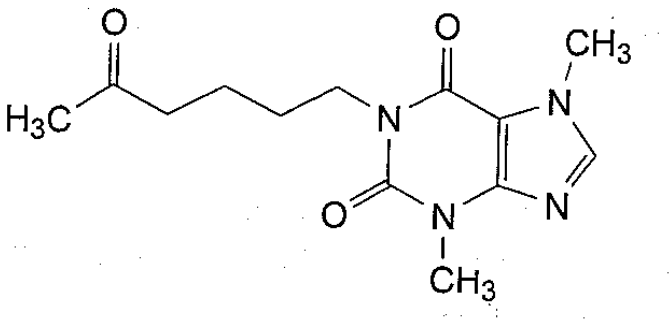


Figure 3: Structure of pentoxiphylline

5.2.4 Budesonide

Budesonide is a potent nonhalogenated synthetic glucocorticosteroid with strong topical and weak systemic effects.
Budesonide has a high topical anti-inflammatory potency and it is rapidly biotransformed in the liver. This favourable separation between topical anti-inflammatory activity and systemic effect is due to strong glucocorticosteroid receptor affinity and an effective first pass metabolism with a short half-life.

5.2.4.1 Physicochemical properties of budesonide

Budesonide is a white or almost white, crystalline powder. It is practically insoluble in water; sparingly soluble in alcohol; freely soluble in dichloromethane. The molecular weight ($C_{25}H_{34}O_6$) is 430.5.

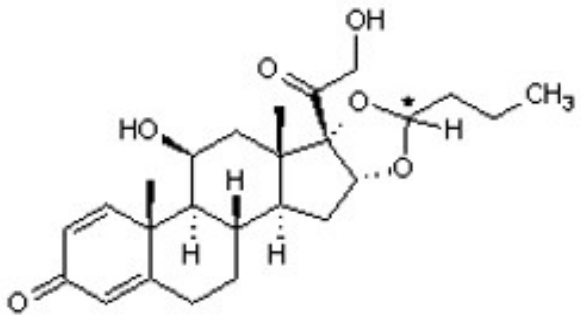


Figure 5: Structure of budesonide.

5.3 Methods

5.3.1 Analytical Methods

5.3.1.1 Particle size and zeta potential determination

The mean diameter, size distribution and zeta potential of the cyclosporin A/(β)-cyclodextrin and the azithromycin/(β)-cyclodextrin nanospheres were determined by laser diffraction using a Malvern Zetasizer (Model Zetasizer 3000 HAS, Malvern Instruments GmbH, Herrenberg, Germany).

Underlying principle

The particle size and zeta potential analysis were performed using Photon Correlation Spectroscopy (PCS). It is applicable to particles suspended in a liquid, which are in a state of random movement due to Brownian Motion (i.e. particles generally of 2 -3 µm diameter and smaller). The pace of the movement is inversely proportional to particle size (the smaller the particles are, the faster they move, or diffuse), and the pace can be detected by analyzing the time dependency of the light intensity fluctuations scattered from the particles when they are illuminated with a laser beam. On the other hand, the measurement of the zeta potential is usually done in three stages. The first stage involves checking instrument settings. The voltage sensed is checked against the voltage applied for the capillary cell. If the drop is too great, there may be a bubble in the system and therefore the measurement is aborted. . If the voltage is different, then this suggests polarisation, normally caused by bubbles at the electrode.
The second stage calculates the apparent mobility when no field is applied. This corrects for particle motion due purely to Brownian motion. The third stage is the measurement itself. The field is applied in short burst to prevent heating of the sample. The frequency is recorded and converted to electrophoretic nobilities and zeta potential.

In order to fit the measurement parameters, the samples were diluted using water as the solvent. In the case of the particle size analysis, each sample was measured in three sets (each containing 10 measurements points) and average values along with the polydispersity index were then obtained. For the zeta potential analysis, a 5 ml sample volume was used.

5.3.1.2 Scanning electron microscopy

The assessment of the form and shape of the cyclosporin A and azithromycin nanospheres was determined by scanning electron microscopy.

Underlying principle

In light microscopy, a specimen is viewed through a series of lenses that magnify the visible-light image. However, the scanning electron microscope (SEM) does not actually view a true image of the specimen, but rather produces an electronic map of the specimen that is displayed on a cathode ray tube (CRT). Electrons from a filament in an electron gun are beamed at the specimen in a vacuum chamber. The beam forms a line that continuously sweeps across the specimen at high speed. This beam irradiates the specimen which in turn produces a signal in the form of either x-ray fluorescence, secondary or backscattered electrons.
By changing the width (w) of the electron beam, the magnification (M) can be changed where

$$\mathbf{M = W/}\boldsymbol{w}$$

and W is the width of the CRT. Since W is constant, the magnification can be increased by decreasing w.
The specimens examined by SEM must be able to withstand the strong electric currents produced by the electron beam. Samples that do not conduct electricity can be damaged by the charges, which can be built up. Non-conductive specimens must first be coated with a thin layer of conductive material. Since most biological samples are non-conductive, they must be coated. The coating produces a nanometre thickness of conductive material on the surface through a cold plasma process that retains the contours of the specimen.
However, many of these samples also need additional treatment prior to sputter coating to prevent the cells from collapsing under the intense electron beam. The types of treatments vary according to the specimens. This usually involves a process that fixes the components of the specimen.
Once the specimen is fixed, it is then glued to a sample holder. The sample holder is placed into the sputter coater until a thin layer of gold is applied to the surface. The specimen is then placed in the SEM vacuum chamber and the electron gun is switched on.

Morphological examination of the suspensions and particle size analysis were carried out using a scanning electron microscope (Jeol JSM-5510 LV, Eching, Germany) after gold coating the samples using a Cressington 108auto (Elektronen-Optik-Service GmbH, Germany).
The normal operation procedure involved:

- **Preparation of the sample:** to a glass slide, one droplet of the sample was added. After allowing the sample droplet to dry, the sample was then gold coated in order to enable it to be electrically conductive;

- **Mounting of the sample into the specimen chamber;**

- **Sample analysis:** an image will appear automatically when the vacuum is ready after a specimen is set in the specimen chamber. The operation conditions are automatically optimized when a standard recipe or a customized recipe is selected. The automatic functions include auto focus, auto stigmator, and auto contrast and brightness make the total operation simple and easy. Nevertheless, manual adjustments are also possible in order to optimize the quality of the image. All the samples were analysed following the previous operations procedures;

Every sample was analysed following the previous procedure. Several images were taken out, at different magnifications, as way of having a more detailed description of the shape and size of the nanospheres.

5.3.1.3 Determination of the Cyclosporin, Azithromycin, Budesonide and Pentoxifylline content

The content of the several drugs investigated in this work was determined by High Performance Liquid Chromatography (HPLC).

Underlying principle

The fundamental basis for HPLC consists in passing a sample (analyte mixture) in a high pressure solvent (called the mobile phase) through a steel tube (called a column) packed with sorbents (called the stationary phase).
As the analytes pass through the column they interact between the two phases (mobile and stationary) at different rates. The difference in rates is primarily due to different polarities for the analytes. The analytes that have the least amount of interaction with the stationary phase or the most amount of interaction with the mobile phase will exit the column faster. Repeated interactions along the length of the column effect a separation of the analytes. Various mixtures of analytes can be analyzed by changing the polarities of the stationary phase and the mobile phase.
As the analytes exit the column, they can be detected by various means. Refractive index, electrochemical, or ultraviolet-absorbance changes in the mobile phase can indicate the presence of an analyte. The amount of analyte leaving the column will determine the intensity of the signal produced in the detector. The detector measures a signal peak as each analyte leaves the column. By comparing the time it takes for the peak to show up (called the retention time) with the retention times for a mixture of known compounds, the components of unknown sample mixtures can be identified. By measuring the signal intensity (response) and comparing it to the response of a known amount of that particular analyte, the amount of analyte in the mixture is then determined

The specifications of the HPLC methods used in the determination of the drug content are described below:

- **Cyclosporin** content was determined by using a HPLC method with a spectrophotometer detector set at 220 nm. The chromatography analysis was performed under the following conditions: column, Waters Sunfire C18 (3.5 µm, 125*3 mm); mobile phase, 0.01% TFA (Trifluoroacetic acid): Acetonitrile (including 0.01% TFA): Iso-Propanol; gradient mode; flow rate, 0.90 ml/min;

- **Azithromycin** content was determined by using a HPLC method with a spectrophotometer detector set at 215 nm. The chromatography analysis was performed under the following conditions: column, phenomenex GeminiC18; 5µm; 110A; 150 x 4.6 mm (L*ID); precolumn, phenomenex GeminiC18; 4 x 3.0 mm; mobile phase, $H_2B_3O_3$ Buffer (pH=10.5): Acetonitrile (35%:65%); gradient mode; flow rate, 1.8 ml/min;

- **Budesonide** content was determined by isocratic reversed phase liquid chromatography using a HPLC method with a spectrophotometer detector set at 245 nm. The chromatography analysis was performed under the following conditions: column, Merck LiChrospher 60, RP Select B; 5µm; 125 x 3mm; precolumn, Merck LiChrospher 60, RP Select B; 5µm; 5 x 4mm; mobile phase, Methanol/Water (63%:37%); isocratic mode; flow rate, 1.0 ml/min;

- **Pentoxifylline** content was determined by using a HPLC method with a spectrophotometric detector set at 273 nm. The chromatography analysis was performed under the following conditions: column, Waters, Symmetry C18; 5µm; 150 x 3.9 mm (L*ID); mobile phase, 600 ml Water pH=3.0/Methanol (60%:40%); isocratic mode; flow rate, 0.8 ml/min.

5.3.1.4 Viscosity determination

The viscosities of the several formulations were determined using a Rheostress 1 from Haake, Karlsruhe, Germany.

Underlying principle

The assessment of the dynamic viscosity is based on the measurement of the cone's rotation speed upon initiation of a given and constant torque. The dynamic viscosity is calculated taking in consideration the shear stress and shear rate values.

The assessment of the dynamic viscosity is based on the measurement of the cone's rotation speed upon initiation of a given and constant torque. The dynamic viscosity is calculated taking in consideration the shear stress and shear rate values.
The rheometer specifications are described in table 2.

Table 2: Rheometer specifications (Lit. [1])

Parameters	**Values**
Angular resolution (µ rad)	0.3
Rotational speed CR-mode (min^{-1})	0.025 – 1200
Rotational speed CS-mode (min^{-1})	0.001 – 1200
Torque (mNm) 0.1 – 50 (1)	0.0005 – 100
Oscillation frequency (Hz)	0.0001 – 100 (1)
Multiwave (Hz)	0.01 – 10
Torsional movement (°)	360
Bearing mechanical	air bearing
Motor type micror	drag cup motor
Optional high shear	0.025 – 3200 rpm

(1) The highest torque is not reached at the highest oscillation frequency.

The determination of the dynamic viscosity was performed under the following conditions:

- Flow Curve with C60-1 “Ti”;
- Shear Rate: 100 to 1000 $^{1/s}$;
- Temperature: 19.5°C (stabilizing time: 50 seconds);
- Regression model: Newtonian;
- Sample volume: approx. 1 ml;

5.3.1.5 Surface tension determination

The surface tension of the several formulations was determined using Sitaonline t60 from Sita, Dresden, Germany.

Underlying principle

The measurement of the dynamic surface tension is based on the bubble-pressure method. In this case, air bubbles are injected into the test sample (liquid) by means of a capillary tube fixed at the measuring head of the tensiometer. The pressure required for this operation is assessed and can be transformed into the dynamic surface tension of the liquid test sample using the Young-Laplace formula taking into account the capillary diameter. At the same time, the bubble frequency, their standing time and the temperature of the solution are measured and stored by the tensiometer.

The determination of the dynamic viscosity was performed in the following conditions:

- Sample temperature: 20°C±1°C
- Sample volume: Approx. 10 ml

- Software parameters[15]:
  Control: Liftime
  Factor: 1.10
  Auto clr data: Off
  Auto save: Off

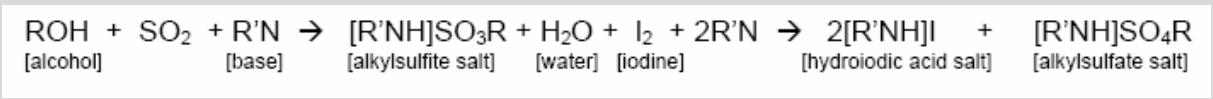
Before the beginning of every the measurement set, the surface tension value of water was determined as a reference.

5.3.1.6 Water content determination

The water content in the cyclosporin A and azithromycin lyophilizates was determined by using the Karl Fischer method.

Underlying principle

Karl Fischer method is a widely used analytical method for quantifying water content in a variety of products. It is based on the following reaction:



Water and iodine are consumed in a 1:1 ratio in the above reaction. Once all of the water present is consumed, the presence of excess iodine is detected voltametrically by the titrator's indicator electrode. Consequently, that signals the end-point of the titration.
The amount of water present in the sample is calculated based on the concentration of iodine in the Karl Fischer titrating reagent and the amount of Karl Fischer Reagent consumed in the titration.

The water content was determined by using a 756 KF Coulometer from Metrohm AG, Herisau, Switzerland. The parameters and reagents used for the measurement were:

a) Rule Parameters

- Rule range:100 mV;
- Max. rate:1500 µg/min;
- Min. rate:15 µg/min;
- Stop criteria: Relative drift;
- Relative Drift:15 µg/min.

[15] The software will allow the recording of data points over the bubble lifetime range of 1-60 secs.

b) Titration Parameters

- Pause: 15 s;
- Extraction time: 30 secs;
- Start drift: 10 µg/min;
- Temperature: 20 °C;
- Time interval: 2 secs;
- Max. tritation: OFF.

c) Reagents

- Trethylamin;
- Hydranal-Coulomat AG;
- Ethanol, denatured.

The determination of the water content was performed by weighting between 50-100 mg for both lyophilizates. The amount of sample used was based on the anticipated water content in the lyophilizates in order to obtain a suitable degree of accuracy.
The measurement was repeated two times in both cases and the obtained average value was then used.

5.3.1.7 Lyophilization

Underlying principle

Lyophilization is the term given to the process whereby water is sublimed from frozen solutions, generally under reduced pressure, leaving a dry porous mass of approximately the same size and shape as the original frozen mass. A typical freeze-drying cycle essentially consists of three stages:

a) Freezing;
b) Primary drying;
c) Secondary drying;

Since freeze-drying takes place at a lower temperature than spray-drying it is normally considered to be less destructive, especially to protein products (Lit. [2]).
In the first stage, the material is cooled until it is completely frozen. This has the effect, at least in part, of separating the water from the solutes. The second stage, usually performed under vacuum and by supplying heat to the product, involves removal of most of the water by sublimation of the ice in the product. The last stage involves the removal of sorbed water and is normally carried out at elevated product temperature to achieve efficient water removal.

Lyophilization of the samples was performed with a Christ Epsilon 2-6D, Osterode am Harz, Germany. The lyophilization parameters are described in the following table.

Table 3: Lyophilization parameters of the cyclosporin A and azithromycin samples.

Process Phase	Time (min)	Temp. (°C)	Vacuum (mbar)	Si. –Pressure (mbar)
Start value	0	0	OFF	OFF
Freezing	1	-38	OFF	OFF
Freezing	44	-38	OFF	OFF
Preparation	15	-38	OFF	OFF
Main drying	1	-38	0.08	OFF
Main drying	15	-25	0.08	0.140
Main drying	704	-25	0.08	0.140
After drying	1	-25	0.08	0.140
After drying	300	15	0.08	0.140
After drying	719	15	0.08	0.140

To the lyophilization vials, it was added 1 ml of the cyclosporin A and azithromycin solutions. At the end of the lyophilization process, the vials were then sealed and kept at 2-8°C.

5.3.1.8 Nebulisation parameters determination

The nebulisation performance was investigated using a novel electronic nebuliser, eFlow® (PARI GmbH, Starnberg, Germany). Droplet sizes of the aerosols were measured by laser diffraction (MasterSizer X, Malvern Instruments, Herrenberg, Germany).

Underlying principle

Laser diffraction is a preferred method for particle size analysis of nebulized aerosols. The method is well established and relies on the fact that particles scatter light at angles in inverse proportion to their size. Laser diffraction systems can measure over a wide dynamic range and have the flexibility to accommodate a variety of sample formats, including liquids, slurries, dry powders and sprays.

The determination of droplet size distribution laser diffraction measurements were performed according to the PARI SOPs:

- Environmental conditions (T = 23 ± 1°C, r.h. = 50 ± 5 %) were controlled and monitored. Test formulation for the characterization of the function of the eFlow® nebulizer heads was an aqueous 0.9% NaCl solution. The droplet size distributions of the formulation were assessed after the eFlow® nebulizer heads have been characterized with 0.9% NaCl solution.
- After the characterisation of the eFlow® nebulizer heads, the sample volume (2 ml) was nebulised for two minutes by using the eflow® under the same environmental conditions previously described.

- At the end, the following parameters determined:
  TOR
  MMD
  GSD
  Percentage of droplets < 5µm
  Percentage of droplets < 2µm

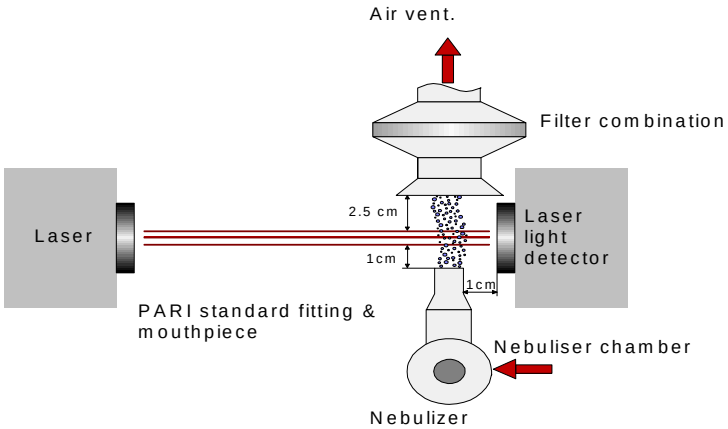


Figure 1: Schematic view of the nebulisation test section.

For a complete view of the test section, please see Figure 2-3.:

Figure 2: View of the nebulisation test section.

Figure 3: Detailed view of the nebulisation chamber containing the eflow®.

5.3.1.9 Taste masking determination

The taste of the formulations were analysed by nebulising and inhaling the formulations or by tasting it with the tongue. The tests were performed with a group of five persons. Although, we could ascertain the taste masking ability of the cyclodextrins regarding the different drug formulations with this method, we can consider it as a subjective test.

5.3.2 Preparation Methods

5.3.2.1 Preparation methods for the azithromycin formulations

5.3.2.1.1 Initial preparation method the azithromycin/CD formulations

In the first set of experiments, the following preparation method was used:

- Preparation of the cyclodextrin solution by dissolving of 2-HP-(β)-CD in water. Afterwards, the suspension is put under sonification until a clear solution is obtained;
- Next, 1 ml of the previous solution was added to each of the vials. Then, an excess of azithromycin is added to the vials. Finally, the vials are continuously stirred at room temperature for 2 hours;
- After 2 hours of stirring, the excipients are added to the vials in accordance to the factorial design;
- Then, the pH of the vials is adjusted to 6.7-7.0. Afterwards, the vials are continuously stirred at room temperature;
- After 3 days, equilibrium time, azithromycin concentration is determined by HPLC.

5.3.2.1.2 Final preparation method for the azithromycin/CD formulations

The following preparation method was used to prepare azithromycin formulations with a superior taste masking ability:

- The azithromycin is weighted into an aqueous solution. The resulting suspension is then stirred;
- Afterwards, HCl (1M) is added until all the azithromycin is solubilized;
- The 2-HP-β-CD is added to the previous solution. Then, the flask is continuously stirred at room temperature;
- Afterwards, NaOH (1M) is added quickly until the pH is between 8.85-9.0;
- Centrifugation at 6000 rpm for 1 min or filtration using a 0.45 μm filter follows;
- To the resulting clear solution, 2-HP-β-CD is added. It is stirred until a clear solution is obtained.
- Finally, the pH is adjusted to 7.5;
- Determination of the azithromycin concentration by HPLC follows.

5.3.2.2 Preparation method for the ethylated-β-cyclodextrin nanospheres

In the case of the ethylated-β-cyclodextrin nanospheres loaded with cyclosporin A, the following experimental procedure was performed:

- The ethylated-β-cyclodextrin is added into an organic solvent (ethanol). Sonification is applied until a clear solution was obtained;
- A specific amount of cyclosporin A is added to the previous solution. In this case, sonification is also applied to obtain a clear solution;
- The ethylated-β-cyclosporin A/cyclodextrin solution was then added to a 10 ml aqueous solution containing 0.88% of Pluronic F68®;
- Consequently, the nanospheres precipitated spontaneously forming an ethylated-β-cyclosporin A/cyclodextrin suspension;
- The organic solvent was then removed by evaporation under vacuum[16];
- A 1.2 μm filter was used to eliminate the large aggregates.

5.3.2.3 Preparation method for the -β-cyclodextrin nanospheres loaded with cyclosporin A

In the case of the β-cyclodextrin nanospheres loaded with cyclosporin A, the following experimental procedure was also performed:

- A specific amount of cyclosporin A was added to a flask containing 25ml or 50ml of ethanol, depending on the set experiment. Sonification was applied until a clear solution was obtained;
- Afterwards, the β-cyclodextrin (c.a. 50 mg or 100 mg) was added to the previous solution. In this case, sonification was also applied;

[16]The equipment used was the Laborata 4003-digital from Heidolph, at a pressure of 150 mbar.

- The β-cyclosporin A/cyclodextrin solution was then added to a certain volume (25 ml or 50 ml) of an aqueous solution containing the following surfactant combinations:
  0.88% of Pluronic F68®
  0.5% Pluronic F68®
  0.53% Pluronic F68® and 0.31% of Tween 80®

- Consequently, the nanospheres precipitated spontaneously forming a β-cyclosporin A/cyclodextrin suspension;
- The ethanol was then removed by evaporation under vacuum;
- A 1.2 µm filter was used to eliminate the large aggregates.

5.3.2.4 Optimisation of preparation method for the β-cyclodextrin nanospheres loaded with cyclosporin A

The method of preparation was:

- The β-CD was added to 25 ml flask. Then, the ethanol is added until the mark was reached. Sonification was applied for 10 minutes;
- Afterwards, the excess of β-CD was removed by using a 0.22 µm filter;
- To the last solution, a specific amount of cyclosporin A is added. After volume correction, the flask was sonificated until a clear solution is obtained;
- To this last solution, 25 ml of an aqueous solution containing 10 mg/ml of Pluronics F68® is added;
- After evaporation of the ethanol, c.a. 18 ml of the β-CD/cyclosporin A is obtained[17];
- Then, the suspension was diluted to 50 ml using a volumetric flask. NaCl was used as a solvent.

5.3.2.5 Preparation method for the β-cyclodextrin nanospheres loaded with azithromycin

For the case of the β-cyclodextrin nanospheres loaded with azithromycin, the following experimental procedure was also performed:

- A specific amount of azithromycin was added to a flask containing 50ml of ethanol. Sonification was applied until a clear solution was obtained;
- Afterwards, the β-cyclodextrin (c.a. 50 mg) was added to the previous solution. In this case, sonification was also applied;
- The β-cyclodextrin/azithromycin solution was then added to a 50 ml of an aqueous solution containing 0.8% of Pluronic F68®;
- Consequently, the nanospheres precipitated spontaneously forming a β-cyclodextrin/azithromycin suspension;

[17] In addition to the evaporation of the ethanol, also a part of the water was removed.

- The ethanol was then separate from the aqueous solution containing the nanospheres by centrifugation (3000 rpm for 2 min.);
- A 1.2 μm filter was used to eliminate the large aggregates.

5.3.2.6 Preparation method for the Pentoxifylline formulations

The preparation method of the pentoxifylline formulations involves:

- Preparation of a 50 mg/ml aqueous solution of pentoxifylline. Sonification is applied until a clear solution was obtained;
- To the previous solution, specific amount of 2-HP-β-CD is added in order to obtain the desired concentration of cyclodextrin. Sonification is applied until a clear solution is obtained;
- Finally, the vials are stirred at room temperature for a defined period of time.

5.3.2.7 Preparation method for the Budesonide formulations

The method of preparation was similar to the one performed for cyclosporin A with the difference that for the budesonide/cyclodextrin system, the equilibrium time and pH were, respectively, 1 day and 4.5. The method consists of:

- Preparation of cyclodextrin aqueous solutions containing different concentrations (for example, between 1-40%);
- To each of the vials containing the cyclodextrin solutions, an excess budesonide is added;
- Afterwards, sodium ascorbate was added to the vials;
- The pH was then corrected to 4.5;
- Then, the vials are stirred at room temperatures for one day;
- Finally, the solutions are filtered (0.45μm and 0.22μm) and analysed by UV-spectrophotometer.

Subchapter references

[1] https://www.thermo.com
[2] Pikal, M.J., "Freeze drying of proteins. Part I: Process design", *BioPharm.* (1990):18–27.
[3] Higuchi, T., Connors, K.A. "Phase-Solubility Techniques, Advances in Analytical Chemistry and Instrumentation", Reilly, C.,N., John Wiley & Sons, Inc., New York, (1965): 117–212.

6 RESULTS

6.1 Determination of the nebulisation parameters of the eflow

In the study performed, a Tween 80® (0.001-0.5% w/v) and glucose (5-40% w/v) aqueous solutions were prepared in order to investigate the effects of solutions with defined viscosities and surface tensions on Mass Median Diameter (MMD) and total output rate (TOR) in the eflow® to predict optimal viscosity and surface tension ranges. The nebulisation performance was investigated in accordance to the 5.3.1.8. analytical method.
The results allowed to obtain the following conclusions:

An increase of viscosity to about 2.6 mPas causes:

- Decrease in MMDs with an increase in the respirable fraction from 59% to 68%.
- Decrease in total output rates from 350 to about 200 mg/min when the viscosity rose by 1.5 mPas.

An decrease in surface tension to about 55 mN/m causes:

- Practically no effect in total output rates.

In order to confirm the previous conclusions, a pentoxifylline formulation was also analysed in terms of the relationship between Viscosity/Surface tension and the MMD/TOR.

Table 1: Physicochemical properties of 5 different pentoxifylline formulations

Formulation composition	Viscosity (mPa s)	Surface Tension (mN/m)	MMD (µm)	TOR (mg/ml)
50 mg/ml Pentoxifylline+ 10 mg/ml 2-HP-β-CD	1.24	55.10	3.36	490
50 mg/ml Pentoxifylline+ 25 mg/ml 2-HP-β-CD	1.28	55.81	3.30	476
50 mg/ml Pentoxifylline+ 50 mg/ml 2-HP-β-CD	1.38	56.59	3.25	485
50 mg/ml Pentoxifylline+ 75 mg/ml 2-HP-β-CD	1.51	56.81	3.21	454
50 mg/ml Pentoxifylline+ 100 mg/ml 2-HP-β-CD	1.64	57.12	3.05	400

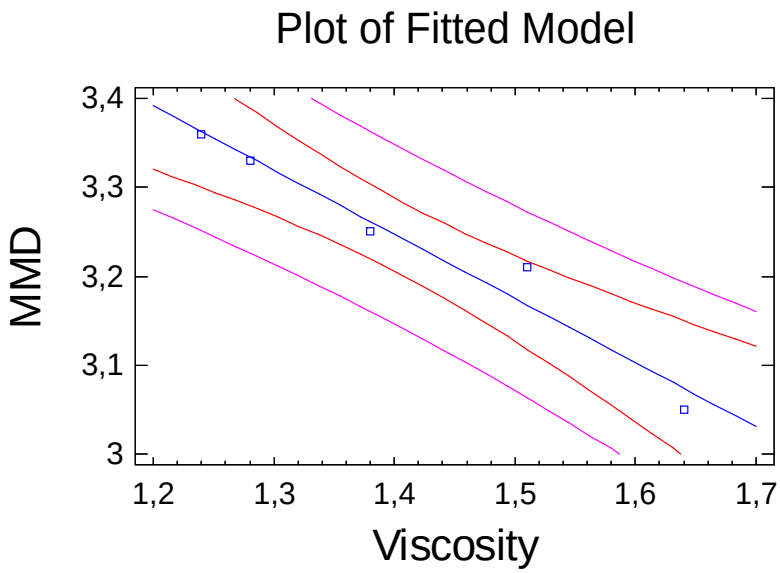


Figure 1: Fitting a linear model to describe the relationship between MMD and Viscosity. The units of viscosity mPa s and MMD are in mPa s and µm, respectively

Table 2: Fitting a linear model to describe the relationship between MMD and Viscosity. The units of viscosity and MMD are in mPa s and µm, respectively.

Parameter	Estimate	Error	Statistic	P-Value
Intercept	4.25762	0.123929	34.3554	0.0001
Slope	-0.721715	0.0874121	-8.25647	0.0037

Table 3: Analysis of Variance of the linear model to describe the relationship between MMD and Viscosity.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	0.0570877	1	0.0570877	68.17	0.0037
Residual	0.00251232	3	0.000837439	---	---
Total (Corr.)	0.0596	4	---	---	---

- Correlation Coefficient = -0.978697
- R-squared = 95.7847 percent
- R-squared (adjusted for d.f.) = 94.3796 percent
- Standard Error of Est. = 0.0289385
- Mean absolute error = 0.0168686
- Durbin-Watson statistic = 2.92116 (P=0.0088)
- Lag 1 residual autocorrelation = -0.576708

The output shows the results of fitting a linear model to describe the relationship between MMD and Viscosity. The equation of the fitted model is:

MMD = 4.25762 – 0.721715*Viscosity

Since the P-value in the ANOVA table is less than 0.01, there is a statistically significant relationship between MMD and Viscosity at the 99% confidence level.

The R-Squared statistic indicates that the model as fitted explains 95.7847% of the variability in MMD. The correlation coefficient equals -0.978697, indicating a relatively strong relationship between the variables. The standard error of the estimate shows the standard deviation of the residuals to be 0.0289385.

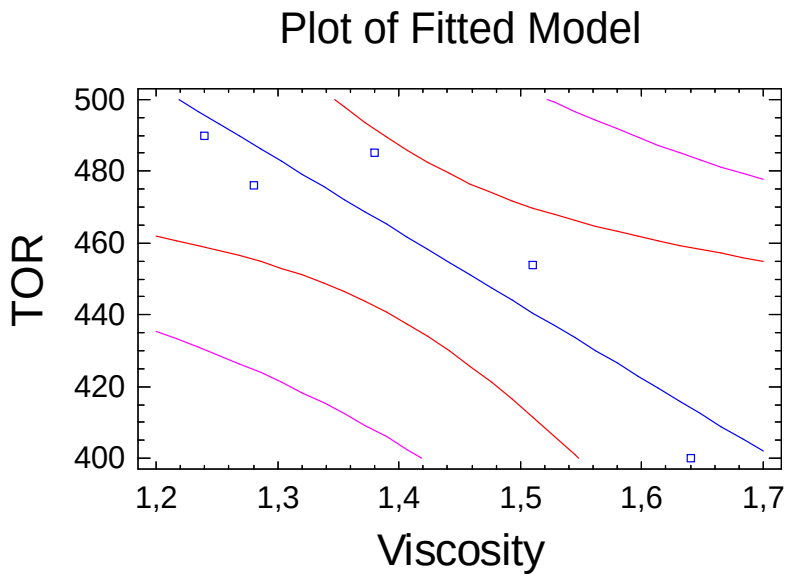


Figure 2: Fitting a linear model to describe the relationship between TOR and Viscosity. The units of viscosity and TOR are in mPa s and mg/ml respectively

Table 4: Fitting a linear model to describe the relationship between TOR and Viscosity. The units of viscosity and TOR are in mPa s and mg/ml, respectively.

Parameter	Estimate	Error	Statistic	P-Value
Intercept	748.275	72.6115	10.3052	0.0019
Slope	-203.741	51.2159	-3.97808	0.0284

Table 5: Analysis of Variance of the linear model to describe the relationship between TOR and Viscosity.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	4549.53	1	4549.53	15,83	0,0284
Residual	862.466	3	287.489	---	---
Total (Corr.)	5412	4	---	---	---

- Correlation Coefficient = -0.916863
- R-squared = 84.0638 %
- R-squared (adjusted for d.f.) = 78.7518 %
- Standard Error of Est. = 16.9555
- Mean absolute error = 12.5047
- Durbin-Watson statistic = 1.94146 (P=0.1683)
- Lag 1 residual autocorrelation = -0.105047

The output shows the results of fitting a linear model to describe the relationship between TOR and Viscosity. The equation of the fitted model is

TOR = 748.275 – 203.741*Viscosity

Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between TOR and Viscosity at the 95% confidence level.
The R-Squared statistic indicates that the model as fitted explains 84.0638% of the variability in TOR. The correlation coefficient equals -0.916863, indicating a relatively strong relationship between the variables. The standard error of the estimate shows the standard deviation of the residuals to be 16.9555.

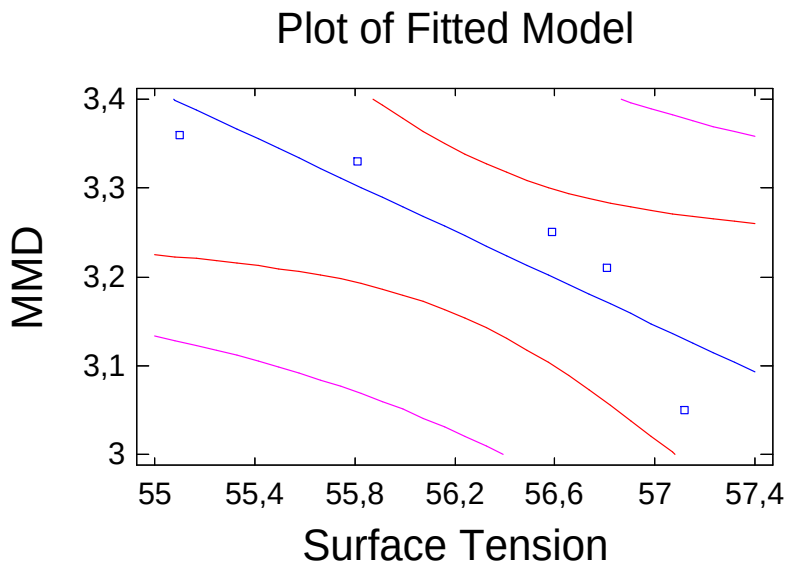


Figure 3: Fitting a linear model to describe the relationship between MMD and Surface Tension. The units of Surface Tension and MMD are in mN/m and µm, respectively.

Table 6: Fitting a linear model to describe the relationship between MMD and Surface Tension. The units of Surface Tension and MMD are in mN/m and µm, respectively.

Parameter	Estimate	Error	Statistic	P-Value
Intercept	10.6795	2.21368	4.8243	0.017
Slope	-0.132172	0.0393528	-3.36096	0.0437

Table 7: Analysis of Variance of the linear model to describe the relationship between Surface Tension and MMD.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	0.0470931	1	0.0470931	11.3	0.0437
Residual	0.0125069	3	0.00416898	---	---
Total (Corr.)	0.0596	4	---	---	---

- Correlation Coefficient = -0.888905
- R-squared = 79.0152 %
- R-squared (adjusted for d.f.) = 72.0203 %
- Standard Error of Est. = 0.0645677
- Mean absolute error = 0.0466099
- Durbin-Watson statistic = 1.51083 (P=0.0555)
- Lag 1 residual autocorrelation = -0.063802

The output shows the results of fitting a linear model to describe the relationship between MMD and Surface Tension. The equation of the fitted model is:

MMD = 10.6795 – 0.132172*Surface Tension

Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between MMD and Surface Tension at the 95% confidence level.

The R-Squared statistic indicates that the model as fitted explains 79.0152% of the variability in MMD. The correlation coefficient equals -0.888905, indicating a moderately strong relationship between the variables. The standard error of the estimate shows the standard, deviation of the residuals to be 0.0645677.

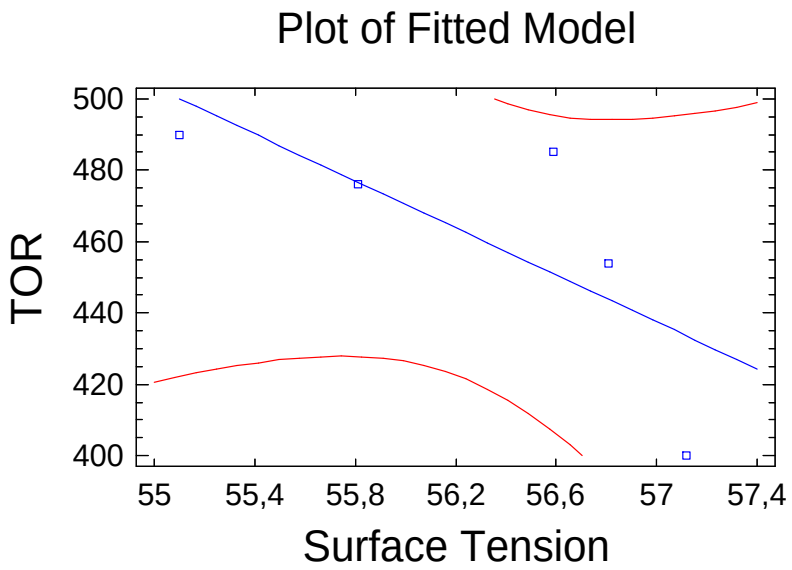


Figure 4: Fitting a linear model to describe the relationship between TOR and Surface Tension. The units of Surface Tension and TOR are in mN/m and mg/ml, respectively.

Table 8: Fitting a linear model to describe the relationship between TOR and Surface Tension. The units of Surface Tension and MMD are in mN/m and mg/ml, respectively.

Parameter	Estimate	Error	Statistic	P-Value
Intercept	2317.71	987.364	24433	0.1008
Slope	-32.9337	17.5404	-1.87759	0.1571

Table 9: Analysis of Variance of the linear model to describe the relationship between Surface Tension and TOR.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	2923.85	1	2923.85	3.53	0.1571
Residual	2488.15	3	829. 382	---	---
Total (Corr.)	5412.0	4	---	---	---

- Correlation Coefficient = -0.735019
- R-squared = 54.0254 5
- R-squared (adjusted for d.f.) = 38.7005 %
- Standard Error of Est. = 28.799
- Mean absolute error = 17.7076
- Durbin-Watson statistic = 1.51647 (P=0.0566)
- Lag 1 residual autocorrelation = -0.00453906

The output shows the results of fitting a linear model to describe the relationship between TOR and Surface Tension. The equation of the fitted model is

TOR = 2314.71 – 32.9337*Surface Tension

Since the P-value in the ANOVA table is greater or equal to 0.10, there is not a statistically significant relationship between TOR and Surface Tension at the 90% or higher confidence level.

The R-Squared statistic indicates that the model as fitted explains 54.0254% of the variability in TOR. The correlation coefficient equals -0.735019, indicating a moderately strong relationship between the variables. The standard error of the estimate shows the standard deviation of the residuals to be 28.799.

Subchapter conclusion

Since excipients and drugs can affect viscosity and surface tension, the physicochemical properties and also the concentration of each formulation component can have an influence on the final viscosity and surface tension values.
Based on this study, recommend values for viscosity and surface tension for formulations can be defined, making it possible to develop optimized formulations for the e-Flow nebuliser[18].

[18] The measurements were performed using an equipment (eflow®) that was still under development. For this reason, slight changes in the values obtained can be expected when using the final version of the device.

6.2 Solutions

6.2.1 Cyclosporin A Cyclodextrin Formulations

6.2.1.1 Introduction

In order to improve the solubility of cyclosporin A (CSA) in an aqueous solution, the possibility of using cyclodextrins as solubility enhancers was investigated.
From previous work, it is known that the smaller cavities of the α-CD molecules are more appropriate for the nonpolar aliphatic parts of the CSA molecule to be held in and, consequently more suitable for the complex formation (Lit. [14]).
As a starting point, the water solubility of CSA was determined at 2 different temperatures:

- Water solubility of Cyclosporin A at 23°C: **10.41µg/ml**
- Water solubility of Cyclosporin A at 36°C: **12.1µg/ml**

Although, the smaller cavity from the α-CD is more suitable for the inclusion complex formation, it is known that cyclodextrins and cyclodextrin complexes self-associate to form aggregates or micelle-like structures. Furthermore, it has been shown that the use of other excipients, like water soluble polymers, can interact with such systems and that these aggregates can solubilise drugs through non-inclusion complex formation.
For the reasons mentioned above, it was decided to investigate the possibility of increasing the water solubility of CSA not only by the formation of inclusion complexes but also through non-inclusion complex formation (aggregates or micelle-like structures).
Initially, the ability of several cyclodextrins to increase the solubility of CSA was investigated using the Higuchi/Connors method (Lit. [15]).

However, due to toxicological and costs issues, the amount and also the type of cyclodextrin that can be used to enhance the water solubility/stability of a drug is limited. Consequently, in order to reduce the amount of cyclodextrin necessary to obtain the desired drug solubilizing/stabilizing effect, it is important to maximize the complexing ability of the cyclodextrins without recurring to an increase of the cyclodextrin concentration.
As previous mentioned, cyclodextrins and cyclodextrins complexes are known to self-associate to form aggregates or micelle-like structures consisting of two to several hundred cyclodextrin molecules and/or cyclodextrins complexes (Lit. [16]). In addition, the drug/cyclodextrin complexes self-association forms water soluble aggregates (or microaggregates) of several drug/cyclodextrin complex units and these aggregates can solubilise lipophilic water insoluble drugs through non-inclusion complexation (Lit.[17]-Lit. 18]). For these reasons, the standard description of drug/cyclodextrin complexes in aqueous solutions is more complex as it might seem.

With this purpose in mind, several classes of substances were investigated, through the use of a factorial design, for their ability to improve the solubility/stability effect of the cyclodextrins.

6.2.1.2 Methods

The following methods were used for the preparation of the cyclosporin A formulations

6.2.1.2.1 The Higuchi Connors method and its variants

The initial preparation method for the cyclosporin A formulations was based on the Higuchi/Connors method (Lit. [3]). It consists of:

1) Preparation of aqueous solutions of cyclodextrin containing different concentrations (for example, between 1-40%);
2) To each of the vials containing the cyclodextrin aqueous solutions, an excess cyclosporin A is added;
3) Afterwards, the vials are stirred at different temperatures and for defined periods of time;
4) Then, the solutions are filtered, using 0.45 µm filters, or centrifuged;
5) Finally, the content of CSA is analysed by UV-spectrophotometer.

A variation of this method consisted in adding the several auxiliary substances used between steps 2 and 3.

One other variation involves adding the defined amounts of the CSA/Lecithin system at step 2 instead of an excess of CSA.

6.2.1.2.2 Preparation method of the CSA/CD lyophilizate

- The 2-HP-(β)-CD was dissolved in an aqueous solution. Sonification was applied until a clear solution was obtained[19];
- In parallel, the CSA/Lecithin was dissolved in an ethanolic solution. Sonification was applied until a clear solution was obtained;
- While stirring the CD solution, the sodium ascorbate was added. Sonification was applied until a clear solution was obtained;
- Afterwards, to the CD/Sodium ascorbate solution, the ethanolic CSA/Lecithin solution was added;
- Sonification was applied for 15 minutes. At the end, trehalose was added to the final solution as a cryoprotector.
- The pH was then corrected to 4.5.
- To the Lyophilization vials, 1 ml of the CSA/CD solution was added. The lyophilization program chosen was 13c GSH_2. A detailed description of the lyophilization parameters is present on table 3.

[19] The equipment used was the Sonorex Super RK 1028 from Bandelin electronics; Sonification was applied for 15 minutes.

6.2.1.3 Results

6.2.1.3.1 Phase solubility tests between CSA and several cyclodextrins

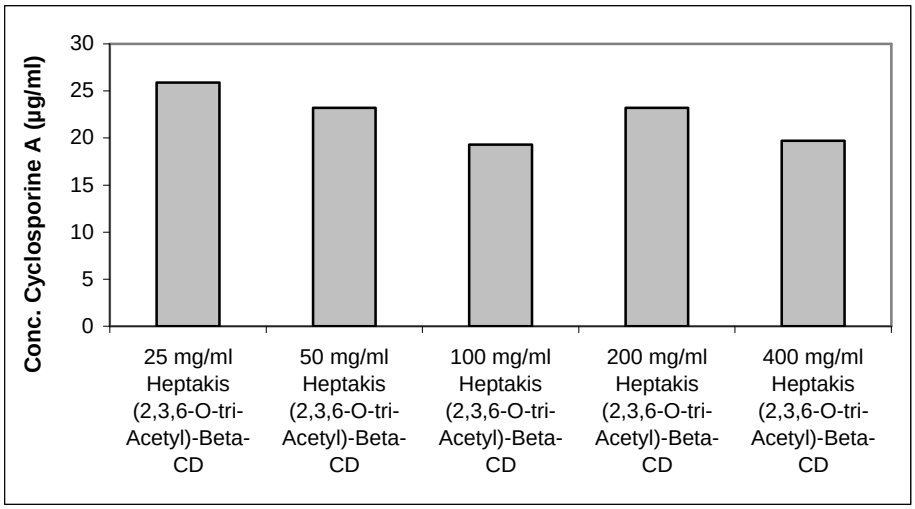


Figure 1: Phase solubility diagram in aqueous heptakis (2, 3, 6-O-tri-Acetyl)-β-CD solutions at ambient temperature.

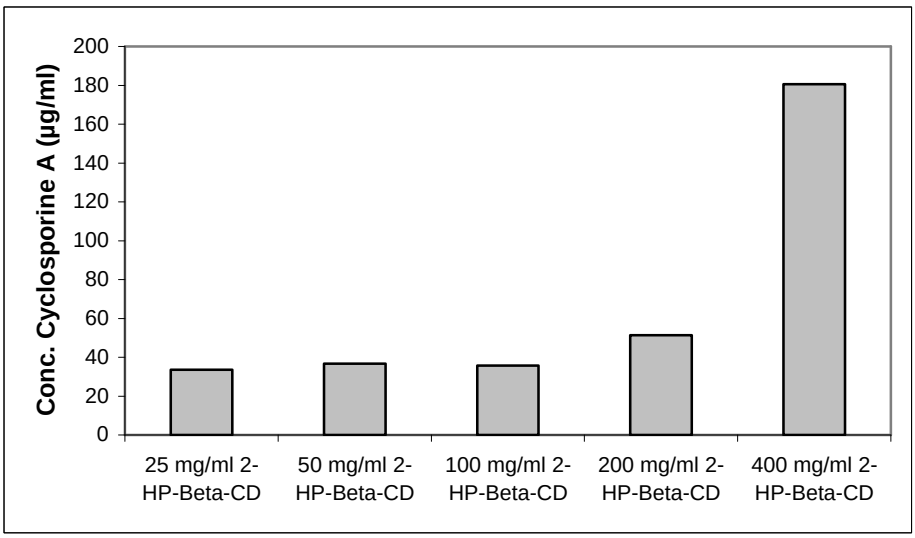


Figure 2: Phase solubility diagram in aqueous 2-HP-β-CD solutions at ambient temperature.

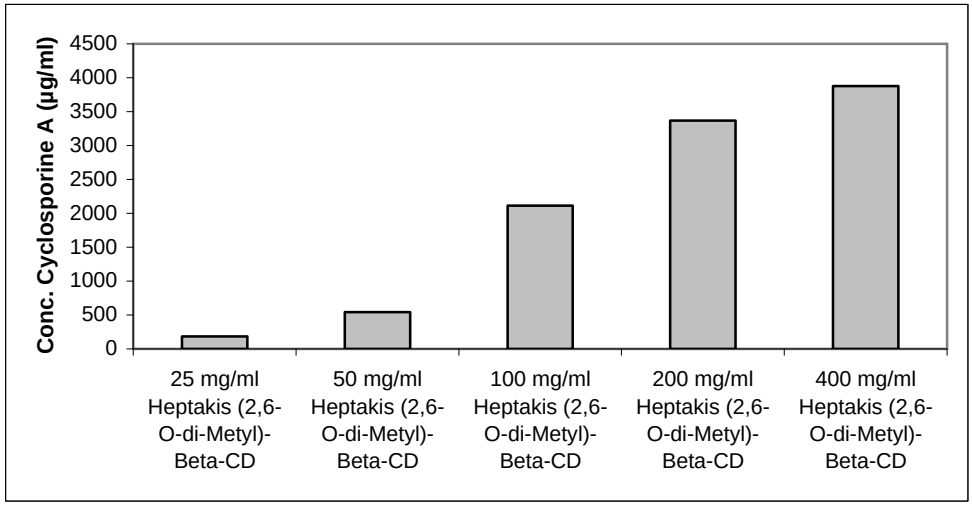


Figure 3: Phase solubility diagram in aqueous heptakis (2, 6-O-di-Methyl)-β-CD solutions at ambient temperature.

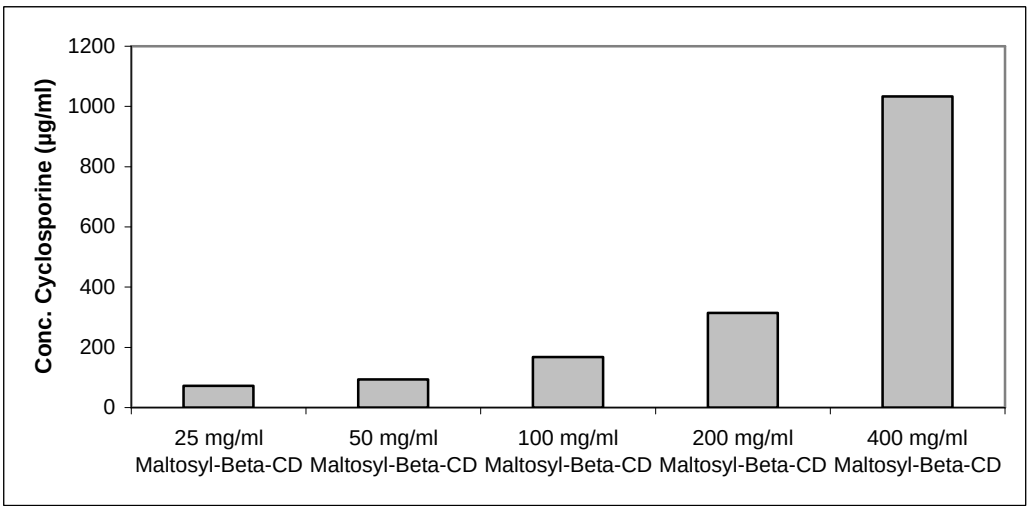


Figure 4: Phase solubility diagram in aqueous heptakis 6-O-Maltosyl-β-CD solutions at ambient temperature.

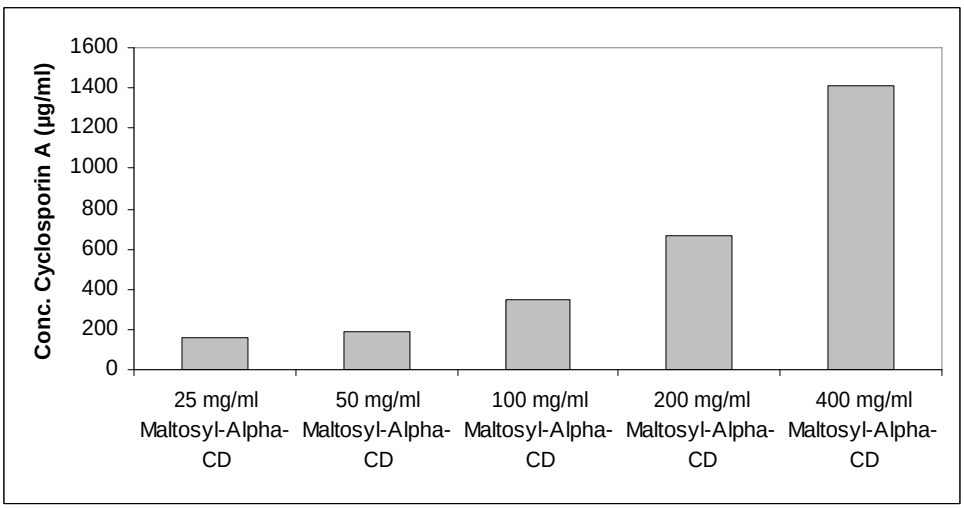


Figure 5: Phase solubility diagram in aqueous heptakis 6-O-Maltosyl-α-CD solutions at ambient temperature.

From the results obtained, it was possible to classify the different cyclodextrins in terms of their solubility profile with cyclosporin A.:

2-HP-β-CD, Heptakis (2, 6-O-di-Metyl)-β-CD, 6-O-Maltosyl-α-CD and Heptakis 6-O-Maltosyl-β-CD: Type A diagram (subtype A_p) where an increase in solubility of the compound occurs as the amount of complexing agent increases.

Heptakis (2, 3, 6-O-tri-Acetyl)-β-CD: Type B diagrams (subtype Bs) where complexes of limited solubility are formed. The curve B_S shows the formation of a complex that increases the total solubility of the compound.

6.2.1.4 Influence of the cavity size on the solubility of Cyclosporin A

In order to study the influence of the cyclodextrin cavity size on the complex formation, a Heptakis 6-O-Maltosyl-α-CD/Cyclosporin A formulation was prepared.

Table 1: Influence of the cavity size (indirectly determined by CD-type) on the cyclosporin A solubility in aqueous maltosyl-β-CD and maltosyl-β-CD solutions at ambient temperature.

Cyclodextrin Type (α or β) and concentration	**Conc. Cyclosporin A (µg/ml)**
25 mg/ml Maltosyl-α-CD	157.2
50 mg/ml Maltosyl-α-CD	192.3
100 mg/ml Maltosyl-α-CD	345.7
200 mg/ml Maltosyl-α-CD	670.3
400 mg/ml Maltosyl-α-CD	1413.8
25 mg/ml Maltosyl-β-CD	72.5
50 mg/ml Maltosyl-β-CD	93.3
100 mg/ml Maltosyl-β-CD	167.6
200 mg/ml Maltosyl-β-CD	314.1
400 mg/ml Maltosyl-β-CD	1033.7

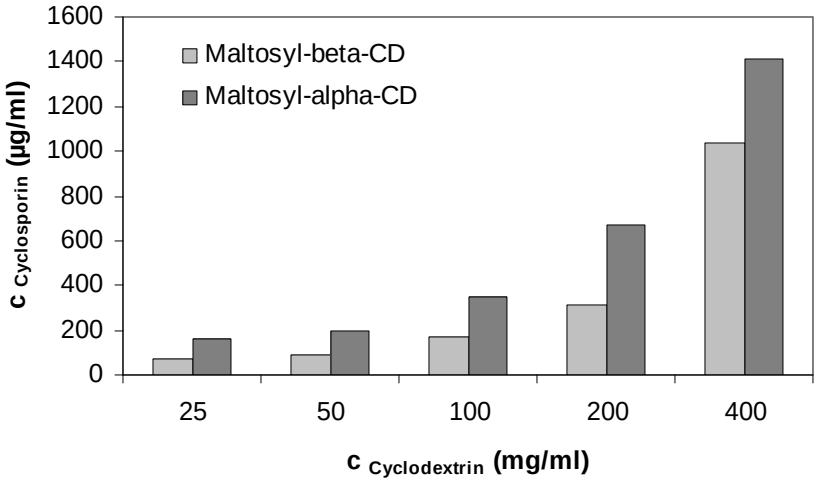


Figure 6: Influence of the cavity size on the cyclosporin A solubility in aqueous maltosyl-α-CD and maltosyl-β-CD solutions at ambient temperature.

From the results obtained, we can ascertain that the cavity size from the maltosyl-α-CD is more suitable for the formation of inclusion complexes rather than the bigger cavity of maltosyl-β-CD. This is due to the fact that the cavity size of the α-CD molecules are more appropriate for the nonpolar aliphatic parts of the CSA to be held in.

6.2.1.4.1 Influence of several excipients on the complexing ability of cyclodextrins

As mentioned before, it is known that cyclodextrins and cyclodextrin complexes self-associate to form aggregates or micelle-like structures. It has also been showed that the use of other excipients, like water soluble polymers, can interact with such systems and that these aggregates can solubilise drugs through non-inclusion complex formation.
With this purpose in mind, 3 different types of water soluble polymers were used to investigate the possibility of increasing the solubility of cyclosporin A through the formation of non-inclusion complexes.

Table 2: Effect of polymers on the solubilisation of cyclosporin A in aqueous cyclodextrins solutions at ambient temperature and with 2 days of equilibrium time.[20]

Cyclodextrin	Polymer	S_1 (µg/ml)	S_2 (µg/ml)	S_R
200 mg/ml Maltosyl-β-CD	1.1 mg/ml HPMC	314.1	324.0	1.03
200 mg/ml Maltosyl-β-CD	2.0 mg/ml HPMC	314.1	371.6	1.18
200 mg/ml Maltosyl-β-CD	2.6 mg/ml HPMC	314.1	344.3	1.10
200 mg/ml Maltosyl-β-CD	1.2 mg/ml PVP K25	314.1	250.1	0.80
200 mg/ml Maltosyl-β-CD	2.0 mg/ml PVP K25	314.1	341.4	1.09
200 mg/ml Maltosyl-β-CD	2.6 mg/ml PVP K25	314.1	271.8	0.87
200 mg/ml Maltosyl-β-CD	1.2 mg/ml PVP K30	314.1	333.7	1.06
200 mg/ml Maltosyl-β-CD	2.0 mg/ml PVP K30	314.1	357.0	1.14
200 mg/ml Maltosyl-β-CD	2.5 mg/ml PVP K30	314.1	430.1	1.37

S1 Solubility in aqueous (200 mg/ml) cyclodextrin solutions. S2 Solubility in aqueous solutions containing different concentrations of a given polymer and 200 mg/ml cyclodextrin. SR Solubility ratio.

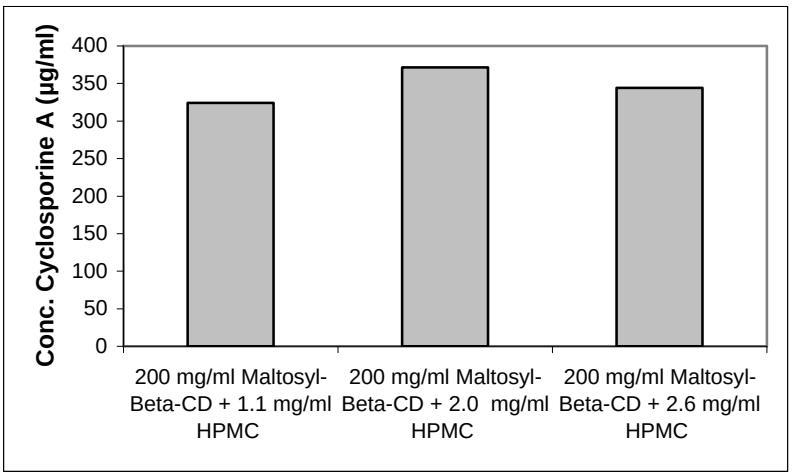


Figure 7: Influence of the HPMC concentration on the cyclosporin A solubility in aqueous maltosyl-β-CD solutions at ambient temperature.

[20] Autoclavation parameters: 120°C for 20 minutes.

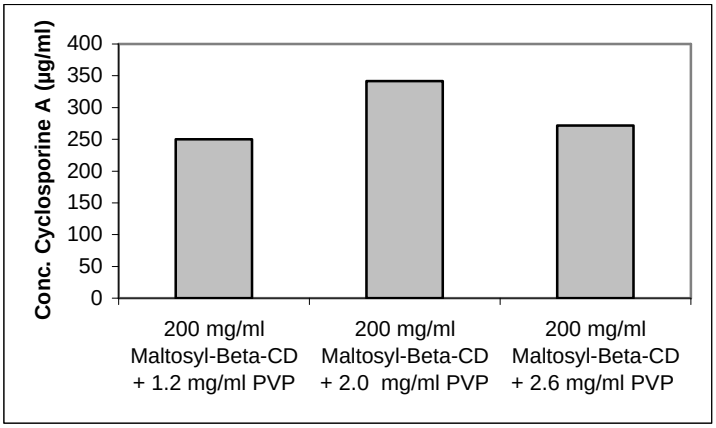


Figure 8: Influence of the PVP concentration on the cyclosporin A solubility in aqueous maltosyl-β-CD solutions at ambient temperature.

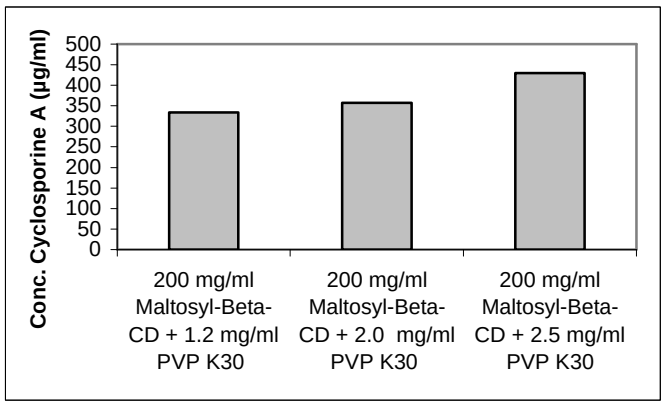


Figure 9: Influence of PVP K30 concentration on the cyclosporin A solubility in aqueous maltosyl-β-CD solutions at ambient temperature.

When the polymer and cyclodextrin are mixed together, it is possible to achieve a larger solubilization enhancement than when the polymer and cyclodextrin are used separately. The solubilization increase is mostly due to a synergistic process rather than a simple additive process.

The optimum amount of the HPMC polymer in the aqueous cyclodextrins solutions appeared to be 2.0 mg/ml. In the case of PVP and PVP K30 polymers, the optimum amount appears to be 2.0 mg/ml and 2.5 mg/ml, respectively.

With the HPMC and PVP polymers, the 2.0 mg/ml polymer concentration seems to be the most appropriate since higher concentrations lead to some decrease on drug solubility. Figures 8 and 9 shows the effect of increasing the polymer concentration on the solubility of cyclosporin A. The solubility increases with the maximum solubility at 2.0 mg/ml and then decreases slowly again.

In the case of the PVP K30 polymer, the solubility of cyclosporin A increases steadily until 2.5 mg/ml of the PVP K30 polymer. However, it is possible that the cyclosporin A solubility can be increased even more by using larger amounts of the PVP K30 polymer.

The driving force for the drug-cyclodextrin formation is the release of enthalpy–rich water molecules from the cyclodextrin cavity. These are promptly replaced by better suitable guest molecules which are less polar than water. The expulsion of the water molecules from the cyclodextrin cavity results in a negative enthalpy. The large negative ΔH° outweighed the

unfavourable negative ΔS° value. The addition of polymers to the aqueous complexation system results in an increase negative enthalpy change, together with an increase negative entropy change. However, the unfavourable ΔS° changes are outweighed by the larger ΔH° values.
Consequently, the complexation is increased upon the addition of polymers to the complexation system.
However, in the case of HPMC and PVP polymers, an increase in the concentration over 2.0 mg/ml seems to result in a decrease of the negative ΔH° value which can lead to a decrease of the cyclosporin solubility.
Nevertheless, a further improvement of the cyclosporin solubility through the use of polymers is possible by changing such parameters as autoclavation time or complexation equilibrium time.
In the next set of experiments the equilibrium and also the autoclavation time were changed in order to study the effect on the solubility of CSA.

Table 3: Effect of HPMC and PVP concentrations on the solubility of cyclosporin A in aqueous cyclodextrins solutions at ambient temperature and with 3 days of equilibrium time.[21]

Cyclodextrin	Polymer	S_1 (mg/ml)	S_2 (mg/ml)	S_R
200 mg/ml Maltosyl-β-CD	2.0 mg/ml HPMC	314	569.9	1.81
200 mg/ml Maltosyl-β-CD	2.0 mg/ml PVP	314	399.6	1.27

S_1 Solubility in aqueous (200 mg/ml) cyclodextrin solutions. **S_2** Solubility in aqueous solutions containing different concentrations of a given polymer and 200 mg/ml cyclodextrin. **S_R** Solubility ratio.

The results show that altering both the equilibrium and also the autoclavation time, it is possible to obtain an even larger increase in the solubility of CSA.

Table 4: Factorial design used to investigate the influence of several classes of excipients on the solubility of CSA.

Vial Number	Amino acids	Vitamins	Metal ions
1	+	+	+
2	+	-	+
3	+	+	-
4	+	-	-
5	-	+	+
6	-	-	+
7	-	+	-
8	-	-	-

[21] Autoclavation parameters: 120°C for 15 minutes.

For the next set of experiments 2-Hydroxyproply-β-cyclodextrin (2-HP-β-CD) was used because it is the most accepted representative of the hydroxyalkylated β-cyclodextrin derivatives, due to its high water solubility and solubilizing power, low cost and low toxicology.

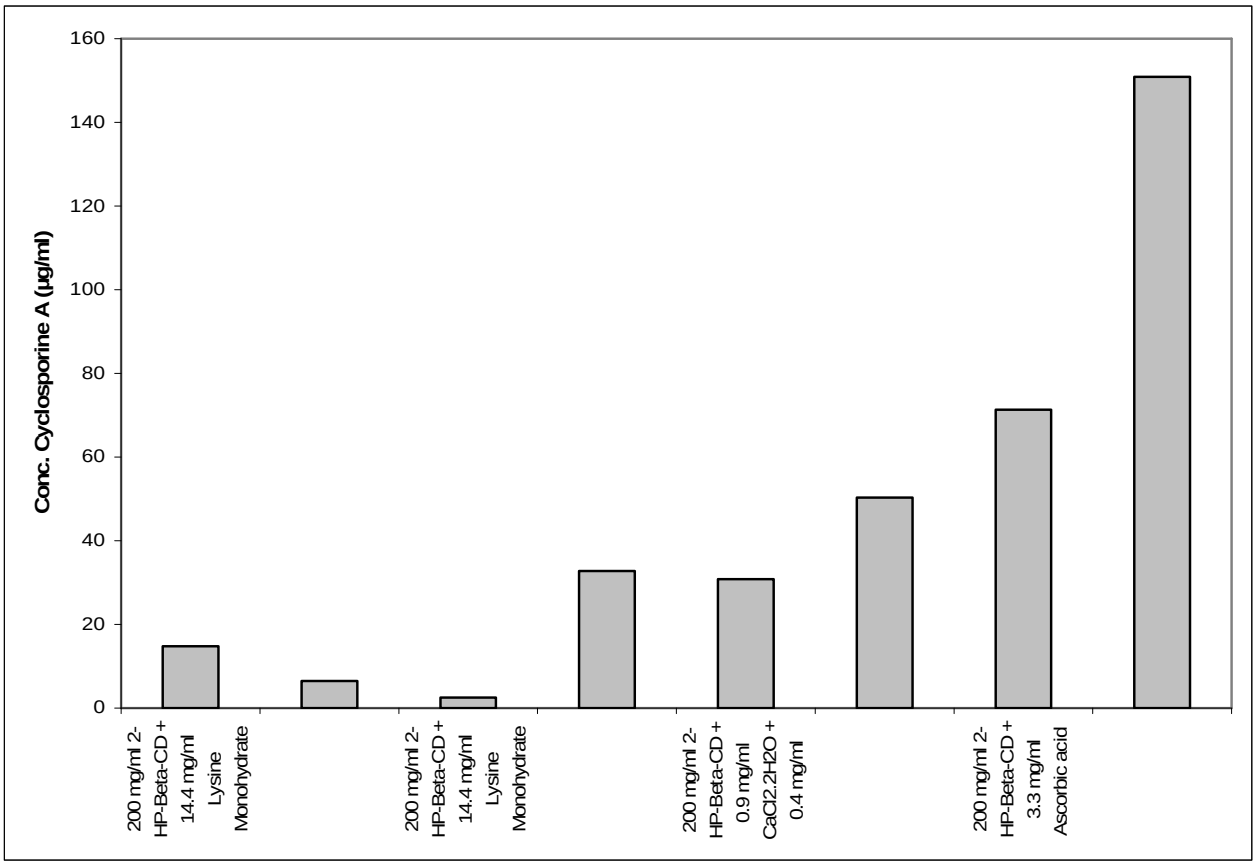


Figure 10: Phase solubility diagram of cyclosporin A in aqueous 2-HP-β-CD solutions at ambient temperature.

6.2.1.4.2 Influence of temperature on the solubility of CSA

An investigation was performed to ascertain the influence of the temperature on the solubility of cyclosporin A. The conditions are described bellow

a) First set of experiments:
Systems containing only cyclosporin A/cyclodextrin were stirred at 70°C[22] for 1 day.
The additives were then added and the system was stirred at room temperature for 3 days.

[22] It was decided to increase the temperature of the cyclosporin A/ cyclodextrin system to 70°C for specific period of time before adding the auxiliary substances.
This was based on the fact that the viscosity of the cyclosporin A/ lecithin system at room temperature is relatively high. Increasing the temperature resulted in a decrease of the viscosity of the cyclosporin A/lecithin system and, consequently, in an increase in the formation of inclusion and non-inclusion complexes.

Table 5: Phase solubility table of cyclosporin A in aqueous 2-HP-β-CD solutions. One day equilibrium time at 70°C with cyclodextrin/cyclosporin A. Afterwards, the additives were and the systems were left under stirring at room temperature for 3 days.

Formulation Composition	**Conc. Cyclosporin A (µg/ml)**
200 mg/ml 2-HP-β-CD + 0.51 mg/ml Lysine monohydrate + 0.05 mg/ml $CaCl_2.2H_2O$ + 0.076 mg/ml $MgSO_4$.6H2O + 0.35 mg/ml Ascorbic acid	192
200 mg/ml 2-HP-β-CD + 0.51 mg/ml Lysine monohydrate + 0.05 mg/ml $CaCl_2.2H_2O$ + 0.076 mg/ml $MgSO_4.6H_2O$	222
200 mg/ml 2-HP-β-CD + 0.51 mg/ml Lysine monohydrate + 0.35 mg/ml Ascorbic acid	249
200 mg/ml 2-HP-β-CD + 0.51 mg/ml Lysine monohydrate	389
200 mg/ml 2-HP-β-CD + 0.05 mg/ml CaCl2.2H2O + 0.076 mg/ml $MgSO_4.6H_2O$ + 0.35 mg/ml Ascorbic acid	a
200 mg/ml 2-HP-β-CD + 0.05 mg/ml $CaCl_2.2H_2O$ + 0.076 mg/ml $MgSO_4.6H_2O$	464
200 mg/ml 2-HP-β-CD + 0.35 mg/ml Ascorbic acid	857
200 mg/ml 2-HP-β-CD	562

a The HPLC measurement of the vial was not possible due to experimental reasons.

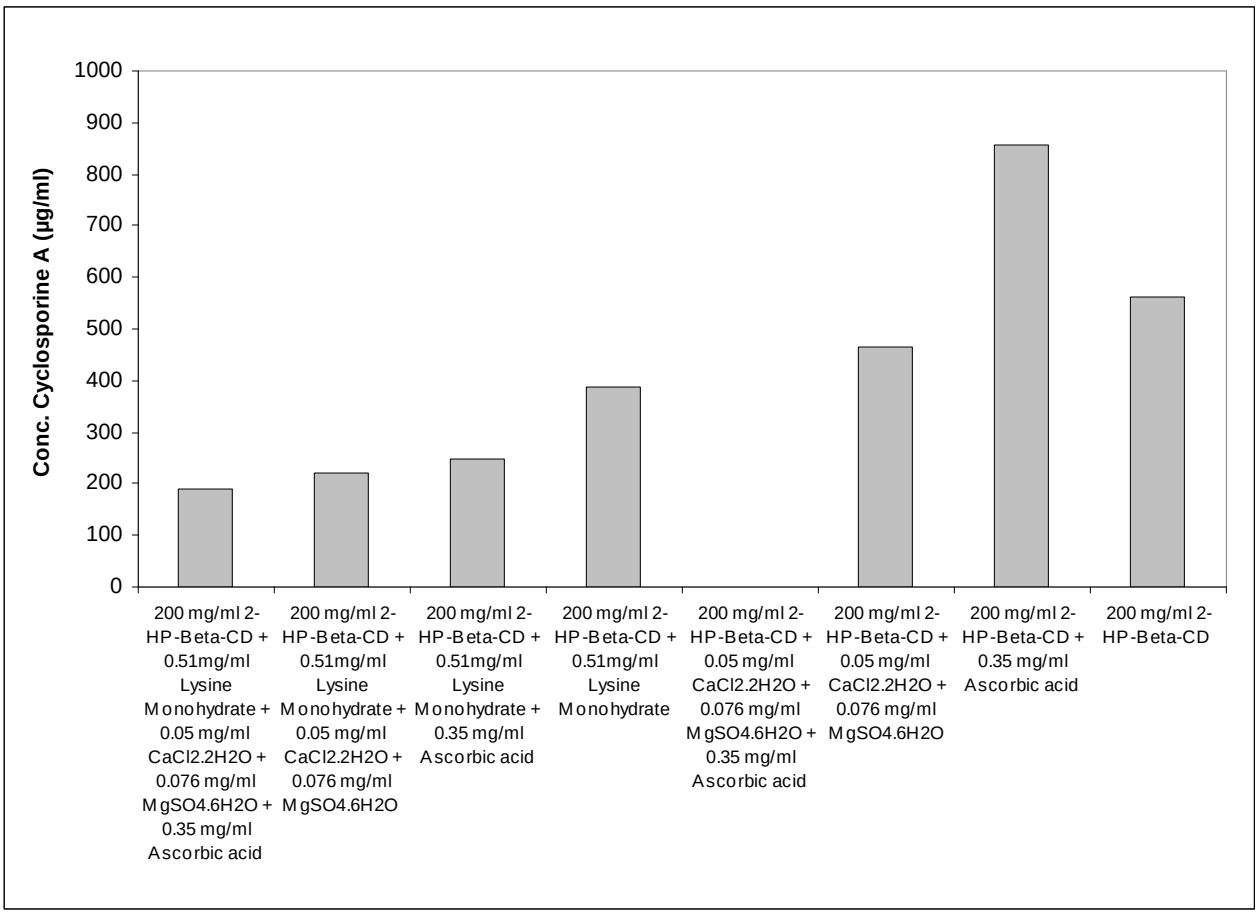


Figure 11: Phase solubility diagram of cyclosporin A in aqueous 2-HP-β-CD solutions. One day equilibrium time at 70°C with cyclodextrin/cyclosporin A. Afterwards, the additives were and the systems were left under stirring at room temperature for 3 days.

b) Second set of experiments:

Systems containing only cyclosporin A/cyclodextrin were stirred at 70°C for 5 hours
The additives were then added and the system was stirred at room temperature for 3 days.

Table 6: Phase solubility table of cyclosporin A in aqueous 2-HP-β-CD solutions. Five hours equilibrium time at 70°C with the cyclosporin A/cyclodextrin system. Afterwards, the additives were and the systems were left under stirring at room temperature for 3 days.

Formulation Composition	**Conc. Cyclosporin A (µg/ml)**
200 mg/ml 2-HP-β-CD + 0.4 mg/ml Lysine monohydrate + 0.05 mg/ml $CaCl_2$.2H2O + 0.076 mg/ml $MgSO_4.6H_2O$ + 0.35 mg/ml Ascorbic acid	1845
200 mg/ml 2-HP-β-CD + 0.4 mg/ml Lysine monohydrate + 0.05 mg/ml $CaCl_2$.2H2O + 0.076 mg/ml $MgSO_4.6H_2O$	56
200 mg/ml 2-HP-β-CD + 0.4 mg/ml Lysine monohydrate + 0.35 mg/ml Ascorbic acid	51
200 mg/ml 2-HP-β-CD + 0.4 mg/ml Lysine monohydrate	69
200 mg/ml 2-HP-β-CD + 0.05 mg/ml $CaCl_2$.2H2O + 0.076 mg/ml $MgSO_4$.6H2O + 0.35 mg/ml Ascorbic acid	898
200 mg/ml 2-HP-β-CD + 0.05 mg/ml $CaCl_2$. 2H2O + 0.076 mg/ml $MgSO_4$. 6H2O	2246
200 mg/ml 2-HP-β-CD + 0.35 mg/ml Ascorbic acid	289
200 mg/ml 2-HP-β-CD	275

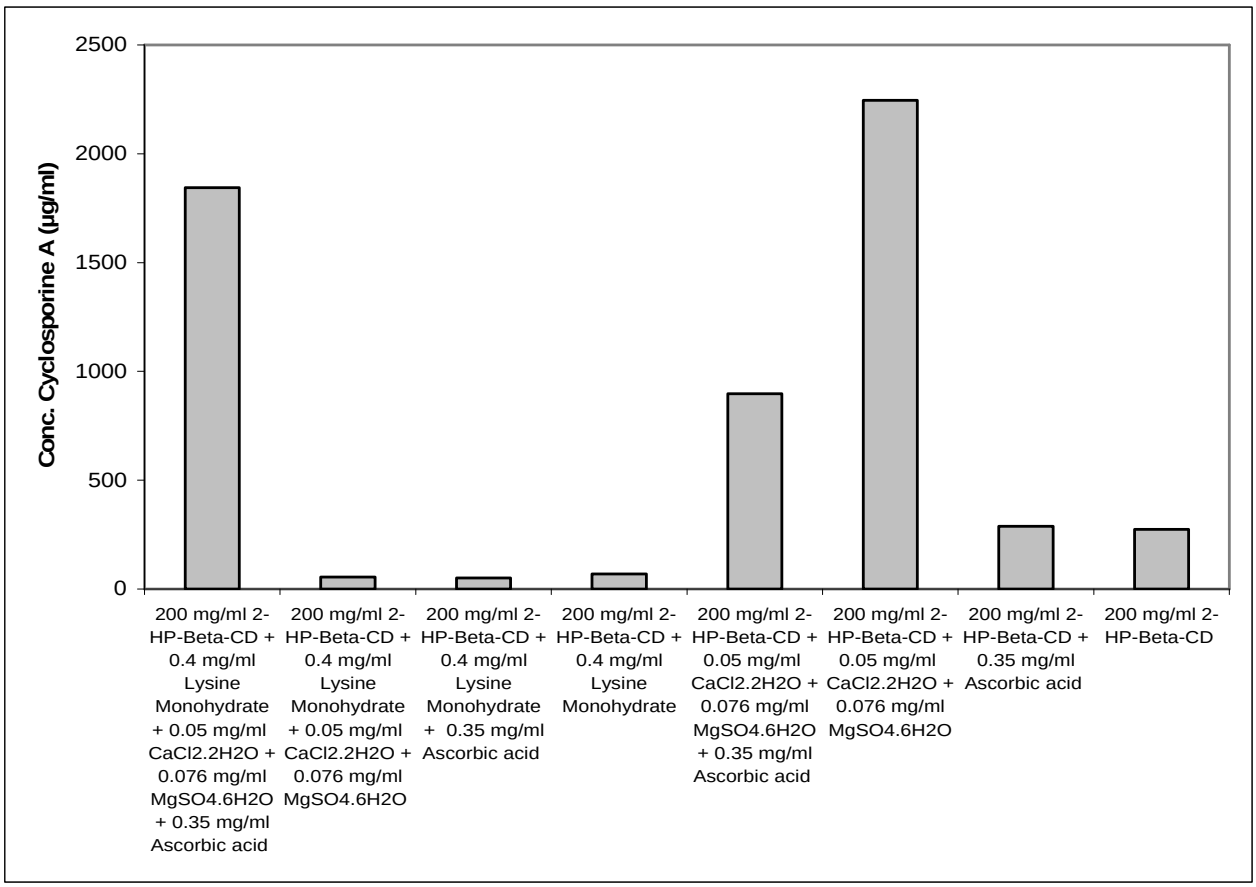


Figure 12: Phase solubility diagram of cyclosporin A in aqueous 2-HP-β-CD solutions. Five hours equilibrium time at 70°C with cyclosporin A/cyclodextrin. Afterwards, the additives were and the systems were left under stirring at room temperature for 3 days.

Based on these results, we could see that the temperature has a very important influence on the solubility of cyclosporin A. It was noticed that shorter equilibrium times at 70°C were more suitable for the increase of the cyclosporin A solubility, contrary to longer times at 70°C or at room temperature. In addition, it was also possible to ascertain that one of the best combinations were the ones containing the metal ions and the ascorbic acid as auxiliary substances.

For this reason, the experiment was repeated for the best combinations changing the preparations conditions due to stability purposes. The parameters were:

a) First set of experiments:

Systems containing only cyclosporin A/cyclodextrin at 70°C for 2 hours.

The additives were added and the system was put under stirring at room temperature for 4 days.

Table 7: Phase solubility table of cyclosporin A in aqueous 2-HP-β-CD solutions. Two hours equilibrium time at 70°C with cyclosporin A/cyclodextrin. Afterwards, the additives were added and the systems were left under stirring at room temperature for 4 days.

Formulation composition	Conc. Cyclosporin A (µg/ml)
200 mg/ml 2-HP-β-CD + 0.05 mg/ml $CaCl_2.2H_2O$ + 0.076 mg/ml $MgSO_4.6H_2O$ + 0.35 mg/ml Ascorbic acid	4136
200 mg/ml 2-HP-β-CD + 0.35 mg/ml Ascorbic acid	7485

b) Second set of experiments:

Systems containing only cyclosporin A/cyclodextrin at 70°C for 30 minutes.

The additives were added and the system was put under stirring at room temperature for 4 days.

Table 8: Phase solubility table of cyclosporin A in aqueous 2-HP-β-CD solutions. 30 minutes equilibrium time at 70°C with cyclodextrin/cyclosporin A. Afterwards, the additives were added and the systems were left under stirring at room temperature for 4 days.

Formulation composition	Conc. Cyclosporin A (µg/ml)
200 mg/ml 2-HP-β-CD + 9.6 mg/ml Ascorbic acid	12904.5
200 mg/ml 2-HP-β-CD + 41.4 mg/ml Ascorbic acid	13076.8
200 mg/ml 2-HP-β-CD + 74.9 mg/ml Ascorbic acid	12825.3
200 mg/ml 2-HP-β-CD + 100 mg/ml Ascorbic acid	10998.6
200 mg/ml 2-HP-β-CD + 119.9 mg/ml Ascorbic acid	6940.4
200 mg/ml 2-HP-β-CD + 136.4 mg/ml Ascorbic acid	9370.3
200 mg/ml 2-HP-β-CD + 150.1 mg/ml Ascorbic acid	9401.0

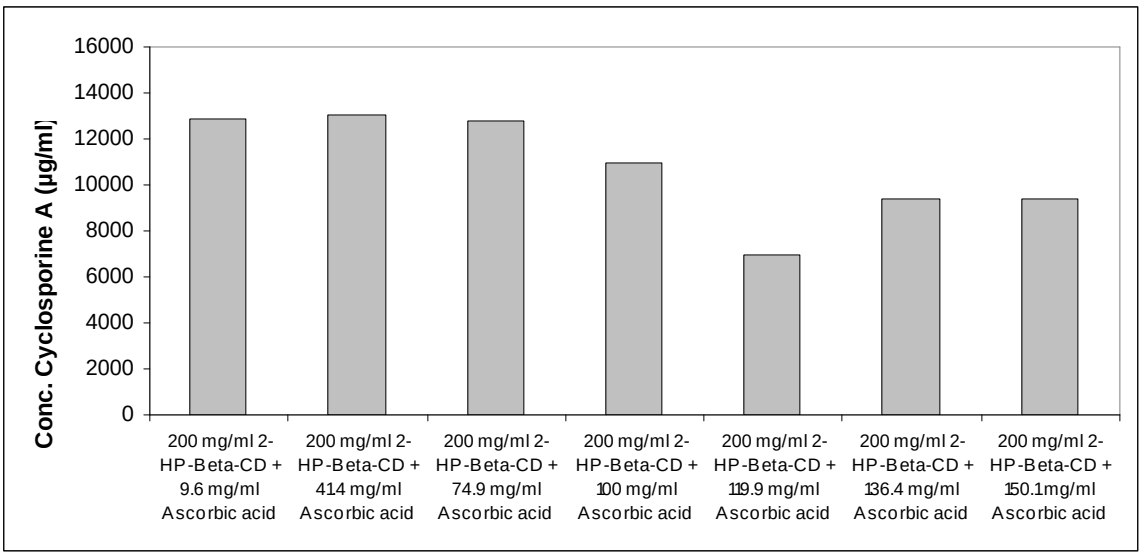


Figure 13: Phase solubility diagram of cyclosporin A in aqueous 2-HP-β-CD solutions. 30 minutes equilibrium time at 70°C with cyclosporin A/cyclodextrin. Afterwards, the additives were added and the systems were left under stirring at room temperature for 4 days.

From the results obtained from the different set of experiments, it was possible to ascertain that temperature plays a very important role on the complex formation and, consequently, on the solubility of CSA. This is mainly due to an increase in the intrinsic solubility and mobility of the lecithin/cyclosporin A system that allows an increase in the formation of inclusion and non-inclusion complexes.

6.2.1.4.3 Influence of pH on the solubility of CSA

From the results previously obtained, we could see that in presence of ascorbic acid the solubility of CSA is greatly improved. For this reason and also to ascertain the effect of the pH on the solubility, the following experiments were performed using ascorbic as the auxiliary substance.

Table 9: Influence of the pH on the solubility of in aqueous 2-HP-β-CD solutions. One day equilibrium time at room temperature with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 4 days.

Formulation Composition	**Conc. Cyclosporin A (µg/ml)**
200 mg/ml 2-HP-β-CD + 1.44 mg/ml Ascorbic acid (pH 4.5)	864
200 mg/ml 2-HP-β-CD + 3.35 mg/ml Ascorbic acid (pH 4.5)	1083
200 mg/ml 2-HP-β-CD + 6.66 mg/ml Ascorbic acid (pH 4.5)	2063
200 mg/ml 2-HP-β-CD + 9.6 mg/ml Ascorbic acid (pH 4.5)	1840
200 mg/ml 2-HP-β-CD + 1.44 mg/ml Ascorbic acid (pH 8.0)	280
200 mg/ml 2-HP-β-CD + 3.35 mg/ml Ascorbic acid (pH 8.0)	249
200 mg/ml 2-HP-β-CD + 6.66 mg/ml Ascorbic acid (pH 8.0)	191
200 mg/ml 2-HP-β-CD + 9.6 mg/ml Ascorbic acid (pH 8.0)	186

From the results we can see that the pH does have an influence on the formation cyclosporin A and 2-HP-β-CD. In the presence of ascorbic acid, the complex formation is favoured by an acidic environment.

6.2.1.4.4 Influence of sodium acetate on the solubility of CSA

The influence of sodium acetate on the solubility of CSA was also investigated. For this purpose, it was added to several vials containing 200 mg/ml 2-HP-β-CD aqueous solution increasing amounts of sodium acetate.

Table 10: Influence of sodium acetate on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 2 days.

Formulation Composition	Conc. Cyclosporin A (µg/ml)
200 mg/ml 2-HP-β-CD + 9.6 mg/ml Sodium acetate	1306
200 mg/ml 2-HP-β-CD + 18.5 mg/ml Sodium acetate	1210
200 mg/ml 2-HP-β-CD + 22.6 mg/ml Sodium acetate	544
200 mg/ml 2-HP-β-CD + 26.5 mg/ml Sodium acetate	1455
200 mg/ml 2-HP-β-CD + 33.9 mg/ml Sodium acetate	2405

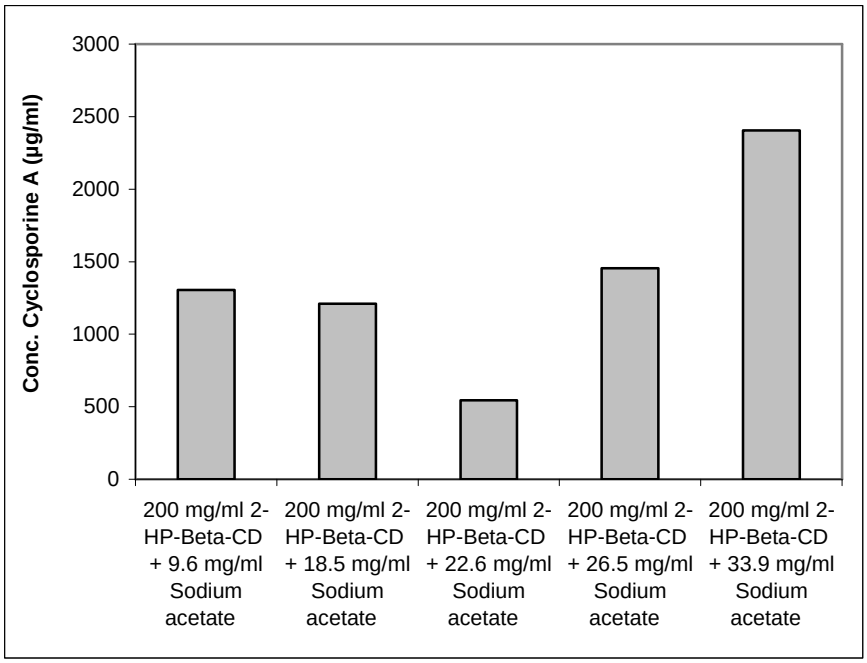


Figure 14: Influence of sodium acetate on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 2 days.

In the case of sodium acetate, the optimum amount that maximizes the complex formation and, consequently, the solubility of CSA is 33.9 mg/ml.
Figure 14 shows the effect of increasing the sodium acetate concentration on the solubility of cyclosporin A. The solubility decreases with the minimum solubility at 22.6 mg/ml and then increases rapidly.

Table 11: Influence of sodium acetate on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

Formulation Composition	Conc. Cyclosporine A (µg/ml)
200 mg/ml 2-HP-β-CD + 18.2 mg/ml Sodium acetate	1200
200 mg/ml 2-HP-β-CD + 29.2 mg/ml Sodium acetate	747
200 mg/ml 2-HP-β-CD + 33.5 mg/ml Sodium acetate	1633
200 mg/ml 2-HP-β-CD + 40.2 mg/ml Sodium acetate	1092
200 mg/ml 2-HP-β-CD + 46.3 mg/ml Sodium acetate	3697

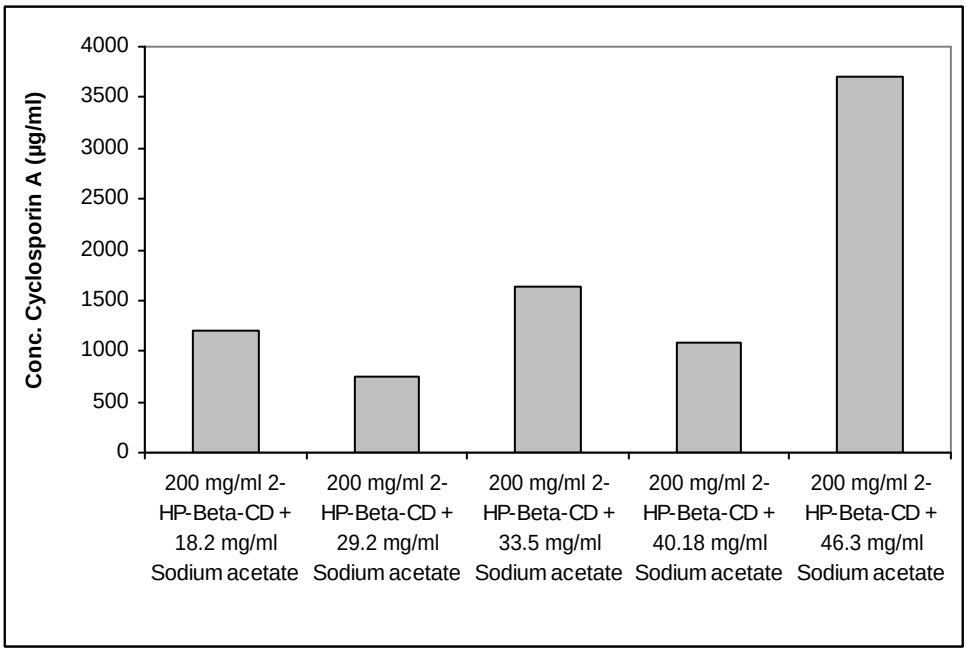


Figure 15: Influence of sodium acetate on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

The aqueous solubility of cyclosporin A at 25°C is 10.4 µg/ml. In the presence of 46.3 mg/ml of sodium acetate and 200 mg/ml of 2-HP-β-CD, the solubility is increased to 3697 mg/ml. The acetate ions are able solubilized the cyclosporin A/cyclodextrin microagregates formed in the aqueous solutions increasing, consequently, the solubility of cyclosporin A.

6.2.1.4.5 Influence of several parameters on the ascorbic acid complexation

From the previous results we could ascertain that ascorbic acid is the best auxiliary substance for the complex formation. However, as previously showed, the complex formation and, therefore, the solubility of cyclosporin A are also influenced by temperature and equilibrium time.

In the next experiment, the system containing only cyclosporin A and cyclodextrin was stirred for 30 minutes at 70°C. Afterwards, the system continued under stirring at room temperature. One day after, the ascorbic acid was added and was stirred at room temperature for 2 more days. At the end, the equilibrium time was 3 days.

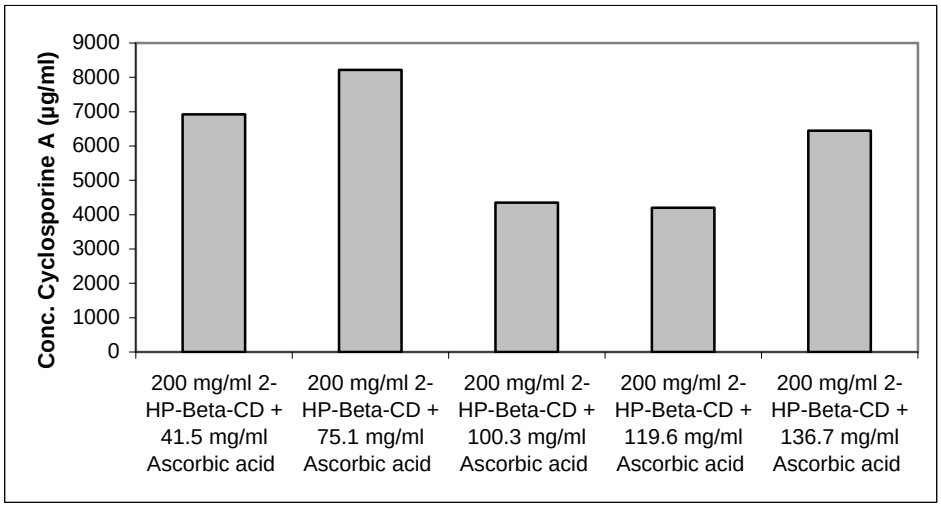


Figure 16: Influence of ascorbic acid on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 2 days.

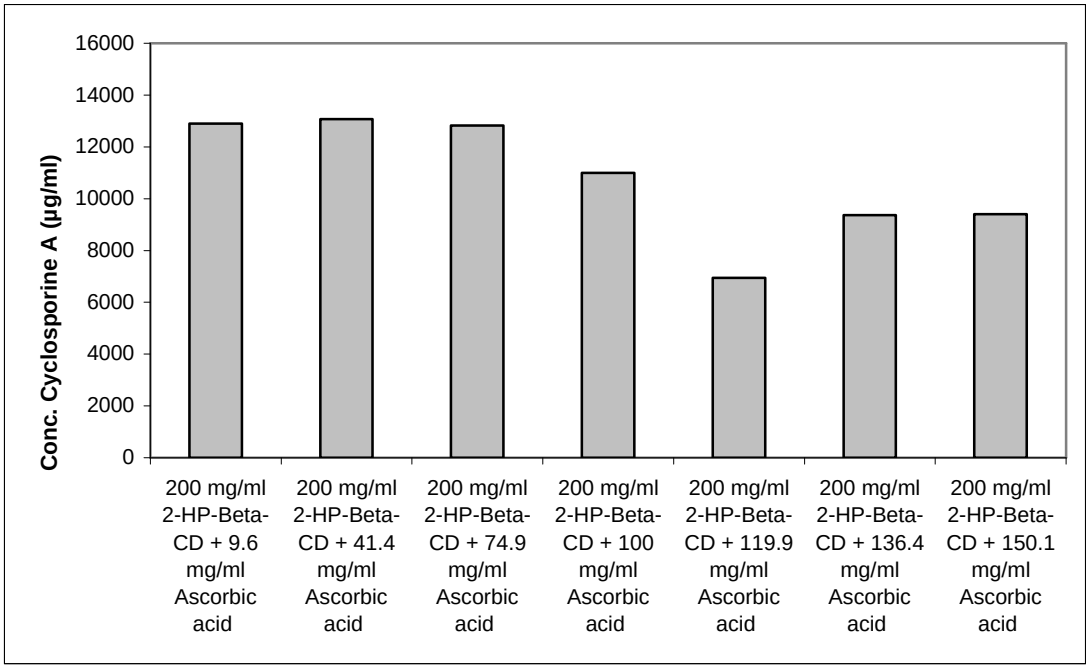


Figure 17: Influence of ascorbic acid on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 2 days.

In addition to the temperature and equilibrium time, also the relationship between the amount of cyclosporin A and the lecithin was investigated.

In the next experiment, the system containing only cyclosporin A and cyclodextrin was stirred for 30 minutes at 70°C. Afterwards, the system continued under stirring at room temperature. One day after, the ascorbic acid was added and was stirred at room temperature for 2 days. At the end, the equilibrium time was 3 days.

For a 4.0g lecithin/0.375g of cyclosporin A relationship:

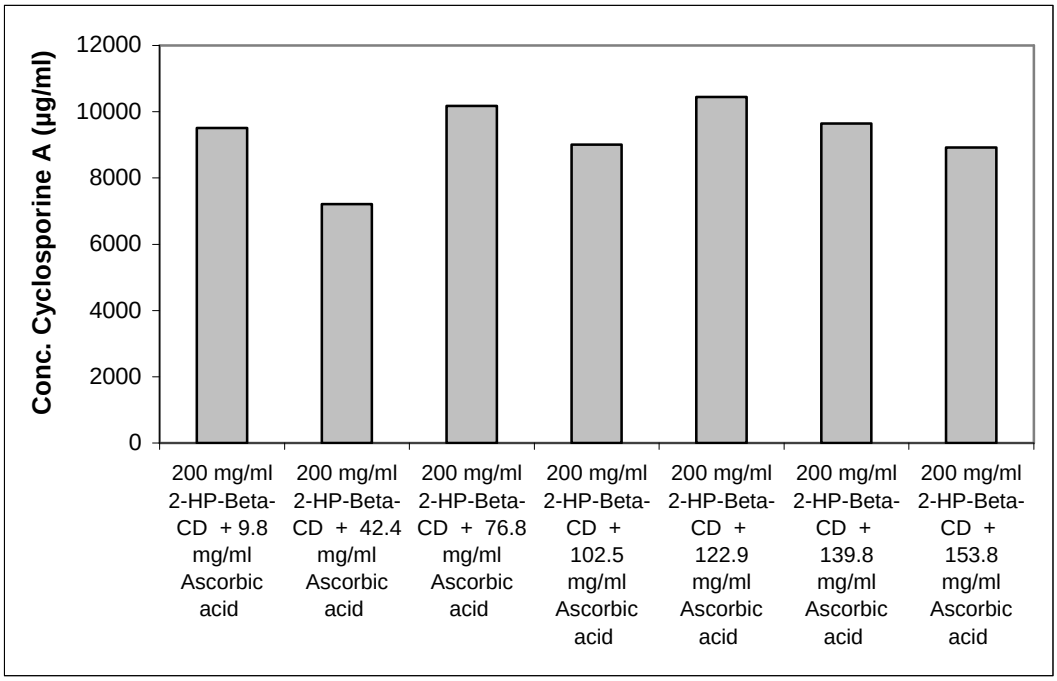


Figure 18: Influence of the relationship between the amount of cyclosporin A and the lecithin on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 2 days.

For a 4.0g lecithin/0.75g of cyclosporin A relationship:

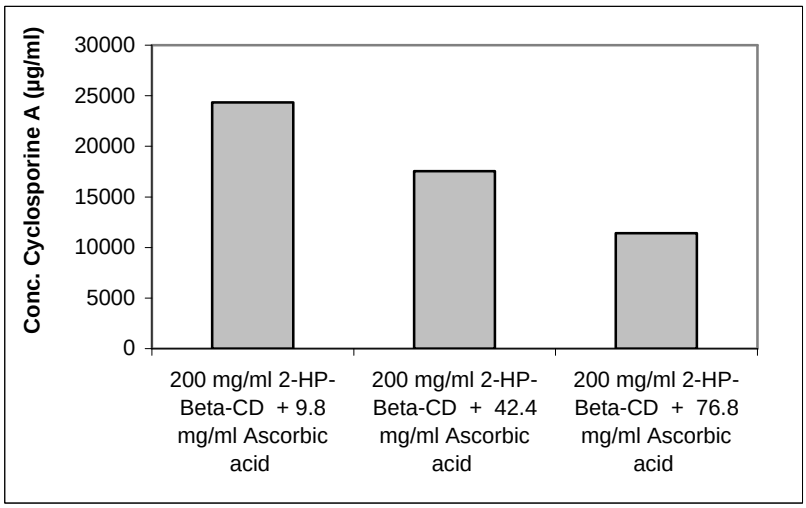


Figure 19: Influence of the relationship between the amount of cyclosporin A and the lecithin on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 2 days.

For a 4.0g lecithin/1.125g of cyclosporin A relationship:

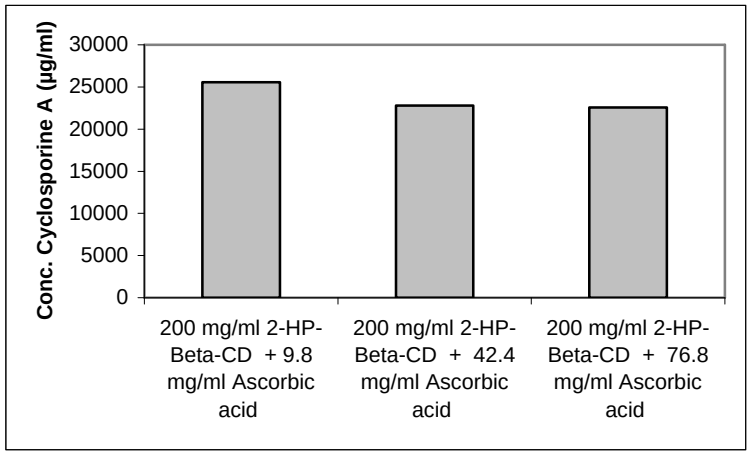


Figure 20: Influence of the relationship between the amount of cyclosporin A and the lecithin on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 2 days.

In order to study the effect of the equilibrium time on the complex formation, the previous experiment was repeated but with an equilibrium time of 7 days.

For a 4.0g lecithin/0.375g of cyclosporin A relationship:

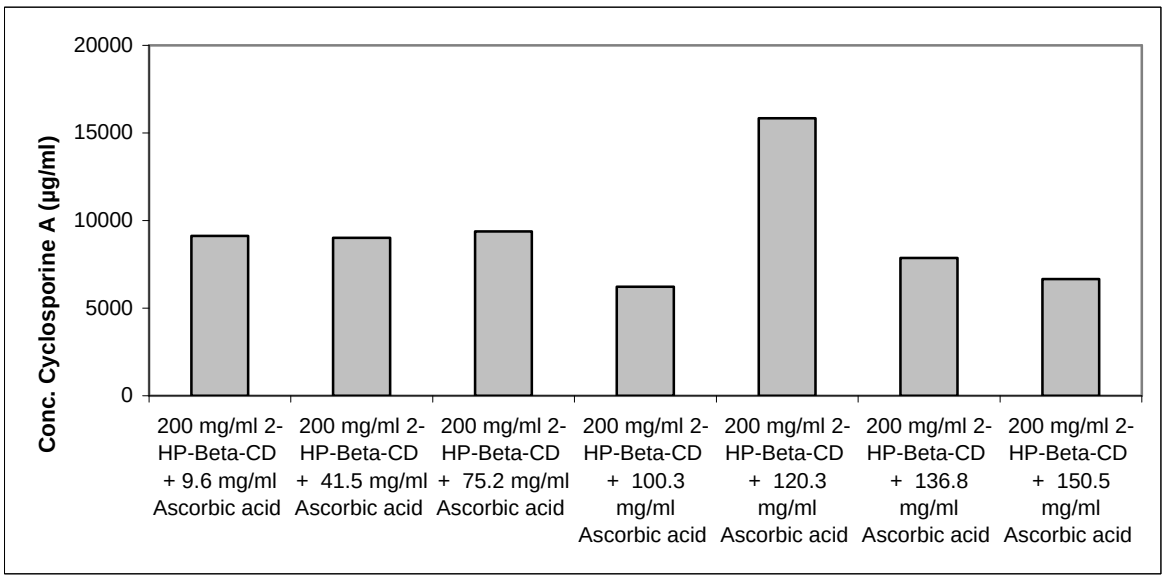


Figure 21: Influence of the relationship between the amount of cyclosporin A and the lecithin on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 7 days.

For a 4.0g lecithin/0.75g of cyclosporin A relationship:

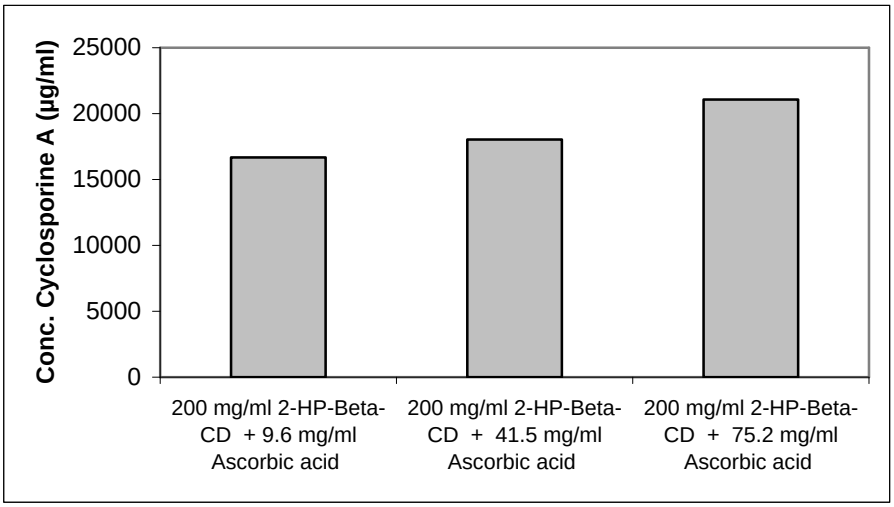


Figure 22: Influence of the relationship between the amount of cyclosporin A and the lecithin on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 7 days.

For a 4.0g lecithin/1.125g of cyclosporin A relationship:

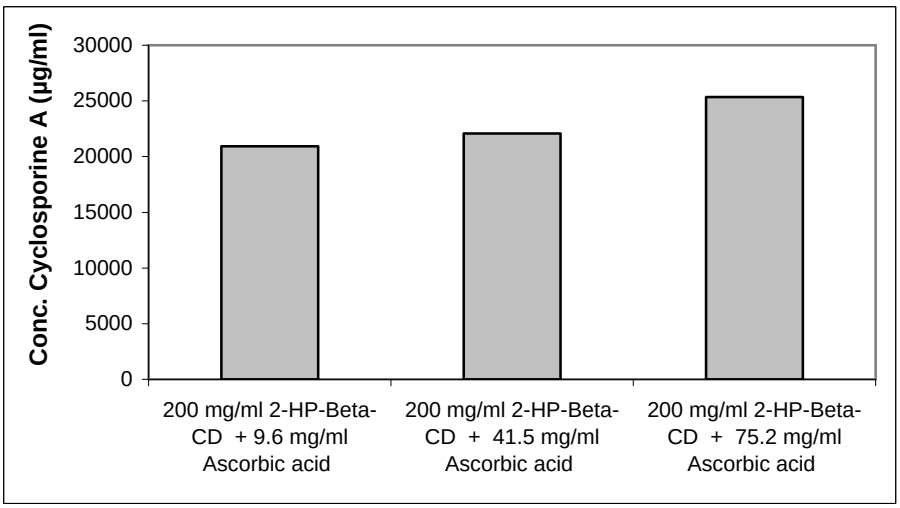


Figure 23: Influence of the relationship between the amount of cyclosporin A and the lecithin on the solubility of CSA in aqueous 2-HP-β-CD solutions. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 7 days.

The results show that an increase of the amount of cyclosporin A in the cyclosporin A/lecithin relationship is directly related to an increase of the cyclosporin solubility.

Based on the previous results, it was decided that the best parameters for the complex formation are:

- **Equilibrium time**: between 3-5 days (in total).
- **Time and temperature for the cyclosporin A/cyclodextrin system**: 70°C for 30 minutes.
- **Auxiliary substance**: ascorbic acid.

Due to toxicological and also cost issues, the amount of cyclodextrin that can be used is limited. Consequently, the next set of experiments focus on the influence of the amount of cyclodextrin used on the solubility of cyclosporin A.

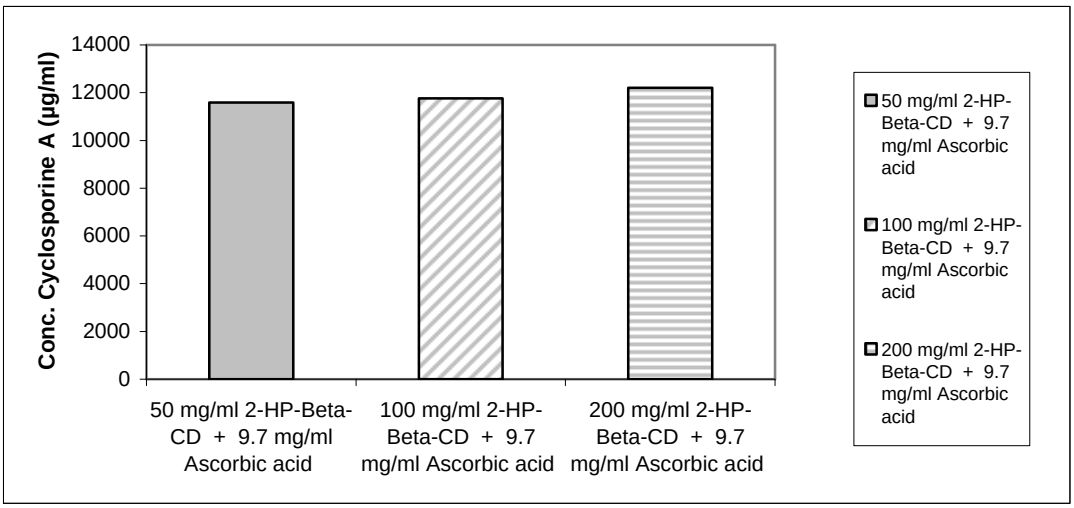


Figure 24: Influence of the 2-HP-β-CD concentration on the cyclosporin A solubility in aqueous 2-HP-β-CD solutions. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

Another combination of cyclosporin A/cyclodextrin was also used:

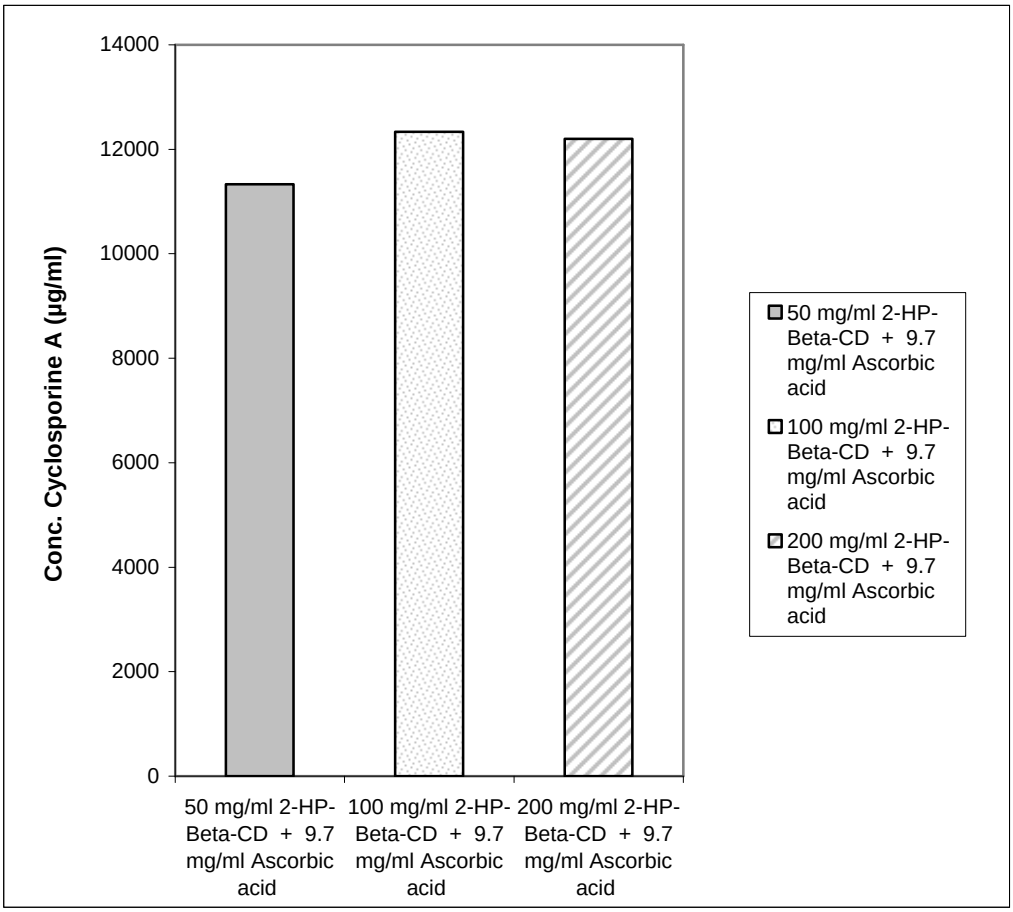


Figure 25: Influence of the 2-HP-β-CD concentration on the cyclosporin A solubility in aqueous 2-HP-β-CD solutions. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

The relationship between the amount of lecithin and cyclosporin A but also the amounts of this system added to the complexing media have a very important influence on the solubility of cyclosporin A.
For this reason, the influence of the amount of lecithin/cyclosporin A added to the system was investigated.

- Adding 0.125 ml of Lecithin/Cyclosporin A :

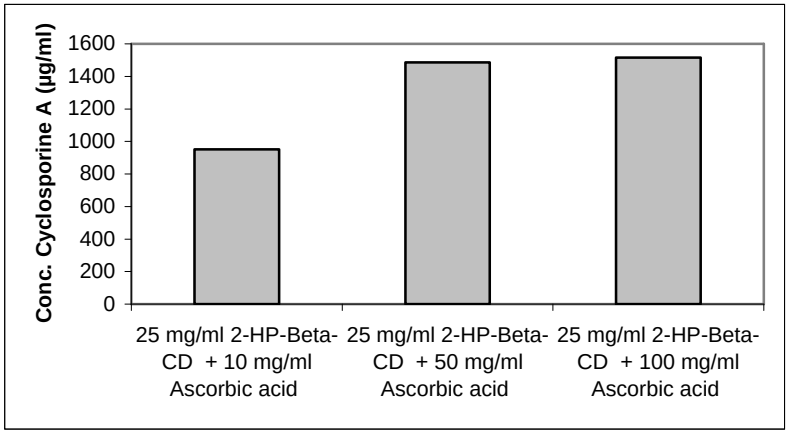


Figure 26: Influence on the solubility of cyclosporin A in aqueous 2-HP-β-CD solutions after adding 0.125 ml of lecithin/cyclosporin A to a aqueous solution containing 25 mg/ml of 2-HP-β-CD. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

- Adding 0.250 ml of Lecithin/Cyclosporin A to an aqueous solution containing 25 mg/ml of 2-HP-β-CD:

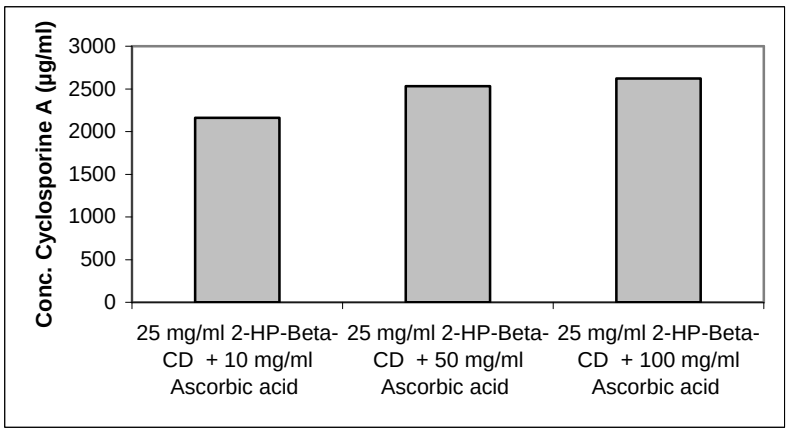


Figure 27: Influence on the solubility of cyclosporin A in aqueous 2-HP-β-CD solutions after adding 0.25 ml of lecithin/cyclosporin A to an aqueous solution containing 25 mg/ml of 2-HP-β-CD. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

- Adding 0.5 ml of Lecithin/Cyclosporin A to an aqueous solution containing 25 mg/ml of 2-HP-β-CD:

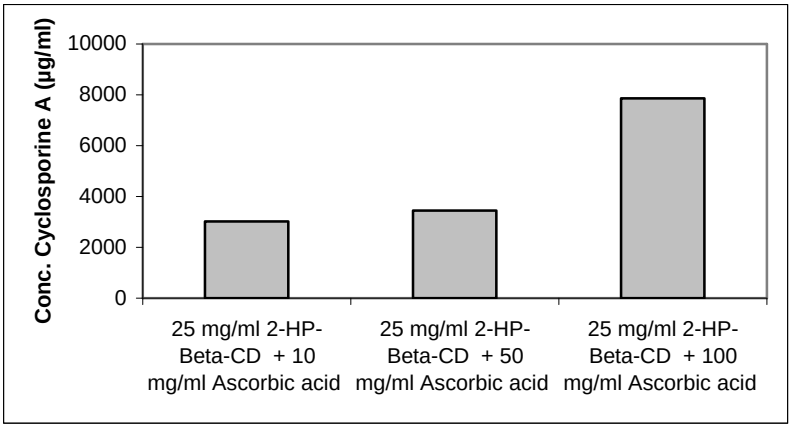


Figure 28: Influence on the solubility of cyclosporin A in aqueous 2-HP-β-CD solutions after adding 0.5 ml of lecithin/cyclosporin A to a aqueous solution containing 25 mg/ml of 2-HP-β-CD. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

- Adding 0.250 ml of Lecithin/Cyclosporin A to an aqueous solution containing 50 mg/ml of 2-HP-β-CD:

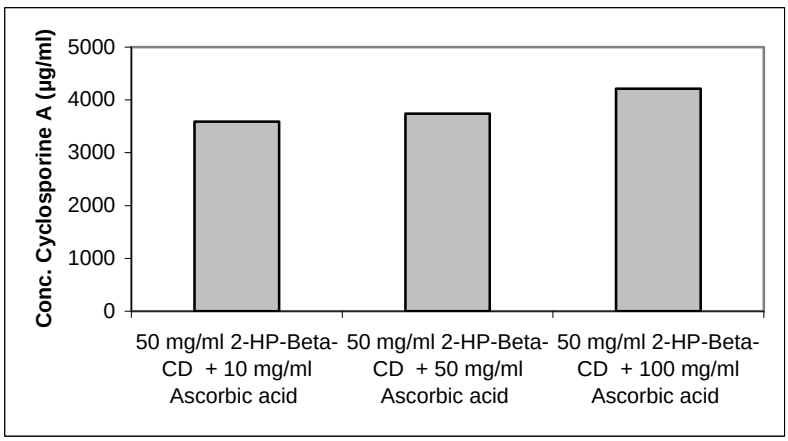


Figure 29: Influence on the solubility of cyclosporin A in aqueous 2-HP-β-CD solutions after adding 0.25 ml of lecithin/cyclosporin A to an aqueous solution containing 50 mg/ml of 2-HP-β-CD. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

- Adding 0.50 ml of Lecithin/Cyclosporin A to an aqueous solution containing 50 mg/ml of 2-HP-β-CD:

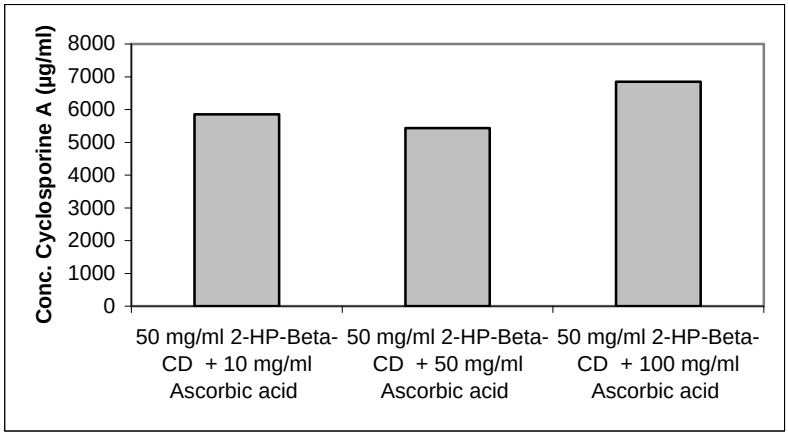


Figure 30: Influence on the solubility of cyclosporin A in aqueous 2-HP-β-CD solutions after adding 0.5 ml of lecithin/cyclosporin A to an aqueous solution containing 50 mg/ml of 2-HP-β-CD. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

- Adding 0.75 ml of Lecithin/Cyclosporin A to an aqueous solution containing 50 mg/ml of 2-HP-β-CD:

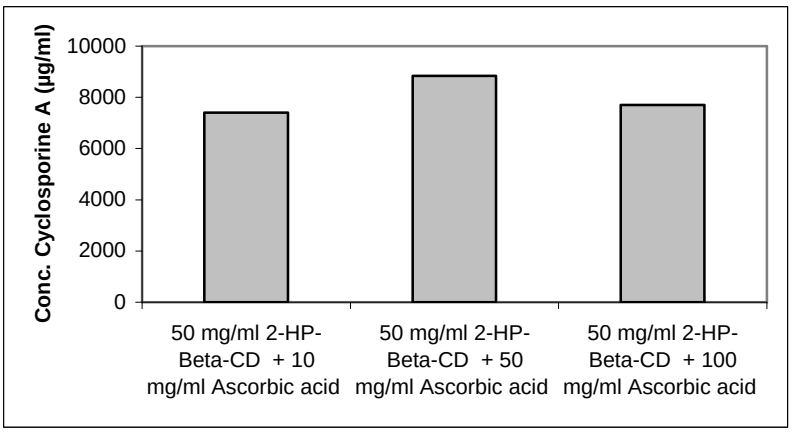


Figure 31: Influence on the solubility of cyclosporin A in aqueous 2-HP-β-CD solutions after adding 0.75 ml of lecithin/cyclosporin A to a aqueous solution containing 50 mg/ml of 2-HP-β-CD. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

- Adding 0.5 ml of Lecithin/Cyclosporin A to an aqueous solution containing 100 mg/ml of 2-HP-β-CD:

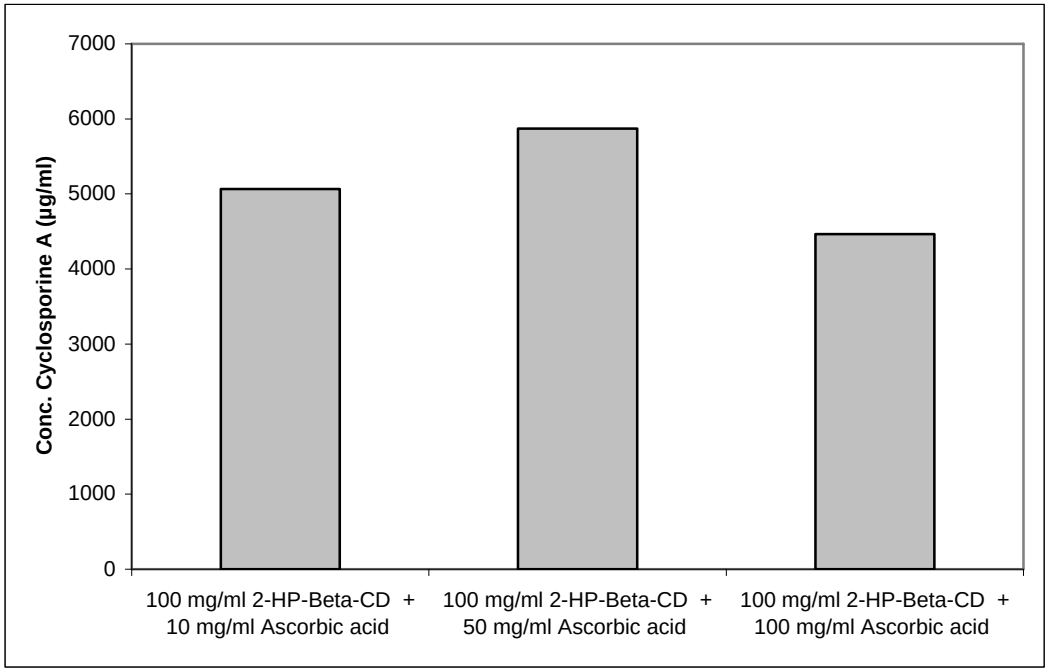


Figure 32: Influence on the solubility of cyclosporin A in aqueous 2-HP-β-CD solutions after adding 0.5 ml of lecithin/cyclosporin A to a aqueous solution containing 100 mg/ml of 2-HP-β-CD. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

- Adding 0.75 ml of Lecithin/Cyclosporin A to an aqueous solution containing 100 mg/ml of 2-HP-β-CD:

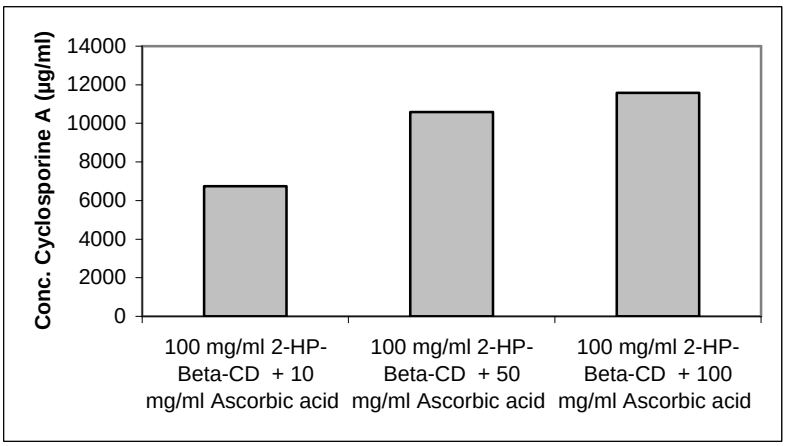


Figure 33: Influence on the solubility of cyclosporin A in aqueous 2-HP-β-CD solutions after adding 0.75 ml of lecithin/cyclosporin A to an aqueous solution containing 100 mg/ml of 2-HP-β-CD. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

- Adding 1.0 ml of Lecithin/Cyclosporin A to an aqueous solution containing 100 mg/ml of 2-HP-β-CD:

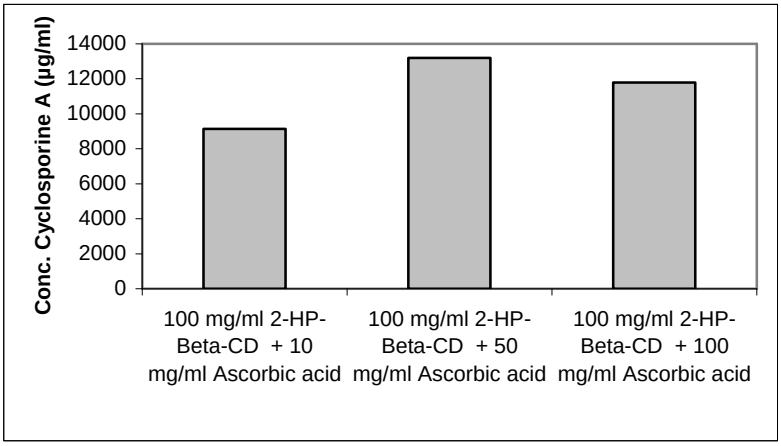


Figure 34: Influence on the solubility of cyclosporin A in aqueous 2-HP-β-CD solutions after adding 1.0 ml of lecithin/cyclosporin A to an aqueous solution containing 100 mg/ml of 2-HP-β-CD. Thirty minutes of equilibrium time at 70°C with cyclosporin A/cyclodextrin system. Afterwards, the additive was added and the system was stirred at room temperature for 3 days.

After analysing the results, it was possible to ascertain that there is a direct relation between the amount of lecithin/cyclosporin A added to the complexing media and the solubility of cyclosporin A.
In all 3 different concentrations of 2-HP-β-CD used, an increase of the amount of lecithin/cyclosporin A added to the complexing media results in a improvement in the solubility of cyclosporin A.
In order to determine which formulation is the most suitable to be nebulized with the eflow (for example, in terms of viscosity and surface tension), it was also taken in consideration the physicochemical characteristics of each of the formulations.

6.2.1.4.6 Physicochemical properties of the cyclosporin A/cyclodextrin formulations

As a starting point, the physicochemical properties of five 2-HP-β-CD formulations containing different concentrations were investigated in order to differentiate the influence of the cyclodextrins from the final physicochemical properties of the cyclosporin A/cyclodextrin formulations.
The results were:

Table 12: Physicochemical properties of five aqueous 2-HP-β-CD formulations containing different concentrations.

Formulation Composition	Surface Tension (mN/m)	Osmolarity (osmol/kg)	Viscosity (mPa s)	pH
50 mg/ml 2-HP-Beta-CD	59.23±0.14	0.031±0.002	1.23±0.05	6.36
100 mg/ml 2-HP-Beta-CD	62.67±0.08	0.077±0.001	1.48±0.03	5.86
200 mg/ml 2-HP-Beta-CD	56.73±0.09	0.266±0.08	2.43±0.03	5.93
400 mg/ml 2-HP-Beta-CD	55.01±0.10	1.28±0.09	9.24±0.24	5.96

The influence of the amount of lecithin/cyclosporin A added to the system on the physicochemical properties of the cyclosporin A/cyclodextrin formulations was also investigated.

Table 13: Physicochemical properties of a cyclosporin A/cyclodextrin formulation in an aqueous solution containing 25 mg/ml of 2-HP-β-CD, after adding 2 different amounts of lecithin/cyclosporin A to the complexing media.

Formulation composition	Surface Tension (N/m)	Osmolarity (osmol/kg)	Viscosity (mPa s)	pH
25 mg/ml 2-HP-Beta-CD + 50 mg/ml Ascorbic acid[A]	33.58±0.35	0.348±0.003	1.32±0.03	2.26
25 mg/ml 2-HP-Beta-CD + 50 mg/ml Ascorbic acid[B]	33.31±0.26	0.435±0.001	1.52±0.04	2.27

A: After adding 0. 125 ml of Lecithin/Cyclosporin A to a aqueous solution containing 25 mg/ml of 2-HP-β-CD

B: After adding 0. 50 ml of Lecithin/Cyclosporin A to a aqueous solution containing 25 mg/ml of 2-HP-β-CD

Table 14: Physicochemical properties of a cyclosporin A/cyclodextrin formulation in an aqueous solution containing 50 mg/ml of 2-HP-β-CD, after adding 2 different amounts of lecithin/cyclosporin A to the complexing media.

Formulation composition	Surface Tension (mN/m)	Osmolarity (osmol/kg)	Viscosity (mPa s)	pH
50 mg/ml 2-HP-Beta-CD + 50 mg/ml Ascorbic acid[A]	33.33±0.7	0.415±0.004	1.46±0.004	2.25
50 mg/ml 2-HP-Beta-CD + 50 mg/ml Ascorbic acid[B]	33.46±0.65	0.443±0.004	1.72±0.04	2.3

A: After adding 0. 25 ml of lecithin/cyclosporin A to a aqueous solution containing 50 mg/ml of 2-HP-β-CD

B: After adding 0. 50 ml of lecithin/cyclosporin A to a aqueous solution containing 50 mg/ml of 2-HP-β-CD

Table 15: Physicochemical properties of a cyclosporin A/cyclodextrin formulation in an aqueous solution containing 100 mg/ml of 2-HP-β-CD, after adding 2 different amounts of lecithin/cyclosporin A to the complexing media.

Formulation composition	Surface Tension (mN/m)	Osmolarity (mosmol/kg)	Viscosity (mPa s)	pH
100 mg/ml 2-HP-Beta-CD + 50 mg/ml Ascorbic acid[A]	33.37±0.59	0.466±0.004	1.72±0.03	2.26
100 mg/ml 2-HP-Beta-CD + 50 mg/ml Ascorbic acid[B]	33.72±0.91	0.492±0.005	2.04±0.04	2.29

A: After adding 0.5 ml of Lecithin/Cyclosporin A to a aqueous solution containing 100 mg/ml of 2-HP-β-CD

B: After adding 0. 75 ml of Lecithin/Cyclosporin A to a aqueous solution containing 100 mg/ml of 2-HP-β-CD

Table 16: Comparison of the physicochemical properties of 2 cyclosporin A/cyclodextrin formulations in an aqueous solution containing 25 mg/ml and 50 mg/ml of 2-HP-β-CD. Two different amounts of lecithin/cyclosporin A were added to the complexing media.

Formulation composition	Surface Tension (mN/m)	Osmolarity (osmol/kg)	Viscosity (mPa s)	pH
25 mg/ml 2-HP-Beta-CD + 10 mg/ml Ascorbic acid [A]	33.25±0.24	0.145±0.004	0.87±0.04	2.64
50 mg/ml 2-HP-Beta-CD + 10 mg/ml Sodium ascorbate[B]	33.26±1.73	0.359±0.003	1.22±0.09	6.26

A: After adding 0.25 ml of lecithin/cyclosporin A to a aqueous solution containing 25 mg/ml of 2-HP-β-CD

B: After adding 1.0 ml of lecithin/cyclosporin A to a aqueous solution containing 50 mg/ml of 2-HP-β-CD

From the results, it was possible to ascertain the following facts:

1. **Surface Tension:** in all formulations there was a decrease in the surface tension values in comparison to the cyclodextrins formulations. However, the addition of increasing amounts of lecithin/cyclosporin A to the complexing media does not have a significant influence on the value of the surface tension on the different formulations.

2. **Viscosity:** regarding the viscosity, all formulations presented an increase in the viscosity values in comparison to the cyclodextrin solutions due to the addition cyclosporin A and the other excipients. Also, the addition of increasing amounts of lecithin/cyclosporin A to the complexing media results in an increase of the value of the viscosity on the different formulations.

3. **Osmolarity:** the osmolarity values were greater as the ones of the cyclodextrin solutions due to the addition cyclosporin A and the other excipients to the cyclodextrins solutions. Furthermore, the addition of increasing amounts of lecithin/cyclosporin A to the complexing media results in an increase of the value of the osmolarity on the different formulations.

4. **pH:** the cyclodextrins solutions have a pH value between 6.0-6.5 while the cyclosporin A/cyclodextrin formulations have a value between 2.25-2.64. The difference can be explained by the presence of the ascorbic acid in the formulations. However, one of the cyclosporin A/cyclodextrin formulations has a value of 6.26 because the ascorbic acid was substituted with sodium ascorbate as excipient.

6.2.1.4.7 Determination of the humidity content of the lyophilizate

The humidity content was determined (Karl-Fischer method), as a way of quantifying the residual water of the product obtained right after the lyophilization process. In the case of the lyophilized amphotericin B microemulsion, the content was just 0.75±0.03% (water content of the lyophilized product should be less than 3%) which is low enough to avoid the hydrolysis reaction by the lecithin phosphatide groups and, therefore, protect the system from any decomposition phenomena related to such processes[23].
As an example, AmBisome® for injection is a sterile, non-pyrogenic lyophilized product for intravenous infusion and it has stability of 36 months at 25°C. [24]
Taking in consideration the previous information, it was decided to lyophilizate the cyclosporin A/cyclodextrin formulation in order to avoid the hydrolysis reaction by the lecithin phosphatide groups and, consequently to obtain a more stable formulation.

[23] For a more detailed explanation see appendix 2.

[24] AmBisome® is a market product containing amphotericin B as active substance. AmBisome® for injection is a sterile, non-pyrogenic lyophilized product for intravenous infusion. Each vial contains 50 mg of amphotericin B, USP, intercalated into a liposomal membrane consisting of approximately 213 mg hydrogenated soy phosphatidylcholine; 52 mg cholesterol, NF; 84 mg distearoylphosphatidylglycerol; 0.64 mg alpha tocopherol, USP; together with 900 mg sucrose, NF; and 27 mg disodium succinate hexahydrate as buffer. Following reconstitution with sterile water for injection, USP, the resulting pH of the suspension is between 5.0-6.0.

6.2.1.4.8 Lyophilization of the Cyclosporin A/Cyclodextrin Formulation

With the purpose of protecting the cyclosporin A/cyclodextrin(CSA/CD) system from any decomposition phenomena related to the hydrolysis reaction by the lecithin phosphatide groups, one cyclosporin A/cyclodextrin formulation was prepared and lyophilized.
The formulation had the following composition:

Table 17: Composition of a lyophilized CSA/CD formulation.

Formulation Composition	**Conc. (mg/ml)**
Cyclosporin A	18[25]
Lecithin	205.9
2-HP-β-CD	100
Sodium ascorbate	10

The vials were filled with 1 ml of the CSA/CD formulation each and placed inside the lyophilizer chamber. Lyophilization of the samples was performed with a Christ Epsilon 2-6D equipment. Conditions of the entire process are shown in Table 23.

Table 18: Lyophilization conditions of a CSA/CD Formulation

Process Phase	**Time (min)**	**Temp. (°C)**	**Vacuum (mbar)**	**Si. –Pressure (mbar)**
Start value	0	0	OFF	OFF
Freezing	1	-38	OFF	OFF
Freezing	44	-38	OFF	OFF
Preparation	15	-38	OFF	OFF
Main drying	1	-38	0.08	OFF
Main drying	15	-25	0.08	0.140
Main drying	704	-25	0.08	0.140
After drying	1	-25	0.08	0.140
After drying	300	15	0.08	0.140
After drying	719	15	0.08	0.140

At the end of the process, the lyophilized obtained was a light yellow coloured product (please see Figure 35).

[25] Value obtained from the delivery dose. The lyophilizate was reconstituted with 3 ml of water and the nebulisation parameters were: nebulisation time of 7.56 min and a 10 l/min flow.

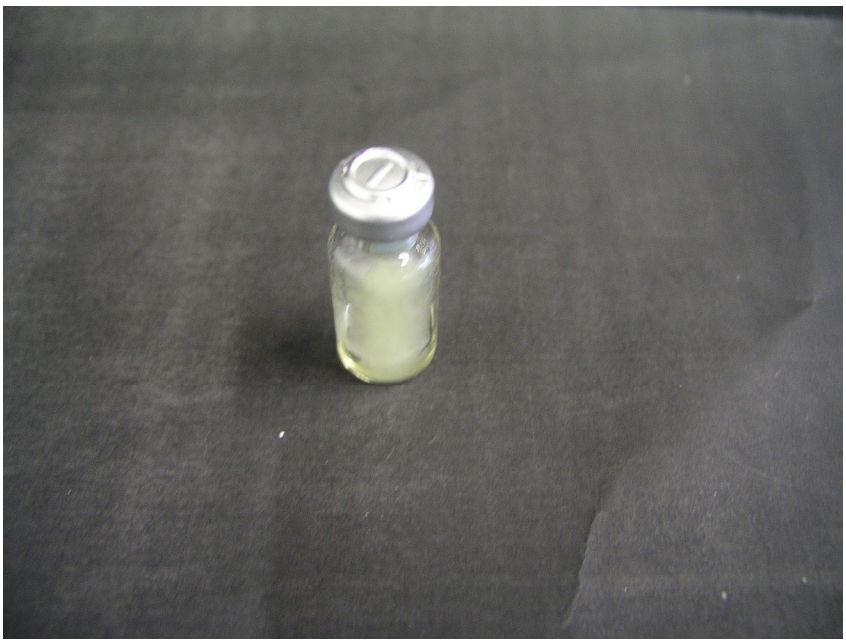


Figure 35: Image of a lyophilizate of a CSA/CD formulation. Image taken 6 days after lyophilization.

The reconstituted lyophilizate of the CSA/CD formulation was a homogeneous, white coloured product (please see Figure 36 and Figure 37).

Figure 36: Image of a reconstituted lyophilizate of a CSA/CD formulation. Reconstitution was made by adding 3 ml and manually shaking the vial. Image taken 6 days after lyophilization.

Figure 37: Reconstituted lyophilizate of a CSA/CD formulation. Reconstitution was performed by adding 3 ml and manually shaking the vial. Image taken 1.5 months after lyophilization.

6.2.1.4.9 Physicochemical properties and nebulisation parameters of cyclosporin/cyclodextrin lyophilizates

Table 20: Physicochemical properties of a CSA/CD formulation in an aqueous solution containing 100 mg/ml of 2-HP-β-CD.

Formulation composition	Surface Tension (mN/m)	Viscosity (mPa s)
100 mg/ml 2-HP-Beta-CD + 10 mg/ml Sodium ascorbate	35.16±0.44	2.4±0.35

Table 21: Nebulization properties of a CSA/CD lyophilizate using eflow ® (Pari GmbH, Germany) as nebuliser .[26]

MMD (µm)	GSD	TOR (mg/ml)	Percent. Particles <5 µm	Percent. Particles <2 µm
3.07	1.62	320	83.79	18.12

[26] MMD (NaCl) = 3.44 µm; Measured at a Malvern Zetasizer (Model Zetasizer 3000 HAS, Germany). The nebulisation trial was performed using a electronic nebulizer, eflow® (Pari GmbH, Germany)

Table 22: Nebulization properties of a CSA/CD lyophilizate using eflow® (Pari GmbH, Germany) as nebuliser [27]

MMD (µm)	GSD	TOR (mg/ml)	Percent. Particles <5 µm	Percent. Particles <2 µm
3.6	1.58	438	77.44	9.08

6.2.1.4.10 Stability trials

In order to ascertain the stability of the formulation, a new CSA/CD formulation was prepared. The Lyophilization was performed under the conditions describe on table 27.
To the CSA/CD system also trehalose was added as a cryoprotectant in order to prevent any degradation caused by the lyophilization process.
The formulation composition of both formulations is described in the following tables:

Table 23: Composition of a lyophilized CSA/CD formulation.

Formulation Composition	Conc. (mg/ml)
Cyclosporin A	2.85[28]
Lecithin	29.8
2-HP-β-CD	101.8
Trehalose	2.1

Table 24: Nebulization properties of a CSA/CD lyophilizate using ® (Pari GmbH, Germany) as nebuliser [29]

MMD (µm)	GSD	TOR (mg/ml)	Percent. Particles <5 µm	Percent. Particles <2 µm
3.58	1.55	415	77.22	8.94

In accordance with the ICH Steering Committee's recommendations, the samples were kept at 25°C and 60% relative humidity for a period of 5 months (Lit. [13]).
Samples were withdrawn in duplicate at 0, 1and 5 months, and assessed for their:

[27] MMD (NaCl) =4.2 µm; Measured at a Malvern Zetasizer (Model Zetasizer 3000 HAS, Germany). The nebulisation trial was performed using a electronic nebulizer, eflow® (Pari GmbH, Germany)

[28] Value obtained from the delivery dose. The lyophilizate was reconstituted with 2 ml of water and the nebulisation parameters were: nebulisation time of 6.06min and a 10 l/min flow.

[29] MMD (NaCl) = 3.93 µm; TOR (NaCl) = 608 mg/ ml. Measured at a Malvern Zetasizer (Model Zetasizer 3000 HAS, Germany). The nebulisation trial was performed using an electronic nebulizer, eflow® (Pari GmbH, Germany).

1) **Chemical stability:** expressed as cyclosporin content, determined by HPLC. Samples were deemed to be stable if presented a cyclosporin A content between 95–105% of the amount declared at the beginning of the study.

2) **Physical stability:** Water content of the lyophilized product should be less than 3%; organoleptic properties of the lyophilized product should be white coloured; macroscopic appearance of the reconstituted lyophilizate should have a homogeneous and with milky appearance.

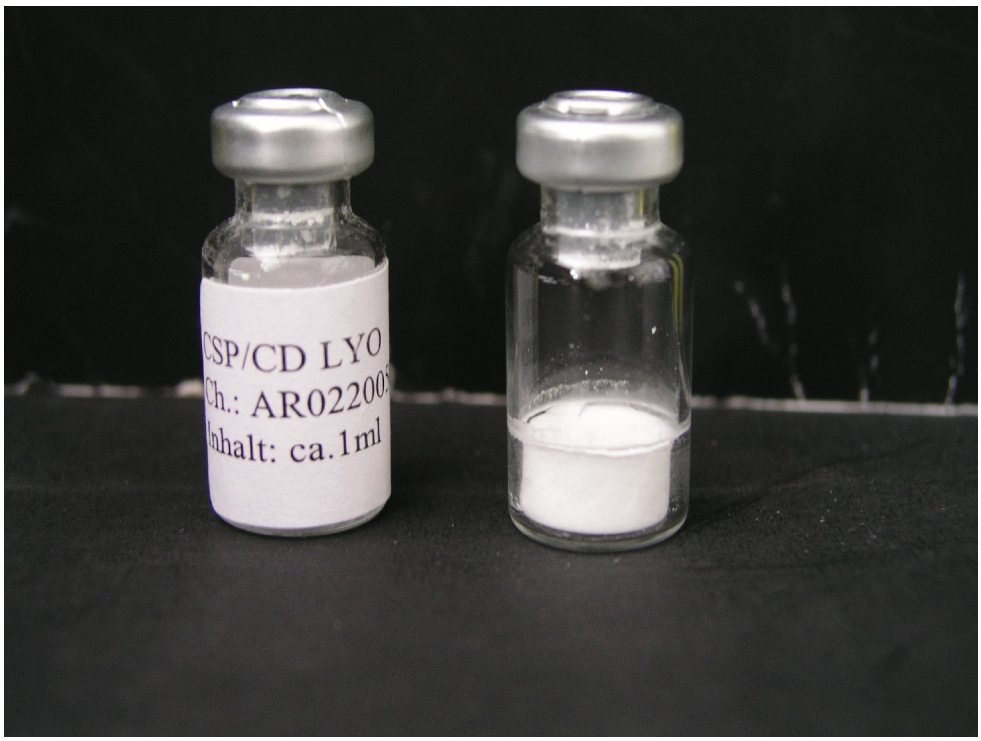


Figure 38: Image of a lyophilizate of a CSA/CD formulation. Image taken 1 day after lyophilization

Figure 39: Image of a reconstituted lyophilizate of a CSA/CD formulation. Reconstituted by adding 2 ml and manual shaking. Image taken 1 day after lyophilization.

Table 25: Nebulization properties of reconstituted lyophilizate of a CSA/CD formulation. Reconstitution was made by adding 3 ml and manually shaking the vial[30].

MMD (µm)	GSD	TOR (mg/ml)	Percent. Particles <5 µm	Percent. Particles <2 µm
3.58	1.55	415	77.22	8.94

The water content of the cyclosporin A lyophilizate was also determined. The results are described on table 27.

Table 26: Water content of a CSA/CD lyophilizate.

Formulation	Water content (%)
Cyclosporin A	2.8

[30] MMD (NaCl): 4.2 µm; TOR (NaCl): 610 mg/ m. Measured at a Malvern Zetasizer (Model Zetasizer 3000 HAS, Germany). The nebulisation trial was performed using a electronic nebulizer, eflow® (Pari GmbH, Germany)

6.2.1.4.10.1 Stability trial of a lyophilized cyclosporin A/cyclodextrin formulation

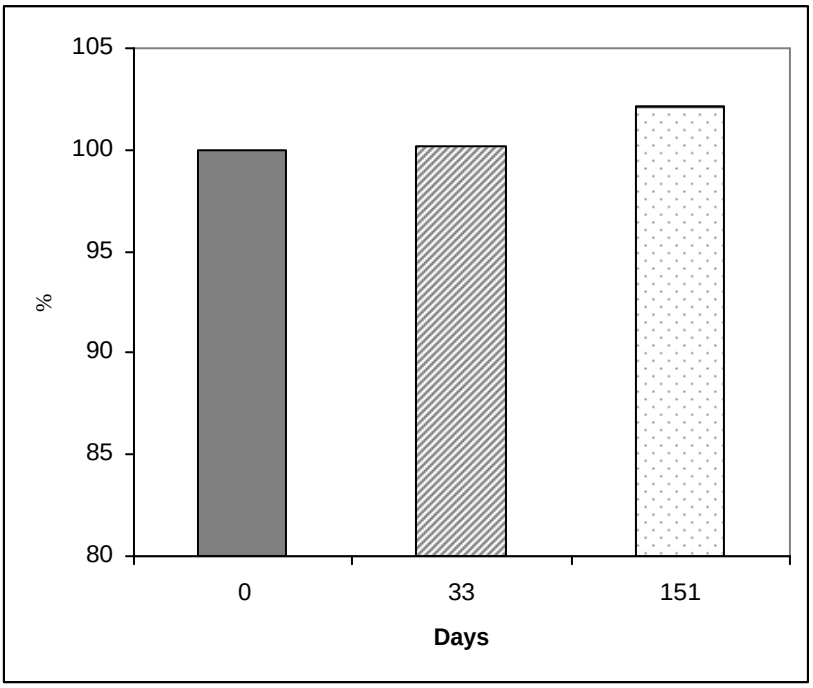


Figure 40: Stability of a lyophilized CSA/CD formulation kept at 25°C and 60% relative humidity.

From the results of the stability trial, we can ascertain that the lyophilized cyclosporin A/cyclodextrin formulation kept at 25°C and 60% relative humidity is stable for at least 5 months.

Subchapter conclusions

Cyclosporin A is a potent immunosuppressive agent widely employed to prevent graft rejection in kidney, liver and bone marrow transplant patients and to delay or prevent disease progression in patients with autoimmune disease and inflammation.
The immunosuppressive effect of CSA is due to a selective and reversible inhibition of T-lymphocytes interleukin-2 driven proliferation of activated T-lymphocytes. Although taking in consideration the important clinical value of the cyclosporin A, its extended use has been often limited by several disadvantages such as low bioavailability, narrow therapeutic window, nephrotoxicity and hepatotoxicity (Lit. [20]- Lit. [21]). It has also been reported that Cremophor EL®, which is a solubilizing agent present in the commercially available intravenous dosage form of CSA, has a risk of anaphylactic shock and nephrotoxicity (Lit. [22]- Lit. [23]). Due to the disadvantages previously mentioned, it is fundamental to develop alternative dosage forms.

Pulmonary delivery can provide an alternative a non-invasive method of delivering drugs that currently can only be delivered orally or intravenously. It is a unique and innovative delivery alternative for therapies that can only be administered by injection (i.v., i.m., s.c.) or by oral

delivery which causes adverse effects or is poorly absorbed. In fact, the main advantage of a therapy via the lungs is the potentially improved therapeutic index, that is, the ratio of therapeutic benefit to adverse effects. This applies mainly to the therapy of pulmonary diseases, but may also be applicable to systemic diseases due to reduced first-pass metabolism that may be associated with hepatocellular injury. One other advantage lies on the very rapid onset of action similar to the i.v. route and quicker than can be achieved with either oral delivery or subcutaneous injections.

However, cyclosporin A is a water insoluble drug which can constitute a problem in terms of delivering it to the lungs due to its poor solubility. For this reason, cyclodextrins were studied as a mean of improving not only the solubility but also the stability of CSA. Cyclodextrins are potential candidates for these functions because of their ability to alter physical, chemical and biological properties of guest molecules through the formation of inclusion and non-inclusion complexes.

The first experiments performed showed that the cavity size of the α-CD molecule is more suitable for the complex formation and, therefore, for the increase in the solubility of CSA.
Nevertheless, due to toxicological and costs issues, it was decided to use 2-HP-β-CD instead of α-CD, or derivatives therefore, because of its high water solubility, high solubilizing power, low cost and low toxicology.
In addition, it is known that cyclodextrin and drug/cyclodextrin complexes self-associate to form water soluble aggregates (or microaggregates) of several drug/cyclodextrin complex units and these aggregates can solubilise lipophilic water insoluble drugs through non-inclusion complexation.
Furthermore, the addition of suitable auxiliary substances can significantly increase the cyclodextrin solubilizing and complexing abilities by multicomponent complex formation.
With this purpose in mind, several classes of substances were investigated, through the use of a factorial design, for their ability to improve the solubility/stability effect of the cyclodextrins.

The first results using 2-HP-β-CD and cyclosporin A in the presence of several auxiliary substances showed that the use of auxiliary substances did not increase the solubilizing ability of the cyclodextrin molecules. This was probably due to the fact that the cyclosporin A molecule was not able to interact with the molecules of the several auxiliary substances added to the complexing system.
Consequently, the major factor in the cyclosporin A/cyclodextrin complexation formation is the cavity size of the cyclodextrin molecule and the cavity size the α-CD molecule is the most appropriate for the nonpolar aliphatic parts of the Cyclosporin A molecules to be held.
Taking in consideration the physical chemical properties of the cyclosporin A molecule, it was decided to investigate the possibility “inserting” the cyclosporin A in another molecule as way of overtaking the natural properties of the cyclosporin A molecule.
Thus, it was decided to use lecithin as the “carrier” molecule for cyclosporin A. It is made from biological phospholipids that are biodegradable, lacking imunogenicity and they exhibit low intrinsic toxicity. In addition, the presence of suitable functional groups in the molecular structure of the lecithin molecules permits the interaction between the several auxiliary substances and the cyclodextrin, allowing this way the formation and stabilization of non-inclusion complexes.

From the auxiliary substances used, we could ascertain that sodium ascorbate is the best auxiliary substance for the complex formation and the best parameters for the complex formation are:

- **Equilibrium time**: between 3-5 days (in total).
- **Time and temperature for the cyclosporin A/cyclodextrin system**: 70°C for 30 minutes.
- **Auxiliary substance**: sodium ascorbate.

Nevertheless, in order to determine if a formulation is suitable to be nebulized with the eflow® (for example, in terms of viscosity and surface tension), we also investigated the physicochemical characteristics of the formulations.
The results showed that formulations containing 25 mg/ml to 100 mg/ml of aqueous 2-HP-β-CD solutions with 5 mg/ml of sodium ascorbate provide the best results in terms of the nebulisation properties.
With the purpose of protecting the CSA/CD system from any decomposition phenomena related to the hydrolysis reaction by the lecithin phosphatide groups, it was decided to prepare a lyophilizate of the formulation. The results obtained from the stability trials showed that the lyophilized cyclosporin A/cyclodextrin formulation kept at 25°C and 60% relative humidity is stable for at least 5 months.

As summary, it was possible to obtain a stable formulation of cyclosporin A through the use of cyclodextrin that can be nebulized which, consequently, constitutes a valid option for the prevention and treatment of graft rejection in the lungs.

6.2.2 Azithromycin/Cyclodextrin Formulations

Azithromycin is a macrolide antibiotic belonging to the azalide group. It is a water insoluble drug and for this reason, cyclodextrins were investigated as a mean of improving the solubility and stability of azithromycin. Cyclodextrins are potential candidates for this function because of their ability to alter physical, chemical and biological properties of guest molecules through formation of inclusion and non-inclusion complexes.
Based on the previous work performed with cyclosporin A and cyclodextrins, it was decided to proceed taking in account this previous experience.

To determine which group of additives can be used to form and stabilize non-inclusion complexes of drug-cyclodextrin and, therefore, enhance the solubility azithromycin, several additives were added to the azithromycin-cyclodextrin complex solutions.

6.2.2.1 Influence of several excipients on the solubility of azithromycin

The tests were performed according to the following factorial design:

Table 1: Factorial design.

Vial Number	Amino acids	Metal ions	Vitamins
1	+	+	+
2	+	+	-
3	+	-	+
4	+	-	-
5	-	+	+
6	-	+	-
7	-	-	+
8	-	-	-

To prepare the following formulations, the preparation method from chapter 5.3.2.1.1 was used.

Table 2: Phase solubility table of azithromycin in aqueous 2-HP-β-CD solutions in the presence of several excipients. Performed at pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

Formulation Composition	CODE	Azithromycin Conc. (mg/ml)
200 mg/ml 2-HP-β -CD + 3.5mg/ml Lysine monohydrate + 3. 5 mg/ml Ascorbic Acid + 0.75 mg/ml $MgSO_4$ 6.H_2O+ 0.5 mg/ml $CaCl_2$.2H_2O	BCD L A M C	8.5
200 mg/ml 2-HP-β -CD + 3. 5mg/ml Lysine monohydrate + 0.75 mg/ml $MgSO_4$ 6.H_2O+ 0.5 mg/ml $CaCl_2$.2H_2O	BCD L M C	7.0
200 mg/ml 2-HP-β -CD + 3.5mg/ml Lysine monohydrate + 3.5 mg/ml Ascorbic Acid	BCD L A	8.7
200 mg/ml 2-HP-β -CD + 3.5mg/ml Lysine monohydrate	BCD L	7.2
200 mg/ml 2-HP-β -CD + 3.5 mg/ml Ascorbic Acid + 0.75 mg/ml $MgSO_4$ 6.H_2O+ 0.5 mg/ml $CaCl_2$.2H_2O	BCD A M C	8.2
200 mg/ml 2-HP-β -CD + 0.75 mg/ml $MgSO_4$ 6.H_2O + 0.5 mg/ml $CaCl_2$.2H_2O	BCD M C	4.3
200 mg/ml 2-HP- β -CD + 3.5 mg/ml Ascorbic Acid	BCD A	8.5
200 mg/ml 2-HP-β-CD	BCD	6.1

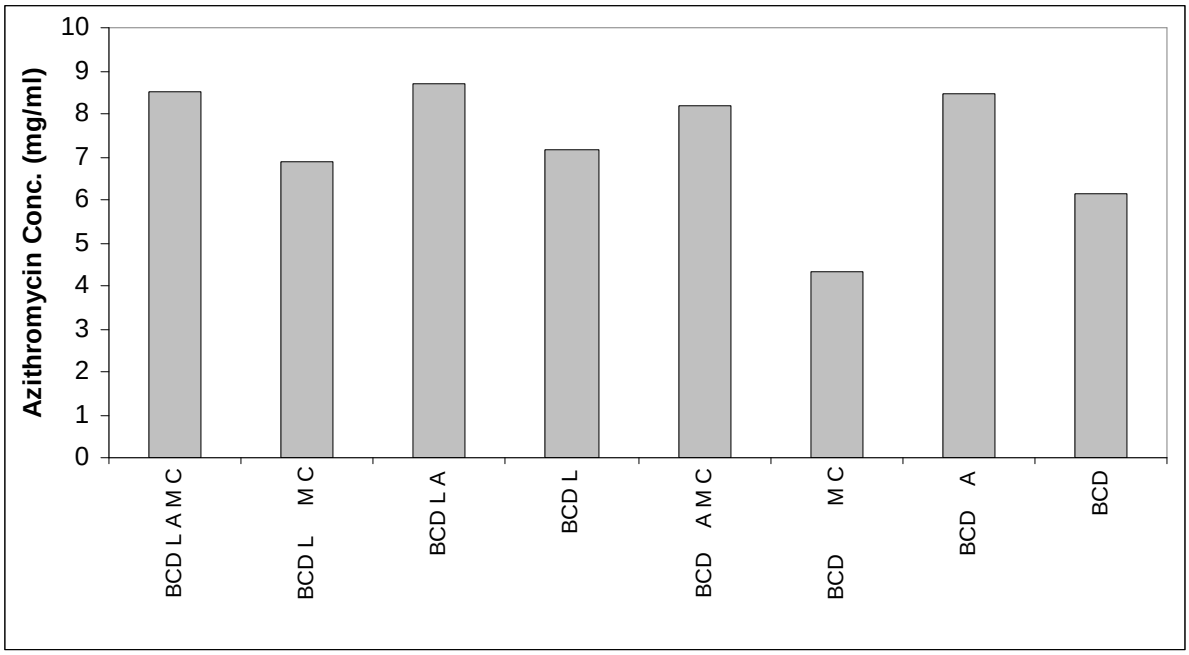


Figure 1: Phase solubility diagram of azithromycin in aqueous 2-HP-β-CD solutions in the presence of several excipients. Performed at pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

From the results, it can be ascertain that in general the combine use of CDs and auxiliary substances can help improve the solubility of azithromycin. The level to which this increase

can be achieved depends on the type and concentration of the auxiliary substances along with the concentration of CDs. Nevertheless, other parameters such as temperature and pH also have a very important influence on the solubility increase of azithromycin.

6.2.2.2 Influence of sodium acetate and citric acid on the solubility of azithromycin

The capacity of sodium acetate and citric acid to form non-inclusion complexes was also investigated. The preparation of each of the formulations was the same as the one performed for the factorial design (c.f. 6.2.2.1).

Table 3: Phase solubility table of azithromycin in aqueous 2-HP-β-CD solutions in presence of increasing concentrations of sodium acetate. Performed at pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

Formulation Composition	Azithromycin Conc. (mg/ml)
200 mg/ml 2-HP-β-CD + 2% Sodium Acetate	7.7
200 mg/ml 2-HP-β-CD + 3% Sodium Acetate	6.5
200 mg/ml 2-HP-β-CD + 4% Sodium Acetate	7.4

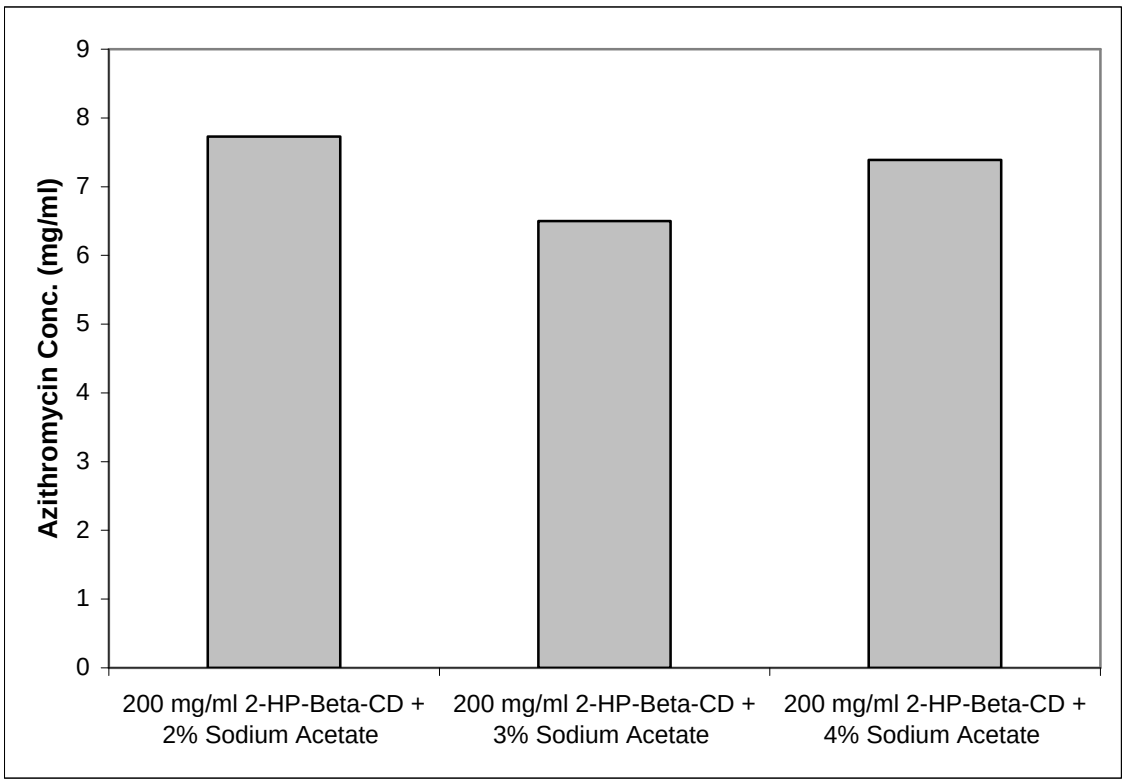


Figure 3: Phase solubility diagram of azithromycin in aqueous 2-HP-β-CD solutions in presence of increasing concentrations of sodium acetate. Performed at pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

Table 4: Phase solubility table of azithromycin in aqueous 2-HP-β-CD solutions in presence of increasing concentrations of citric acid. Performed at pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

Formulation Composition	Azithromycin Conc. (mg/ml)
200 mg/ml 2-HP-β –CD + 6.67 mg/ml Citric Acid	6.7
200 mg/ml 2-HP-β –CD + 13.3 mg/ml Citric Acid	8.1

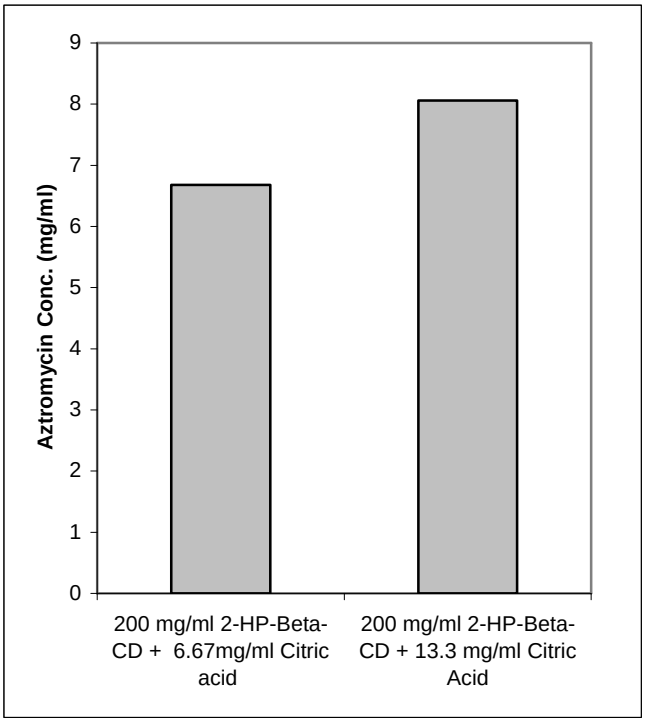


Figure 3: Phase solubility diagram of azithromycin in aqueous 2-HP-β-CD solutions in presence of increasing concentrations of citric acid. Performed at pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

After analysing the results, it can be concluded that for the case of sodium acetate, a maximum solubility of azithromycin can be achieved with 2% sodium acetate. On the other hand, a concentration of 13.3 mg/ml of citric acid allows a higher increase of the azithromycin solubility in comparison with 6.6 mg/ml citric acid.

6.2.2.3 Influence of pH on the solubility of azithromycin

In order to ascertain the effect of pH on the azithromycin solubility, 3 formulations were chosen based on their improved solubility. In this experiment, the pH was adjusted to 4.5-5.5 and 6.5-7.0 respectively.

Table 5: Phase solubility table of azithromycin in 200 mg/ml 2-HP-β-CD aqueous solution in presence of citric acid at 2 different pH values. Performed at pH 4.5-5.5 and pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

Formulation Composition	Azithromycin Conc. (mg/ml)
200 mg/ml 2-HP-β-CD + 6.67mg/ml Citric acid (pH 4.5-5.5)	8.94
200 mg/ml 2-HP-β-CD + 6.67mg/ml Citric acid (pH 6.5-7.0)	6.80

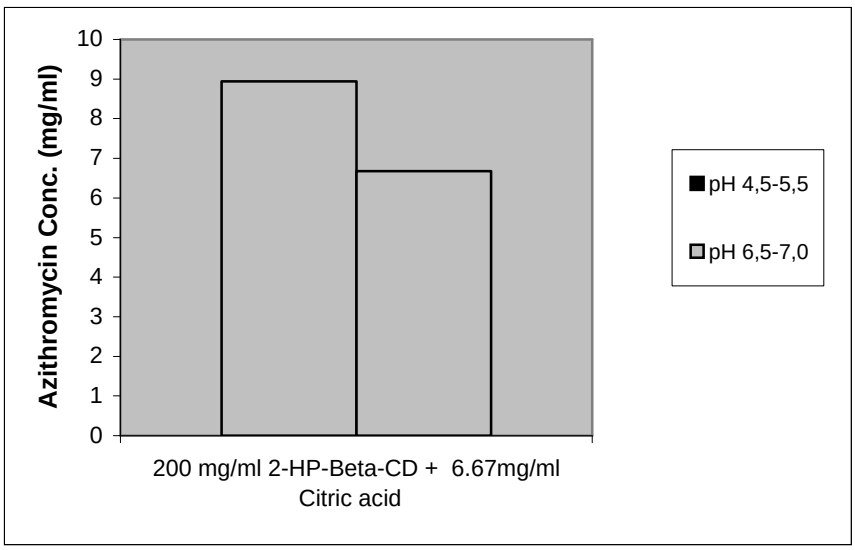


Figure 4: Phase solubility diagram of azithromycin in 200 mg/ml 2-HP-β-CD aqueous solution in presence of citric acid, at 2 different pH values. Performed at pH 4.5-5.5 and pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

Table 6: Phase solubility table of azithromycin in 200 mg/ml 2-HP-β-CD aqueous solution in presence of ascorbic acid, at 2 different pH values. Performed at pH 4.5-5.5 and pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

Formulation Composition	Azithromycin Conc. (mg/ml)
200 mg/ml 2-HP-β-CD + 3.5 mg/ml Ascorbic Acid (pH 4.5-5.5)	7.0
200 mg/ml 2-HP-β CD + 3.5 mg/ml Ascorbic Acid (pH 6.5-7.0)	8.5

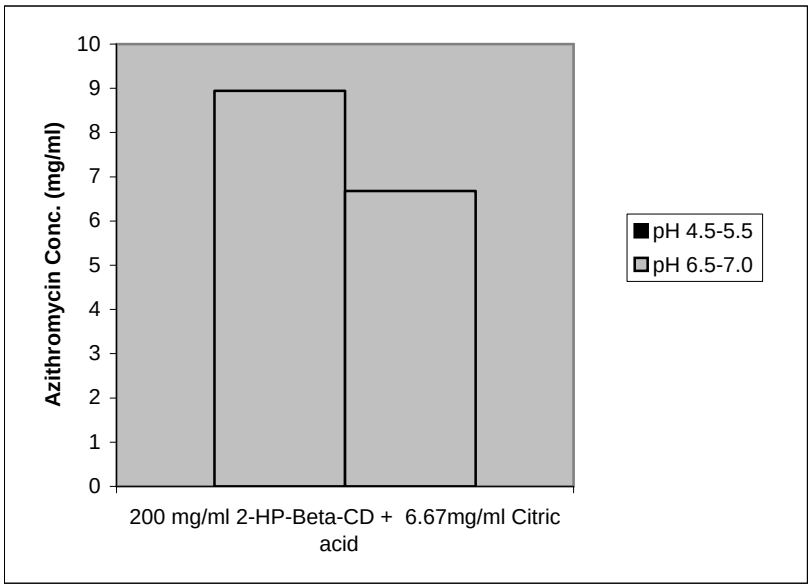


Figure 5: Phase solubility diagram of azithromycin in 200 mg/ml 2-HP-β-CD aqueous solution in presence of ascorbic acid at 2 different pH values. Performed at pH 4.5-5.5 and pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

Table 7: Phase solubility table of azithromycin in 200 mg/ml 2-HP-β-CD aqueous solution in presence of sodium acetate at 2 different pH values. Performed at pH 4.5-5.5 and pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

Formulation Composition	**Azithromycin Conc. (mg/ml)**
200 mg/ml 2-HP-β CD + 2% Sodium Acetate (pH 4.5-5.5)	5.77
200 mg/ml 2-HP-β CD + 2% Sodium Acetate (pH 6.5-7.0)	7.73

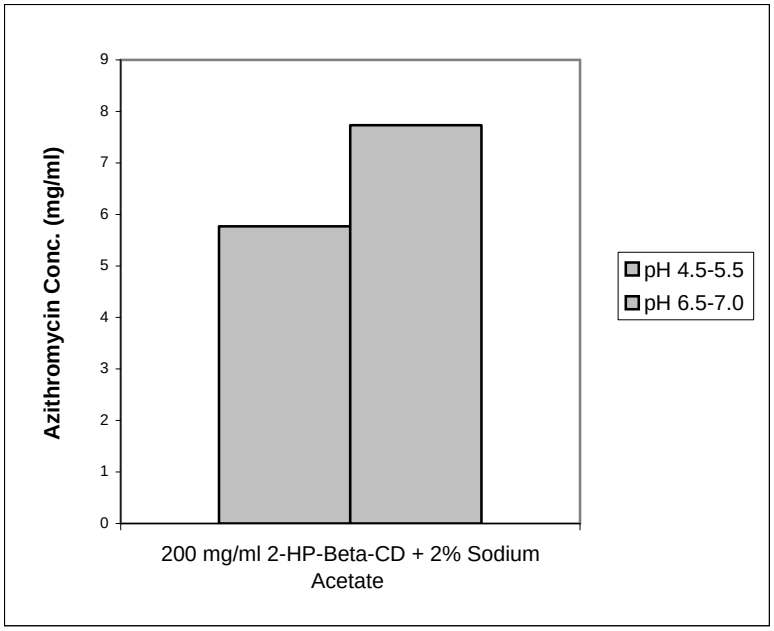


Figure 6: Phase solubility diagram of azithromycin in 200 mg/ml 2-HP-β-CD aqueous solution in presence of sodium acetate, at 2 different pH values. Performed at pH 4.5-5.5 and pH 6.5-7.0, with 3 days of equilibrium time at room temperature.

For each of the excipients used, there is a pH range in which the solubility of azithromycin can reach its maximum. For example, in the case of citric acid, a pH range between 4.5-5.5 is more suitable than a pH range between 6.5-7.0. Contrary, in the case of sodium acetate and ascorbic acid, a pH range 6.5-7.0 produces a higher solubility increase than a pH range 4.5-5.5. This is due to the fact that at a specific pH range, the complex formation between the several components of the formulation is promoted.

6.2.2.4 Influence of lactic acid, nicotine amide and vitamin E acetate on the solubility of azithromycin

In addition, three more excipients were tested in order to determine their ability to solubilise the azithromycin.
For the next set of experiments it was decided to present the results in terms of the percentage of azithromycin recovered as way of comparing the complexing ability of the several auxiliary substances used. This way it was easier to compare the effects of each of the excipients on the solubility of azithromycin.
The experiment was performed at pH 4.5-5.0, with 1 day of equilibrium time at room temperature.

Table 8: Percentage of azithromycin recovered in the presence of different lactic acid concentrations. Performed at pH 4.5-5.0, with 1 day of equilibrium time at room temperature.

Formulation Composition	Azithromycin Recovered (%)
200 mg/ml 2-HP-β-CD + 10 mg/ml Lactic Acid	92.1
200 mg/ml 2-HP-β-CD + 50 mg/ml Lactic Acid	70.5

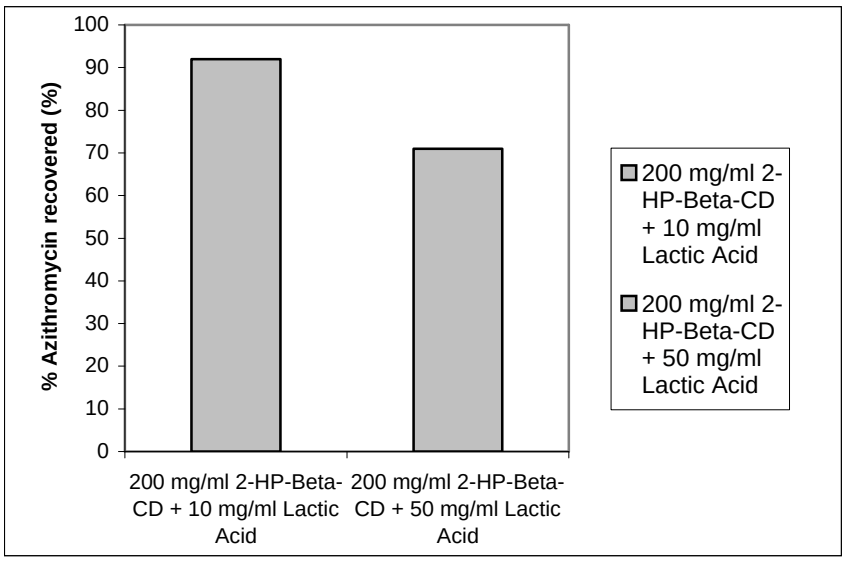


Figure 7: Percentage of azithromycin recovered in the presence of different lactic acid concentrations. Performed at pH 4.5-5.0, with 1 day of equilibrium time at room temperature.

Table 9: Percentage of azithromycin recovered in the presence of different nicotine amide concentrations. Performed at pH 4.5-5.0, with 1 day of equilibrium time at room temperature.

Formulation Composition	Azithromycin recovered (%)
200 mg/ml 2-HP-β-CD + 10 mg/ml Nicotine Amide	82
200 mg/ml 2-HP-β-CD + 50 mg/ml Nicotine Amide	78

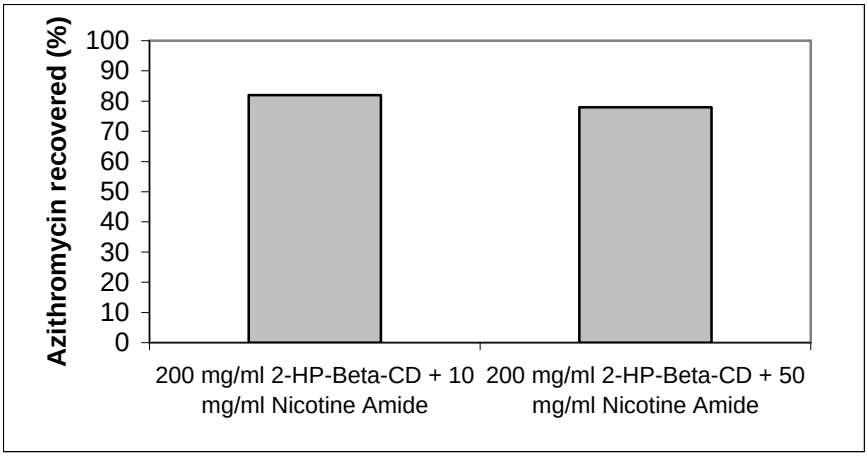


Figure 8: Percentage of azithromycin recovered in the presence of different Nicotine amide concentrations. Performed at pH 4.5-5.0, with 1 day of equilibrium time at room temperature.

Table 10: Percentage of azithromycin recovered in the presence of different Vitamin E acetate concentrations. Performed at pH 4.5-5.0, with 1 day of equilibrium time at room temperature.

Formulation Composition	Azithromycin recovered (%)
200 mg/ml 2-HP-β-CD + 10 mg/ml Vitamin E acetate	66
200 mg/ml 2-HP-β-CD + 50 mg/ml Vitamin E acetate	58

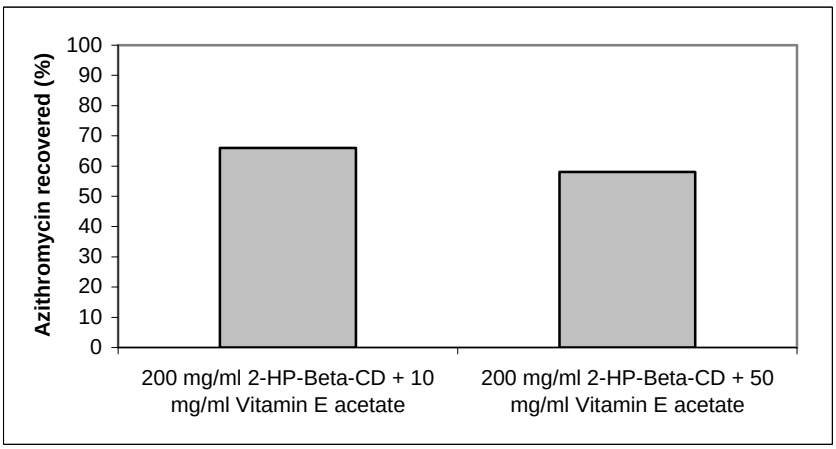


Figure 9: Percentage of azithromycin recovered in the presence of different Vitamin E acetate concentrations. Performed at pH 4.5-5.0, with 1 day of equilibrium time at room temperature.

From the results it was possible to ascertain that there is a relationship between the concentration of each of the excipients and the solubility of azithromycin.
For example, in the case of vitamin E acetate, the increase of the concentration had a negative effect on the solubility of azithromycin.

6.2.2.5 Influence on the solubility of azithromycin of a formulation containing ascorbic acid, 2-HP-β-cyclodextrin

With the purpose of finding the best relationship between the concentration of ascorbic acid, 2-HP-β-cyclodextrin and azithromycin, the following experiments were performed. The experimental procedure for this experiment was similar to the previous experiments but in this case, the equilibrium time was 1 day.

Table 11: Percentage of recovered azithromycin in the presence different concentrations of 2-HP-β-Cyclodextrin, ascorbic acid and azithromycin. Performed at pH 6.5-7.0, with 1 day of equilibrium time at room temperature.

Formulation Composition	Azithromycin recovered (%)
200 mg/ml 2-HP-β-CD + 10 mg/ml Ascorbic Acid	84
200 mg/ml 2-HP-β-CD + 50 mg/ml Ascorbic Acid	93
200 mg/ml 2-HP-β-CD + 100 mg/ml Ascorbic Acid	92
100 mg/ml 2-HP-β-CD + 10 mg/ml Ascorbic Acid	99
100 mg/ml 2-HP-β-CD + 50 mg/ml Ascorbic Acid	95
100 mg/ml 2-HP-β-CD + 100 mg/ml Ascorbic Acid	93
50 mg/ml 2-HP-β-CD + 10 mg/ml Ascorbic Acid	100
50 mg/ml 2-HP-β-CD + 50 mg/ml Ascorbic Acid	97
50 mg/ml 2-HP-β-CD + 100 mg/ml Ascorbic Acid	98

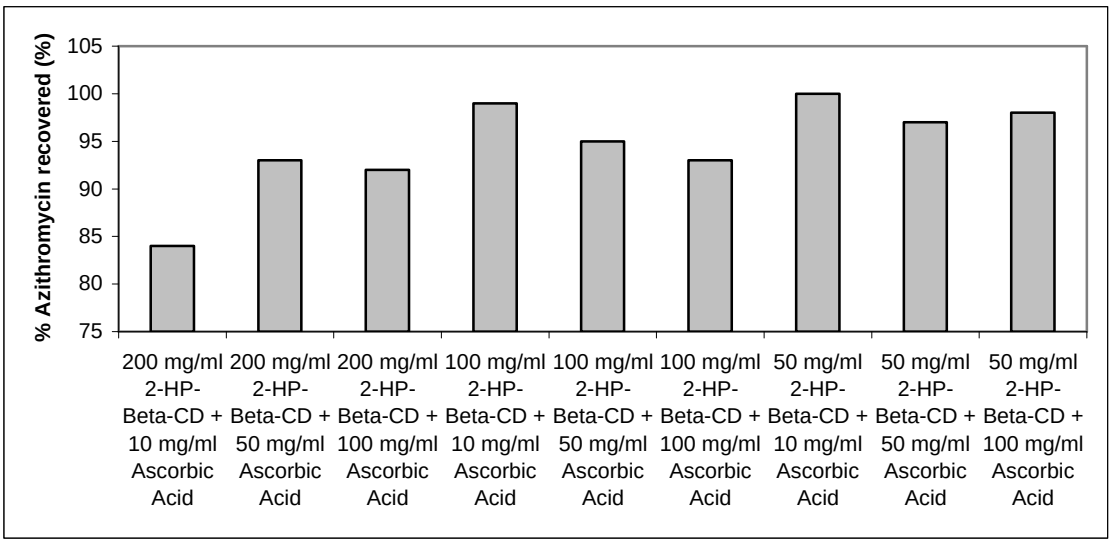


Figure 10: Percentage of recovered azithromycin in the presence of different concentrations of 2-HP-β-Cyclodextrin, ascorbic acid and azithromycin. Performed at pH 6.5-7.0, with 1 day of equilibrium time at room temperature.

For each of the 2-HP-β-CD concentrations, there is a specific concentration of ascorbic acid that maximizes the solubility of azithromycin:

1. 200 mg/ml 2-HP-β-CD → 50 mg/ml Ascorbic Acid
2. 100 mg/ml 2-HP-β-CD → 10 mg/ml Ascorbic Acid
3. 50 mg/ml 2-HP-β-CD → 10 mg/ml Ascorbic Acid

Based on these results, it is possible to improve the solubility of azithromycin by optimising other parameters.
In the next set of experiments, the effect of the temperature, the pH and the equilibrium time was analysed.

6.2.2.5.1 Influence of equilibrium time on the solubility of azithromycin

In this of experiment, the effect of the equilibrium time was analysed. Regarding the equilibrium time, it was observed:

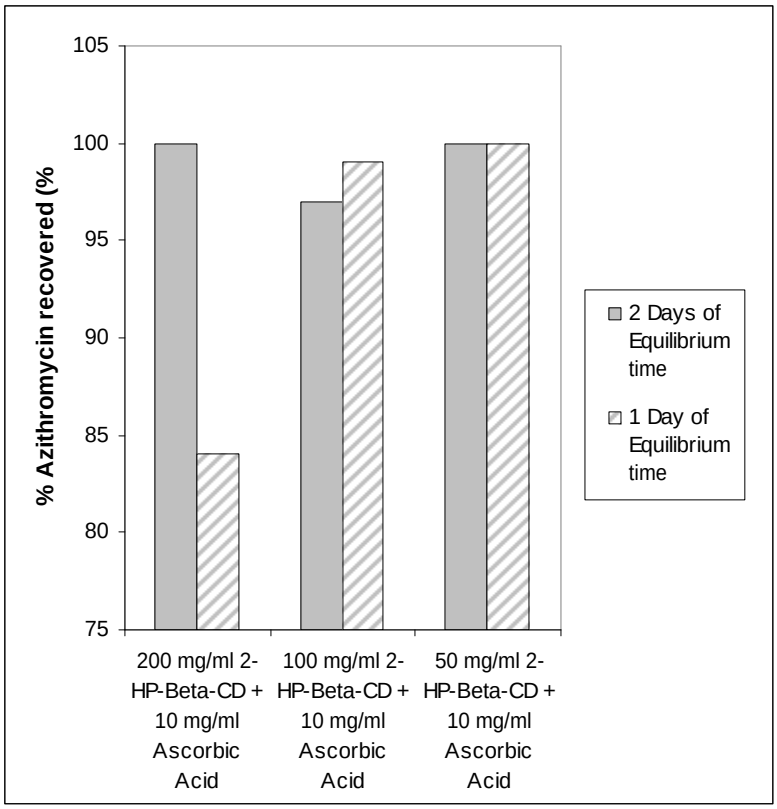


Figure 11: Comparison of the percentage of azithromycin recovered based on the equilibrium time, at a pH of 6.5-7.0.

From the results, it could be ascertained that the equilibrium time has an influence on the solubility of azithromycin. In the formulation with the higher concentration of 2-HP-β-CD, the maximum solubility of azithromycin was obtained at 2 days equilibrium time. This can be explained based on the fact that the complex formation process requires more time due to the higher amount of 2-HP-β-CD present in the formulation.
On the other hand, the formulations containing 50 mg/ml and 100mg/ml 2-HP-β-CD require only 1 day equilibrium time to achieve the maximum solubility of azithromycin.

6.2.2.5.2 Influence of temperature on the solubility of azithromycin

To analyse the effect of the temperature, two identical formulations were prepared using two different temperatures. One of the formulations was continuously stirred at room temperature, while the other was stirred for 30 minutes at 70°C. Afterwards, the vial containing the formulation was continuously stirred at room temperature. The equilibrium time in this experiment was 1 day.

Table 12: Percentage of azithromycin recovered based on the temperature effect. Performed at pH 6.5-7.0, with 1 day of equilibrium time at room temperature.

Formulation Composition	Azithromycin Recovered (%)
50 mg/ml 2-HP-β-CD + 10 mg/ml Ascorbic Acid (RT)	100
50 mg/ml 2-HP-β-CD + 10 mg/ml Ascorbic Acid (t=70°C, for 30 min)	100

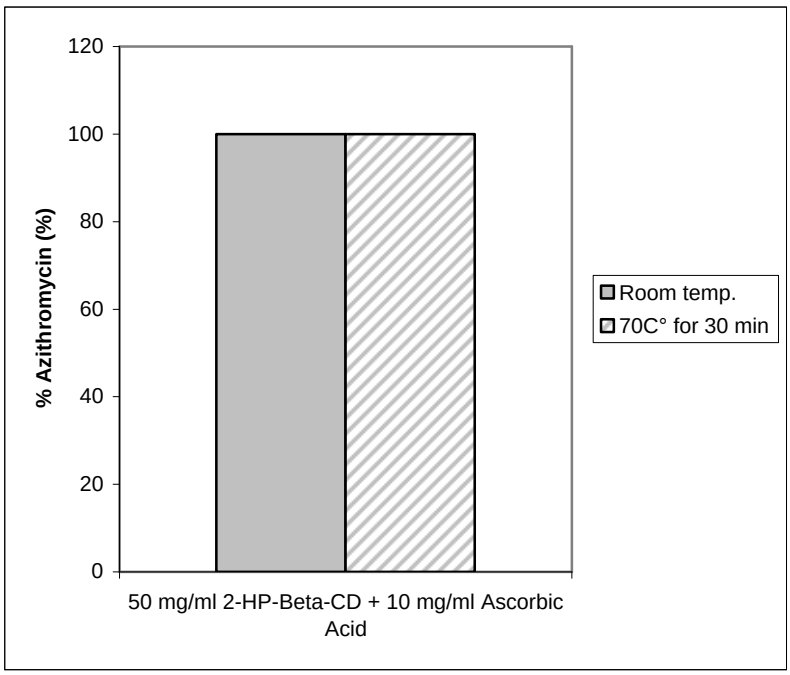


Figure 12: Percentage of azithromycin recovered based on the temperature effect. Performed at pH 6.5-7.0, with 1 day of equilibrium time at room temperature.

Based on the results, it can be concluded that the temperature has no influence in increasing the solubility of azithromycin.

6.2.2.5.3 Influence of pH on the solubility of azithromycin

To ascertain the pH effect, two identical formulations were prepared and the pH from these formulations was adjusted at two different values: 6.5-7.0 and 4.5-5.5. The equilibrium time in this experiment was 1 day, at room temperature.

Table 13: Percentage of azithromycin recovered based on the pH effect. Performed at pH 4.5-5.5 and pH 6.5-7.0, with 1 day of equilibrium time at room temperature.

Formulation Composition	Azithromycin recovered (%)
50 mg/ml 2-HP-β-CD + 10 mg/ml Ascorbic Acid (pH 6.5-7.0)	100
50 mg/ml 2-HP-β-CD + 10 mg/ml Ascorbic Acid (pH 4.5-5.5)	84

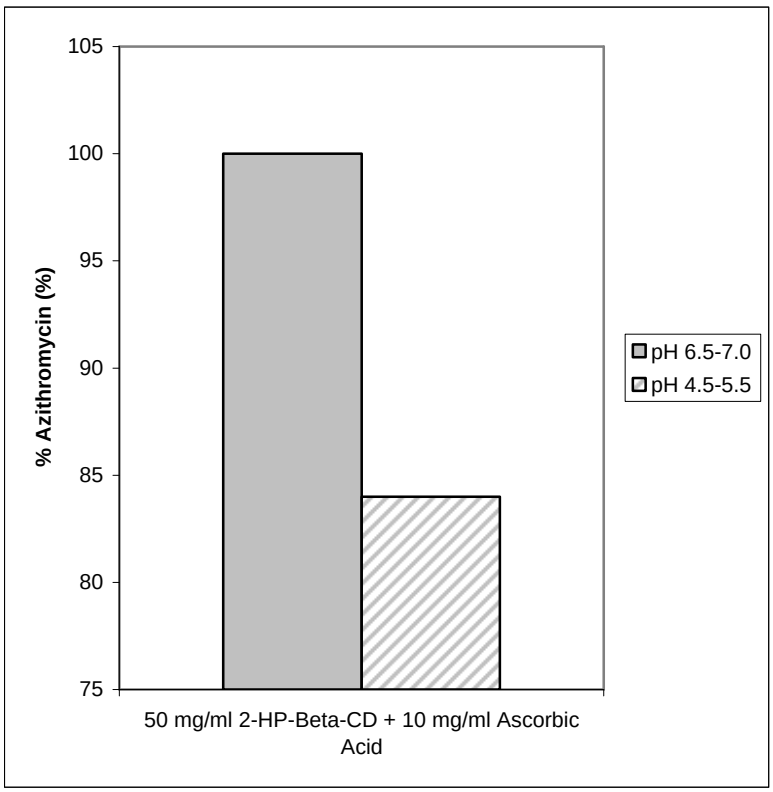


Figure 13: Percentage of Azithromycin recovered based on the pH effect. Performed at pH 4.5-5.5 and pH 6.5-7.0, with 1 day of equilibrium time at room temperature.

After analysing the results, it was possible to ascertain that the pH plays a very important role on the solubility of azithromycin. The pH range 6.5-7.0 is the most suitable for the solubility increase of azithromycin.

6.2.2.5.4 Influence of 2-HP-β-CD concentrations on the solubility of azithromycin

To study the effect of the 2-HP-β-CD concentrations on the solublization of azithromycin, two formulations were prepared. In this experiment, two concentrations were used: 25 mg/ml and 50 mg/ml. As excipient, ascorbic acid was also used. This experiment was performed at pH 6.5-7.0, with 2 days of equilibrium time at room temperature.

Table 14: Percentage of azithromycin recovered in the presence of different 2-HP-β-CD concentrations. Performed at pH 6.5.7.0, with 2 days of equilibrium time at room temperature.

Formulation Composition	Azithromycin recovered (%)
50 mg/ml 2-HP-B-CD + 50 mg/ml Ascorbic Acid	91
25 mg/ml 2-HP-B-CD + 50 mg/ml Ascorbic Acid	89

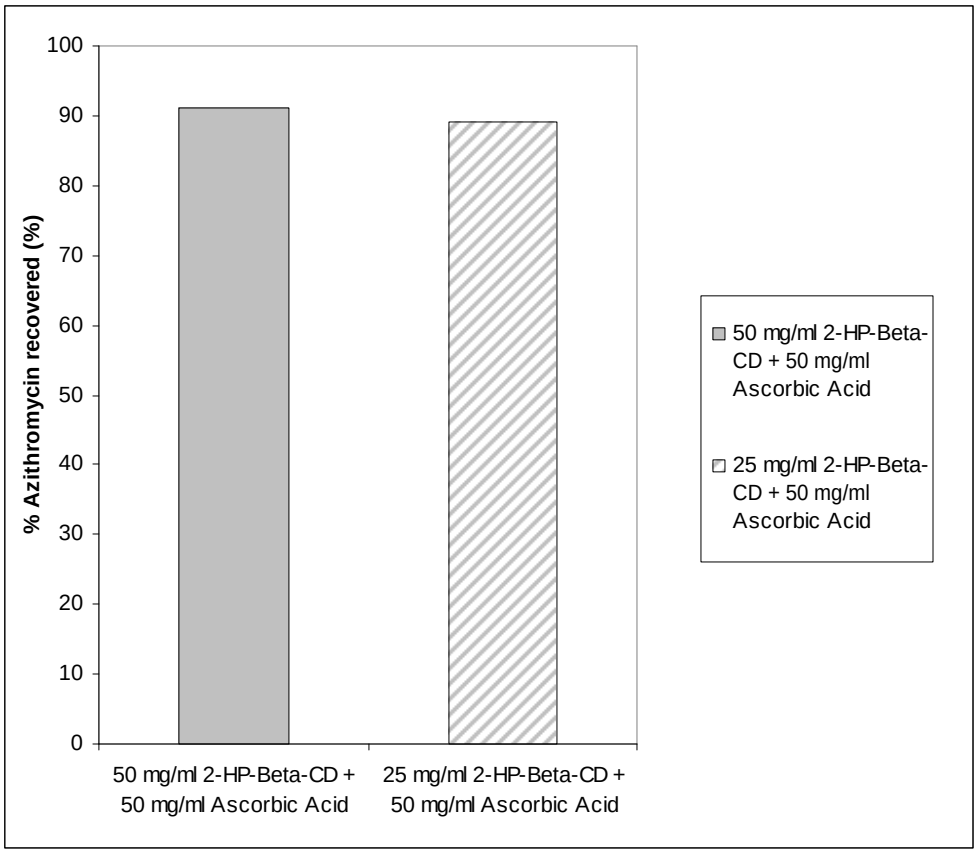


Figure 14: Percentage of azithromycin recovered in the presence of different 2-HP-β-CD concentrations. Performed at pH 6.5.7.0, with 2 days of equilibrium time at room temperature.

From the results, it can be concluded that increasing the concentration of 2-HP-β-CD has a very small effect on the solubility of azithromycin. Based in terms of toxicology and cost issues, it is advisable to use smaller concentrations of 2-HP-β-CD.

6.2.2.5.5 Influence of sodium ascorbate concentrations on the solubility of azithromycin

In the next set of experiments, it was investigated the solubility effect of sodium ascorbate. The reason of using this excipient was mainly based on its better stability and also on its similar molecular structure to ascorbic acid.
This experiment was performed at pH 6.5-7.0, with 2 days of equilibrium time at room temperature.

Table 15: Percentage of azithromycin recovered in the presence of Sodium Ascorbate. Performed at pH 6.5.7.0, with 2 days of equilibrium time at room temperature.

Formulation Composition	Azithromycin recovered (%)
200 mg/ml 2-HP-B-CD + 10mg/ml Sodium Ascorbate	96
100 mg/ml 2-HP-B-CD + 10 mg/ml Sodium Ascorbate	98

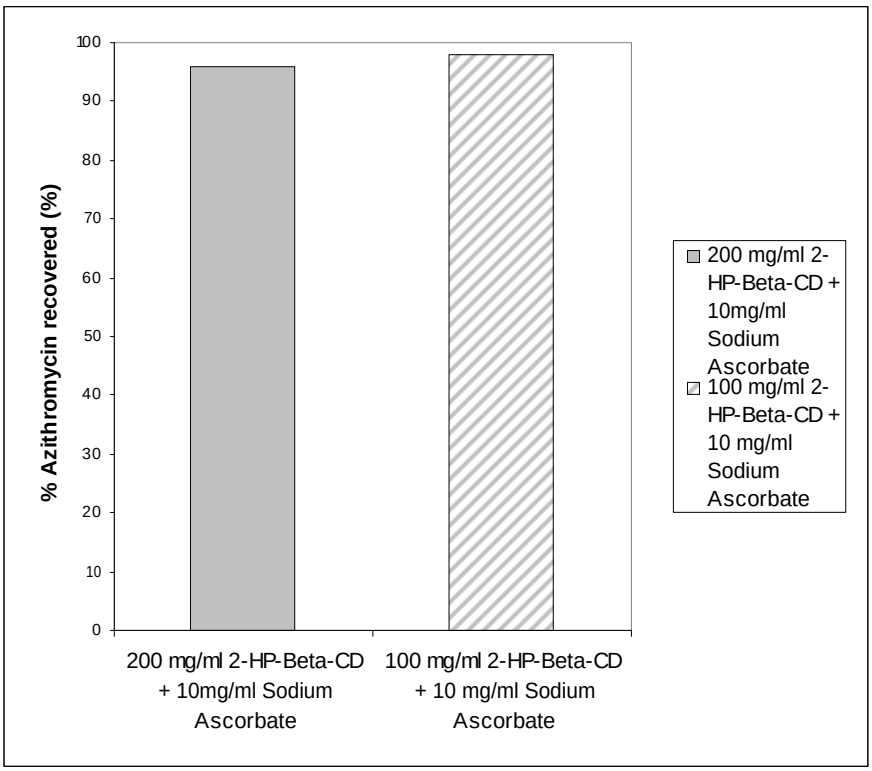


Figure 15: Percentage of azithromycin recovered in the presence of sodium ascorbate. Performed at pH 6.5-7.0, with 2 days of equilibrium time at room temperature.

Based on the results, increasing the 2-HP-β-CD concentration has little influence on the solubility of azithromycin, as previously explained.

One of the reasons for using cyclodextrins is their ability to improve the solubility and stability of azithromycin. On the other hand, taste masking is another important feature to take in consideration; especially in the case of azithromycin molecules due to their very bitter taste. If a patient, particularly young patients, do not like the taste, it is more difficult to persuade them to continue with the therapy. Consequently, taste masking is an important factor in patient compliance.

From the work performed, we could increase the solubility of azithromycin in aqueous solutions, but the taste of the formulations was still bitter. This is mainly due to the presence of azithromycin outside of the cyclodextrin cavity, in non-inclusion complexes.

With the objective of improving the solubility and also the taste of the azithromycin formulation, a new approach was developed. It involves increasing the intrinsic solubility of azithromycin with the help of pH adjustments in order to maximize the solubilization capacity of the cyclodextrins without the formation of non-inclusion complexes.

The resulting formulation showed an improvement in the taste masking although the bitter was not entirely removed. However, the taste of the formulation was tolerable for inhalation.
The concentration of azithromycin of the last formulation was 17 mg/ml.

From the previous data, it was possible to ascertain that the pH plays a very important role in the solubilization of azithromycin. In addition, the concentration of the 2-HP-β-Cyclodextrin also influences the solubility of azithromycin. Finally, the type and also the concentration of the excipients used can also play a part in the solubilization of azithromycin.
However, the effect of the pH is the most important parameter in the solubilization of azithromycin.

6.2.2.6 Nebulization trials

In order to test nebulisation characteristics of the azithromycin/cyclodextrin formulations, three different formulations were analysed, in terms of MMD, TOR, percentage of particles below 5% and 2%.

Table 16: Nebulization trials using 3 different 2-HP-β-CD aqueous solutions in the presence of sodium ascorbate.

Formulation Composition	**MMD NaCl (µm)**	**MMD Formulation (µm)**	**GSD**	**TOR (mg/min)**	**%<5 µm**	**%< 2µm**
200 mg/ml 2-HP-Beta-CD + 10mg/ml Sodium ascorbate	4.2	2.97	1.6	252	84.5	17.6
100 mg/ml 2-HP-Beta-CD + 10 mg/ml Sodium ascorbate	4.2	3.21	1.6	335	81.03	16
25 mg/ml 2-HP-Beta-CD + 50 mg/ml Ascorbic acid	4.2	3.28	1.63	380	79.95	15.1

The results show that it is possible to obtain particle sizes (2.5-3.5 µm) suitable for the pulmonary administration of azitromycin by nebulising the azithromycin/cyclodextrin formulations with the eflow device.

6.2.2.7 Lyophilization of the Azithromycin/Cyclodextrin Formulation

With the purpose of protecting the azithromycin/cyclodextrin system from any decomposition phenomena related to hydrolysis reactions, an azithromycin/cyclodextrin formulation was prepared and lyophilizated.
The formulation had the following composition:

Table 17: Composition of a lyophilized azithromycin/cyclodextrin formulation

Formulation Composition	Conc. (mg/ml)
Azithromycin	4.3[31]
2-HP-β-CD	51.5

The vials were filled with 1 ml of the azithromycin/cyclodextrin formulation each and placed inside the lyophilizer chamber. Lyophilization of the samples was performed with a Christ Epsilon 2-6D (Germany) equipment. Conditions of the entire process are shown in Table 18

Table 18: Lyophilization conditions of an azithromycin/cyclodextrin formulation.

Process Phase	Time (min)	Temp. (°C)	Vacuum (mbar)	Si. –Pressure (mbar)
Start value	0	0	OFF	OFF
Freezing	1	-38	OFF	OFF
Freezing	44	-38	OFF	OFF
Preparation	15	-38	OFF	OFF
Main drying	1	-38	0.08	OFF
Main drying	15	-25	0.08	0.140
Main drying	704	-25	0.08	0.140
After drying	1	-25	0.08	0.140
After drying	300	15	0.08	0.140
After drying	719	15	0.08	0.140

At the end of the process, the lyophilized obtained was a white coloured product (see Figure 16).

[31] Amount present in 19mg of the lyophilizate.

Figure 16: Image of a lyophilizate of an azithromycin/cyclodextrin formulation. Image taken 1 day after lyophilization.

Figure 17: Image of a reconstituted lyophilizate of an azithromycin/cyclodextrin formulation. Reconstitution was made by adding 3 ml and manually shaking the vial. Image taken 1 day after lyophilization.

The determination of the nebulisation properties was investigated using the eflow® device (Pari GmbH, Germany) as nebuliser.

Table 19: Nebulization characteristics reconstituted lyophilizate of an azithromycin/cyclodextrin formulation. Reconstitution was made by adding 3 ml and manually shaking the vial[4].

MMD (µm)	GSD	TOR (mg/ml)	Percent. Particles <5 µm	Percent. Particles <2 µm
3.75	1.55	583	74.3	8.3

The water content present in the lyophilizate was also determined:

Table 20: Water content of a lyophilizate of azithromycin/cyclodextrin

Formulation	Water content (%)
Azithromycin	4.08

The low water content of the formulation allows for a more stable formulation because it reduces of possibility from any decomposition phenomena related to hydrolysis reactions to occur.

6.2.2.8 Stability Batches

It is known from literature (Lit. [24]) that azithromycin can degrade in aqueous solutions, especially in the presence of acids.
In order to investigate the stability of the azithromycin formulation, six stability batches (STB), five solutions and one lyophilizate were prepared:

STB 1: 3.5g azithromycin and 10g 2-HP-β-CD were stirred in 100ml water; pH was continuously observed. 85% phosphoric acid, in quantities of 10 µl, was added when pH was above 7.5. After 18h in total, 320µl H3PO4 were needed to obtain a clear solution with a pH of 7.4.

STB 2: 3.5g azithromycin and 10g 2-HP-β-CD were solubilized in 50ml methanol. After stirring over night, methanol was removed using a rotary evaporator at 30°C to a final pressure of 52 mbar in about 60 minutes. Reconstitution in 120 ml water resulted, surprisingly, in a stable suspension.

STB 3: 3g azithromycin were solubilized in 100ml water and 470µl 85% H3PO4. Then 8g of 2-HP-β-CD were added. After 15 minutes stirring, 9.5ml 1M NaOH were added to pH 8.8 causing precipitation. Suspension was filtrated (0.45µm); pH of the clear solution was adjusted to 7.8 with H3PO4.

STB 4: 2.5g azithromycin were added to 100 ml water and solubilized by adding approx. 4ml 1M HCl. 8g of 2-HP-β-CD were added and solution was stirred for 30 minutes. 1M NaOH was quickly added till pH reached 8.9. Resulting suspension was centrifuged twice at 6000 Rpm for one minute.

STB 5: 3g azithromycin were solubilized in 100 ml water containing 450µl 85% H3PO4, resulting in a clear solution with pH 6.2. This preparation served as reference solution.

STB 6: 3g azithromycin, 10g 2-HP-β-CD and 350µl H3PO4 were solubilized similarly to batch STB 1 in 60 ml water. 2 ml were pipetted into vials and freeze dried according to following program 1

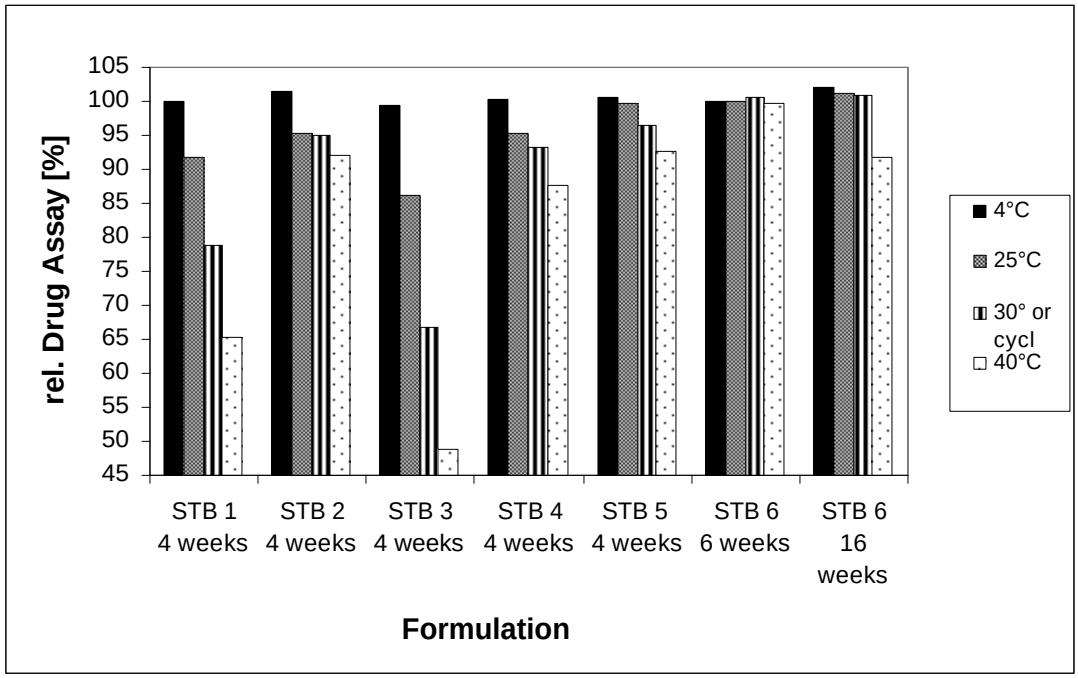


Figure 18: Stability batches of 6 different azithromycin formulations.

Table 21: Percentage of recovered azithromycin in 6 different azithromycin formulations stability batches.

Storage Conditions	STB 1 4 weeks(%)	STB 2 4 weeks(%)	STB 3 4 weeks (%)	STB 4 4 weeks (%)	STB 5 4 weeks (%)	STB 6 6 weeks (%)	STB 6 16 weeks (%)
4°C	100.1	101.4	99.4	100.4	100.6	100.0	102.1
25°C	91.8	95.4	86.3	95.2	99.6	100.0	101.3
30° / cycling	78.7	95.0	66.7	93.2	96.5	100.5	100.8
40°C	65.3	92.1	48.8	87.7	92.6	99.8	91.8

From the results we could ascertain that all aqueous formulations were unstable. It seems to appear that cyclodextrins could have an influence in accelerating the drug degradation, as STB 5, consisting only of drug and acid, was the most stable among the solutions.

However, the lyophilizate showed no significant degradation after 6 weeks, even at 40°C / 75% r.h. storage conditions. Only after 16 weeks under 40°C / 75% r.h. storage conditions it was possible to see some degradation. Although the difference between the 6 weeks and 16

weeks value is above the analytical error (8%), it is not possible to conclude that the difference is statistic significant due to low number of data points

Subchapter conclusion

It was decided to use cyclodextrins as mean of improving not only the solubility/stability properties of the azithromycin but also to taste mask its bitter taste.
From the results obtained, we could ascertain that the pH plays a very important role in the solubility of azithromycin. However, this increase in the solubility of azithromycin was not accompanied by an improvement of the taste of the formulations. This was due to the formation of not only inclusion complexes between the azithromycin and the cyclodextrins, but also due to the formation of non-inclusion complexes.
With the objective of improving the solubility and also the taste of the azithromycin formulation, a new approach was developed. It involved increasing the intrinsic solubility of azithromycin with the help of pH adjustments in order to maximize the solubilization capacity of the cyclodextrins without the formation of non-inclusion complexes. Consequently, the resulting formulation showed an improvement in the taste masking although the bitter was not entirely removed. However, the taste of the formulation was tolerable for inhalation.

Based on the results, we can ascertain that the best excipients which promote the formation of non-inclusion complexes and, consequently, increase the solubility azithromycin solubility are ascorbic acid and citric acid.

With the purpose of obtaining a stable azithromycin formulation it was investigated the stability five solutions and one lyophilizate.
From the results we could ascertain that all aqueous formulations were unstable. However, the lyophilizate showed no significant degradation after 16 weeks. Only after 16 weeks under 40°C / 75% r.h. storage conditions we could see some degradation although not very significant, taking in consideration the storage conditions.

In conclusion, it was possible to obtain a stable formulation of azithromycin with a taste suitable to inhalation purposes.

6.2.3 Pentoxifylline

Pentoxifylline is a water soluble drug[32] used in the field of oncology due its reported potential ability to ameliorate radiation- and chemotherapy-induced toxicity. However, it possesses a very bitter taste which has a very important influence in patient compliance, especially in young patients. For this reason, it was decided to use cyclodextrins as a mean of taste masking the bitter taste of the pentoxifylline formulations.
To five vials containing 50 mg/ml of pentoxifylline, different concentrations of 2-HP-β-CD were added.

Table 1: Formulation composition of 5 different pentoxifylline formulations.

Formulation composition	Code
50 mg/ml Pentoxifylline+ 10 mg/ml 2-HP-Beta-CD	P10
50 mg/ml Pentoxifylline+ 25 mg/ml 2-HP-Beta-CD	P25
50 mg/ml Pentoxifylline+ 50 mg/ml 2-HP-Beta-CD	P50
50 mg/ml Pentoxifylline+ 75 mg/ml 2-HP-Beta-CD	P75
50 mg/ml Pentoxifylline+ 100 mg/ml 2-HP-Beta-CD	P100

These 5 formulations were then analysed not only in terms of their physicochemical properties but also for their nebulisation parameters.

Table 2: Physicochemical properties of 5 different pentoxifylline formulations.

Formulation Comde	pH	Osmolarity (osmol/Kg)	Viscosity (mPa s)	Surface Tension (mN/m)
P10	2.52	0.114±0.002	1.24±0.05	55.1±0.17
P25	2.7	0.118±0.003	1.28±0.03	55.81±0.19
P50	3.23	0.129±0.002	1.38±0.03	56.59±0.28
P75	3.44	0.142±0.004	1.51±0.03	56.81±0.12
P100	3.46	0.161±0.001	1.64±0.04	57.12±0.1

[32] The water solubility of pentoxifylline is > 43 mg/ml.

Table 3: Nebulization parameters of 5 different pentoxifylline formulations[33]

Formulation code	MMD (µm)	GSD	TOR (mg/ml)	Particles <5 µm (%)	Particles <2 µm (%)
P10	2.08	1.64	151	95.4	46.5
P25	2.13	1.66	157	98.4	44.1
P50	2.31	1.76	142	98.7	49.5
P75	2.01	1.7	125	98.1	49.62
P100	2.25	1.69	110	81.7	40.2

Table 4: Nebulization properties of 5 different pentoxifylline formulations[34]

Formulation Composition	MMD (µm)	GSD	TOR (mg/ml)	Particles <5 µm (%)	Particles <2 µm (%)
P10	3.36	1.58	490	80.22	12.48
P25	3.33	1.57	476	80.8	12.64
P50	3.25	1.58	485	81.82	13.52
P75	3.21	1.57	454	82.38	13.63
P100	3.05	1.55	400	85.47	15.1

The nebulisation of the formulations produced particle size droplets suitable for inhalation. For example, using a nebulizer head of 2.75 µm it was possible to produce droplets size between 2.08-2.25 µm. With this particle size it possible to reach 45-55% deposition in the alveolar region. However, the output rate was low, between 110-150 mg/ml, which means longer nebulisation times (table3).

On the other, using a nebulizer head of 3.8 µm, it is possible to obtain a higher output rate (between 400-490 mg/ml) and, consequently, lower nebulisation times. In addition, a droplet size between 3.05-3.36 µm also allows a 45-55% deposition in the alveolar region (table 4).

The taste masking ability of the cyclodextrin in the different pentoxifylline formulations was also investigated.

In order to ascertain the taste of formulations, all five formulations were nebulized (after pH and osmolarity correction) using the electronic nebuliser eflow. All formulations showed a neutral to a slight sweet taste which indicates that cyclodextrins were able to taste mask the bitter taste of the pentoxifylline[35].

[33] MMD (NaCl) = 2.75 µm; Measured at a Malvern Zetasizer (Model Zetasizer 3000 HAS, Germany). The nebulisation trial was performed using an electronic nebulizer, eflow® (Pari GmbH, Germany).

[34] MMD (NaCl) = 3.8 µm; Measured at a Malvern Zetasizer (Model Zetasizer 3000 HAS, Germany). The nebulisation trial was performed using an electronic nebulizer, eflow® (Pari GmbH, Germany).

[35] The taste of all formulations was evaluated subjectively by a group of 5 persons.

Table 5: Taste description of 5 different pentoxifylline formulations.

Formulation composition	Taste Description
50 mg/ml Pentoxifylline+ 10 mg/ml 2-HP-β-CD	Neutral
50 mg/ml Pentoxifylline+ 25 mg/ml 2-HP-β-CD	Neutral
50 mg/ml Pentoxifylline+ 50 mg/ml 2-HP-β-CD	Neutral
50 mg/ml Pentoxifylline+ 75 mg/ml 2-HP-β-CD	Neutral to Sweet
50 mg/ml Pentoxifylline+ 100 mg/ml 2-HP-β-CD	Neutral to Sweet

Thus, the use of cyclodextrins can help improve the patient compliance in treatments were pentoxifylline plays a major role.

Subchapter conclusion

Pentoxifylline is a xanthine derivate that has been used in the field of oncology based on its reported potential ability to ameliorate radiation- and chemotherapy-induced toxicity.
It is a water soluble molecule which, however, possesses a very bitter taste. In order to improve patient compliance, it was decided to use cyclodextrins as a mean of taste masking the bitter taste of the Pentoxifylline molecules.
The results obtained show that it is possible to prepare a pentoxifylline formulation with a taste suitable for pulmonary administration. In addition, the formulations can be also efficiently nebulized through the use of eflow®.

6.2.4 Budesonide

Budesonide is a corticosteroid used for the treatment of asthma, non-infectious rhinitis (including hay fever and other allergies) and for the treatment and prevention of nasal polyposis. However, budesonide is water insoluble drug which constitutes a challenge not only in terms of increasing its solubility but also its stability.
Based on the previous work devolved for cyclosporin A and azithromycin, it was decided to investigate the possibility of using cyclodextrins as a way of increasing the solubility and stability of budesonide.
The purpose behind this approach was to investigate the plausibility of using a common platform to increase to solubility of water insoluble drugs.
Consequently, taking in consideration the previous work preformed with cyclosporin A/azithromycin and cyclodextrin and also the physicochemical properties of budesonide, it was decide to prepare a budesonide/2-HP-β-CD formulation.
The method of preparation was similar to the one performed for cyclosporin A with the difference that for the budesonide/cyclodextrin system, the equilibrium time and pH were, respectively, 1 day and 4.5[36]. The formulation composition and the results obtained are as follows:

Table 1: Formulation composition of a budesonide/cyclodextrin formulation.

Formulation composition	Conc. Budesonide (µg/ml)
100 mg/ml 2-HP-Beta-CD + 5.0 mg/ml sodium ascorbate	1008

Table 2: Physicochemical properties of a budesonide/cyclodextrin formulation.

Property	value
Surface Tension (σ) [mN/m]	59.05±0.12
Osmolarity [osmol/kg]	0.169±0.001
pH	4.59
Viscosity [mPa s]	1.4±0.04

[36] A complete description of the preparation method is on point 5.3.2.7 of the method chapter.

Table 3: Nebulization properties of a budesonide/cyclodextrin formulation[37]

MMD (µm)	GSD	TOR (mg/ml)	Percent. Particles <5 µm	Percent. Particles <2 µm
3.00	1.58	330	85.24	17.47

In addition to the previous formulation, the influence of the amount cyclodextrin in the solubility of budesonide was also investigated.

Table 4: Formulation composition of a budesonide/cyclodextrin formulation.

Formulation composition	Conc. Budesonide (µg/ml)
50 mg/ml 2-HP-Beta-CD + 5.0 mg/ml Sodium ascorbate	584

From the results obtained, we could ascertain that the solubility of budesonide is also directly dependent from the amount of cyclodextrin used.

It is known that budesonide can degrade in aqueous solutions. For this reason, a budesonide/cyclodextrin formulation was prepared in order to investigate the stability of budesonide in aqueous solutions at 2 different temperatures.

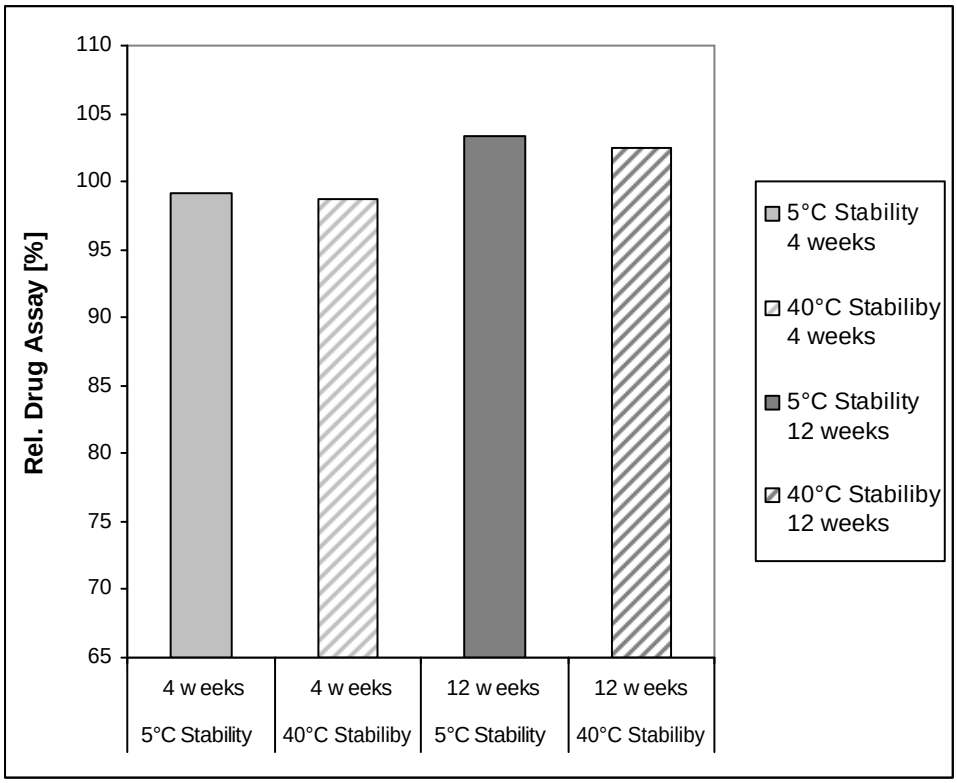


Figure 1: Stability test of a budesonide/cyclodextrin formulation.

[37] MMD (NaCl) = 3.44 µm; Measured at a Malvern Zetasizer (Model Zetasizer 3000 HAS, Germany). The nebulisation trial was performed using an electronic nebulizer, eflow® (Pari GmbH, Germany).

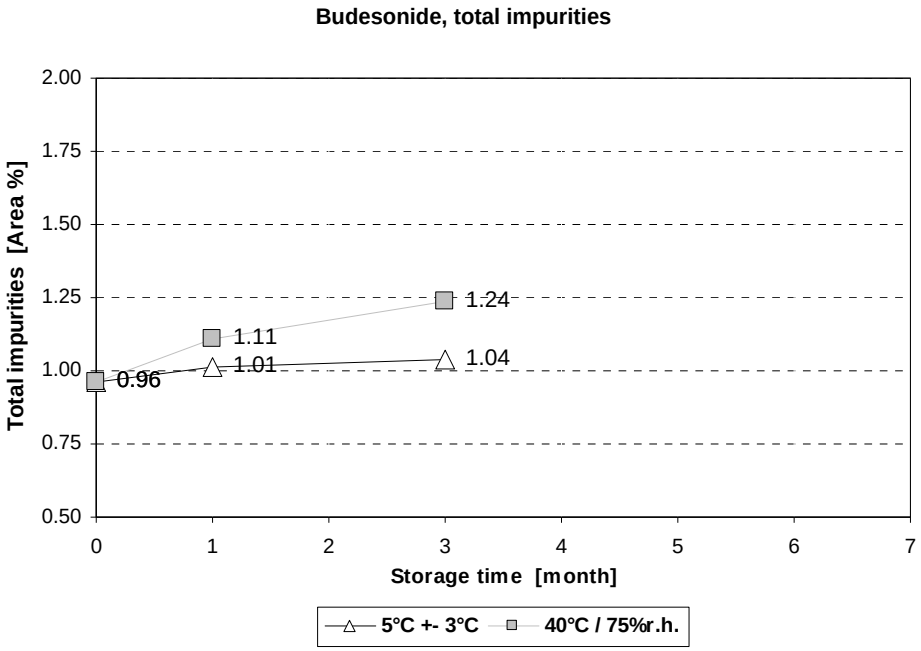


Figure 2: Stability test of a budesonide/cyclodextrin.

The results showed that the budesonide/cyclodextrin formulation is stable in aqueous solutions.
In addition, if required the budesonide/cyclodextrin formulation can be sterilised. For this reason and only as an example, the influence of filtration on the solubility of budesonide was investigated. Two different filters, 0.45 µm and 0.22 µm, were used.

Table 5: Comparison of the budesonide solubility between unfiltered and filtered aqueous solutions of budesonide.

Formulation composition	**Conc. Budesonide (µg/ml)**
100 mg/ml 2-HP-Beta-CD + 5.0 mg/ml Sodium Ascorbate (unfiltered)	299.7
100 mg/ml 2-HP-Beta-CD + 5.0 mg/ml Sodium ascorbate (0.45 µm filtered)	299.5
100 mg/ml 2-HP-Beta-CD + 5.0 mg/ml Sodium Ascorbate (0.22 µm filtered)	299.5

The results proved that the filtration has no influence in the solubility of budesonide and that, consequently, it is possible to obtain a sterile solution without affecting the solubility of budesonide.

Subchapter conclusion

Taking in consideration the work previously done with cyclosporin A and azithromycin, it was decide to prepare a budesonide/2-HP-β-CD formulation using a method of preparation similar to the one performed for cyclosporin A.
The results show that it is possible to apply the preparation method used for cyclosporin A to other water insoluble drugs.
As a conclusion, the use of cyclodextrins allows the preparation of a sterile and stable aqueous solution of budesonide.

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6.3 SUSPENSIONS

The preparation of water insoluble/cyclodextrin solutions and the possibility of using suspensions as a way of increasing the solubility of water insoluble drug, such as cyclosporin A and azithromycin were investigated.
However, it was decided to try a new approach using cyclodextrin in the preparation of the suspensions.

Amphiphilic and hydrophobic esters of cyclodextrins have been used to prepare colloidal suspensions of nanospheres which can be used as carriers for hydrophobic drugs. The method currently used to prepare these nanospheres suspensions is called nanoprecipitation Lit [1].
It is based on the spontaneous assembling of the cyclodextrin molecules previously dissolved in a water-miscible organic solvent when it is added into an aqueous phase. Normally, the hydrophobic drug is added to the organic phase of the formulation.
Another type of method, called emulsification solvent evaporation, is usually used to encapsulate hydrophobic drugs in polymeric matrices. It requires the emulsification of water-immiscible organic solutions of preformed polymers in aqueous phases containing soluble surfactants Lit [2]. This method has been widely applied to prepare biodegradable microspheres (Lit. [3], Lit. [4] and Lit. [5]).
Nevertheless, to obtain submicronic particles it is necessary to make modifications on the emulsification procedure, such as the salting-out process, high-pressure emulsification, or emulsification solvent diffusion (Lit. [6-11]).

In this work, we first investigated the feasibility of preparing nanospheres from hydrophobic cyclodextrins, instead of using amphiphilic cyclodextrins, with the nanoprecipitation method. However, several other cyclodextrins with different water solubility values can also be used, although different results can be expected. As an example, γ-cyclodextrin and α-cyclodextrin are more water soluble molecules as the β-cyclodextrin but it is also possible to prepare nanosuspensions from the γ and α cyclodextrins. Nonetheless, the method of preparation is different from the more hydrophobic cyclodextrins. Thus, it was decided to use hydrophobic cyclodextrins mainly due to availability and experimental reasons although the list of cyclodextrins that can be used is quite large.

The influence of the cyclodextrin concentration on the loading capacity of a hydrophobic drug was also investigated. Finally, it was also studied the feasibility nebulisation of these formulations using a novel electronic nebuliser , the eFlow® (PARI GmbH, Starnberg, Germany).The eFlow® uses a vibrating perforated membrane to generate aerosols of a well defined droplet size distribution.
Cyclosporin A and azithromycin were used in this study as hydrophobic model drugs.

6.3.1 Methods of preparation

6.3.1.1 Preparation of cyclodextrin nanospheres loaded with insoluble drugs

The preparation method of the cyclodextrin nanospheres consists in dissolving the cyclodextrin in an organic solvent miscible in water (acetone or ethanol) and adding this solution in an aqueous phase, with or without the presence of a surfactant, under stirring at room temperature. Consequently, the nanospheres precipitate spontaneously. Afterwards, the organic solvent, and also a part of the water, are removed by evaporation under vacuum.
The preparation of drug-loaded nanospheres can be obtained by following the previous procedure, with water soluble or water insoluble drugs dissolved in the aqueous phase or the organic solvent, respectively.
Nanospheres of cyclodextrin can also be prepared by using the emulsion solvent evaporation method [Lit12].
For the preparation of the cyclodextrin nanospheres, two types of cyclodextrin were used:

- β-Cyclodextrin;
- Ethylated-β-Cyclodextrin.

As model drugs, two water-insoluble drugs were chosen:

- **Cyclosporin A;**
- **Azithromycin.**

6.3.1.1.1 Cyclosporin A/Cyclodextrin and Azithromycin/Cyclodextrin suspensions

The preparation methods of the cyclosporin A/cyclodextrin and azithromycin/cyclodextrin nanosuspensions are described in detail in chapter 3.2.2. .

6.3.1.2 Cyclosporin A Suspensions

In this work, we first evaluated the feasibility of producing hydrophobic cyclodextrin nanospheres by the nanoprecipitation method. It is based on the spontaneous assembling of the cyclodextrin molecules previously dissolved in a water-miscible organic solvent (with the hydrophobic drug present) when it is added into an aqueous phase.
With this purpose in mind, a cyclosporin A suspension containing 20.2 mg/ml of ethylated-β-cyclodextrin and 8.86 mg/ml Pluronic F68® in an aqueous system was prepared.
As we can see from Figure 1, it was possible to produce ethylated-β-cyclosporin A/cyclodextrin nanospheres. The content of cyclosporin A present in the suspension was 0.51 mg/ml.

Table 1: Formulation composition of an ethylated-β-cyclosporin A/cyclodextrin nanospheres suspension.

Formulation composition	Conc. (mg/ml)
Cyclosporin A	0.51
Ethylated-β-cyclodextrin	20.2
Pluronic F68®	8.86

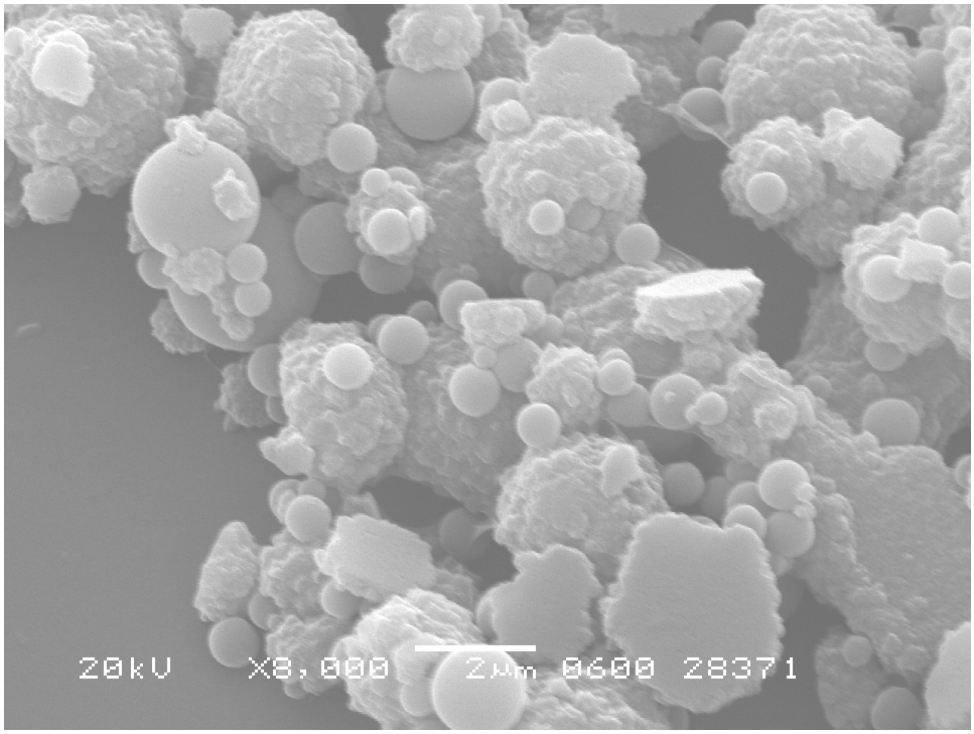

Figure 1: Magnified view of a cyclosporin A suspension containing 20.2 mg/ml of ethylated-β-cyclodextrin, 0.51 mg/ml of cyclosporin A and 8.86 mg/ml Pluronic F68® in an aqueous system. Magnified x8000.

After ascertaining the feasibility of forming hydrophobic cyclodextrin nanospheres, one other type of hydrophobic cyclodextrin was investigated for its ability on forming nanospheres, β-cyclodextrin.

Also in his case, it was possible to form a β-cyclosporin A/cyclodextrin nanospheres suspension (see Figure 2 to Figure 6).

Table 2: Formulation composition of a β-cyclosporin A/cyclodextrin nanospheres suspension.

Formulation composition	Conc. (mg/ml)
β-cyclodextrin	2.34
Pluronic F68®	8.86

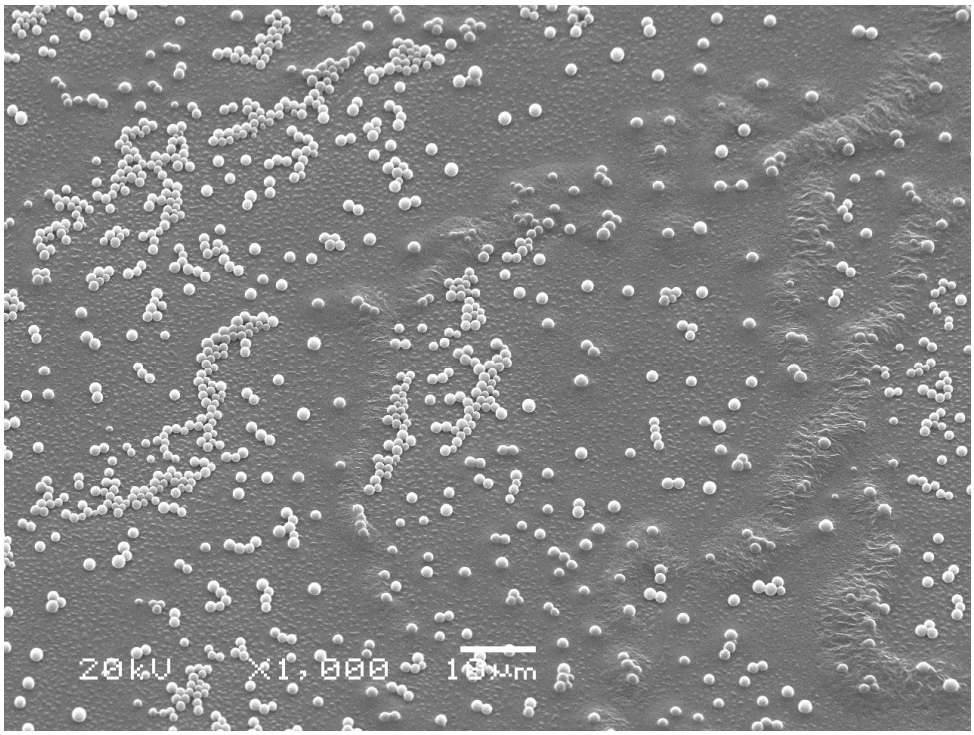

Figure 2: Overall view of a cyclosporin A suspension containing 2.3 mg/ml of β-cyclodextrin and 8.86 mg/ml Pluronic F68® in an aqueous system. Magnified x1000.

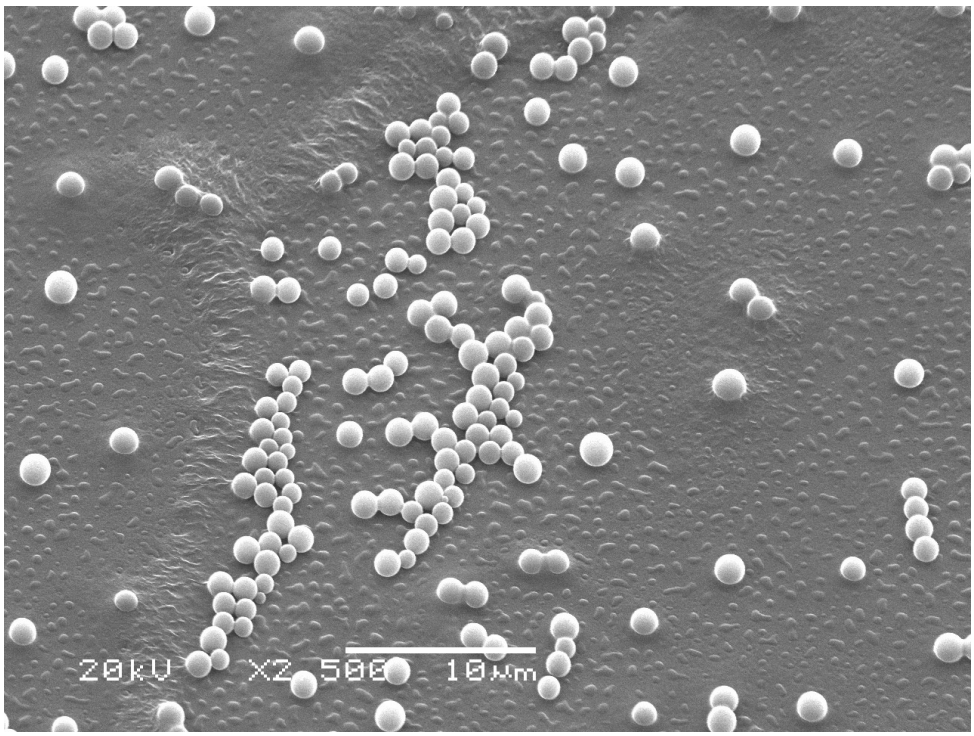

Figure 3: Overall view of a cyclosporin A suspension containing 2.3 mg/ml of β-cyclodextrin and 8.86 mg/ml Pluronic F68® in an aqueous system. Magnified x2500.

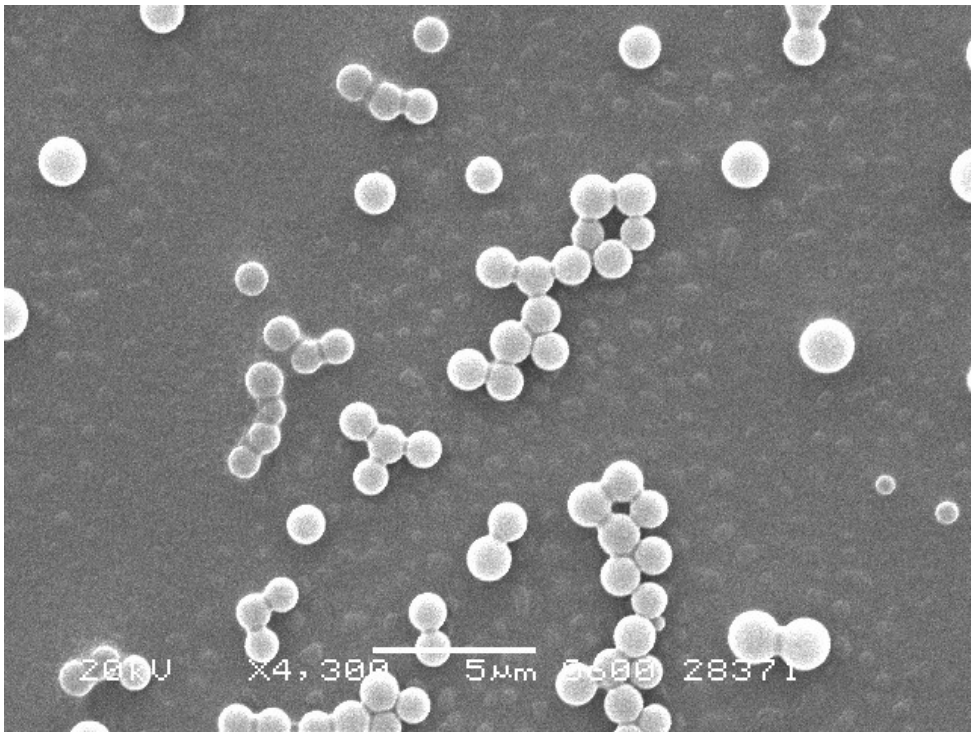

Figure 4: Overall view of a cyclosporin A suspension containing 2.3 mg/ml of β-cyclodextrin and 8.86 mg/ml Pluronic F68® in an aqueous system. Magnified x4300.

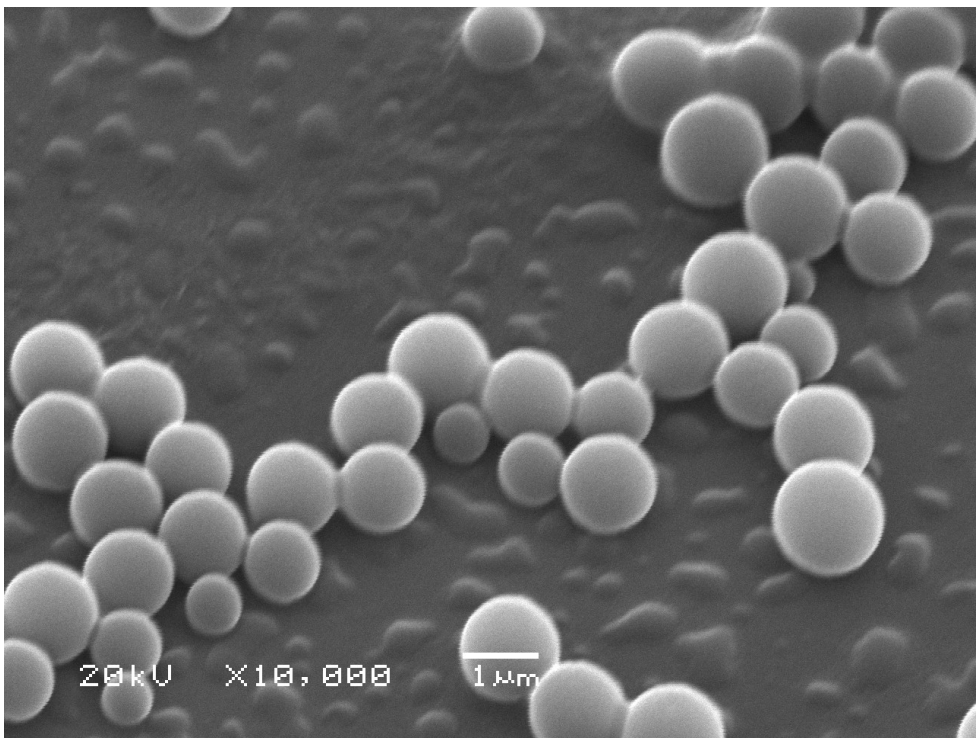

Figure 5: Magnified view of a cyclosporin A suspension containing 2.3 mg/ml of β-cyclodextrin and 8.86 mg/ml Pluronic F68® in an aqueous system. Magnified x10000.

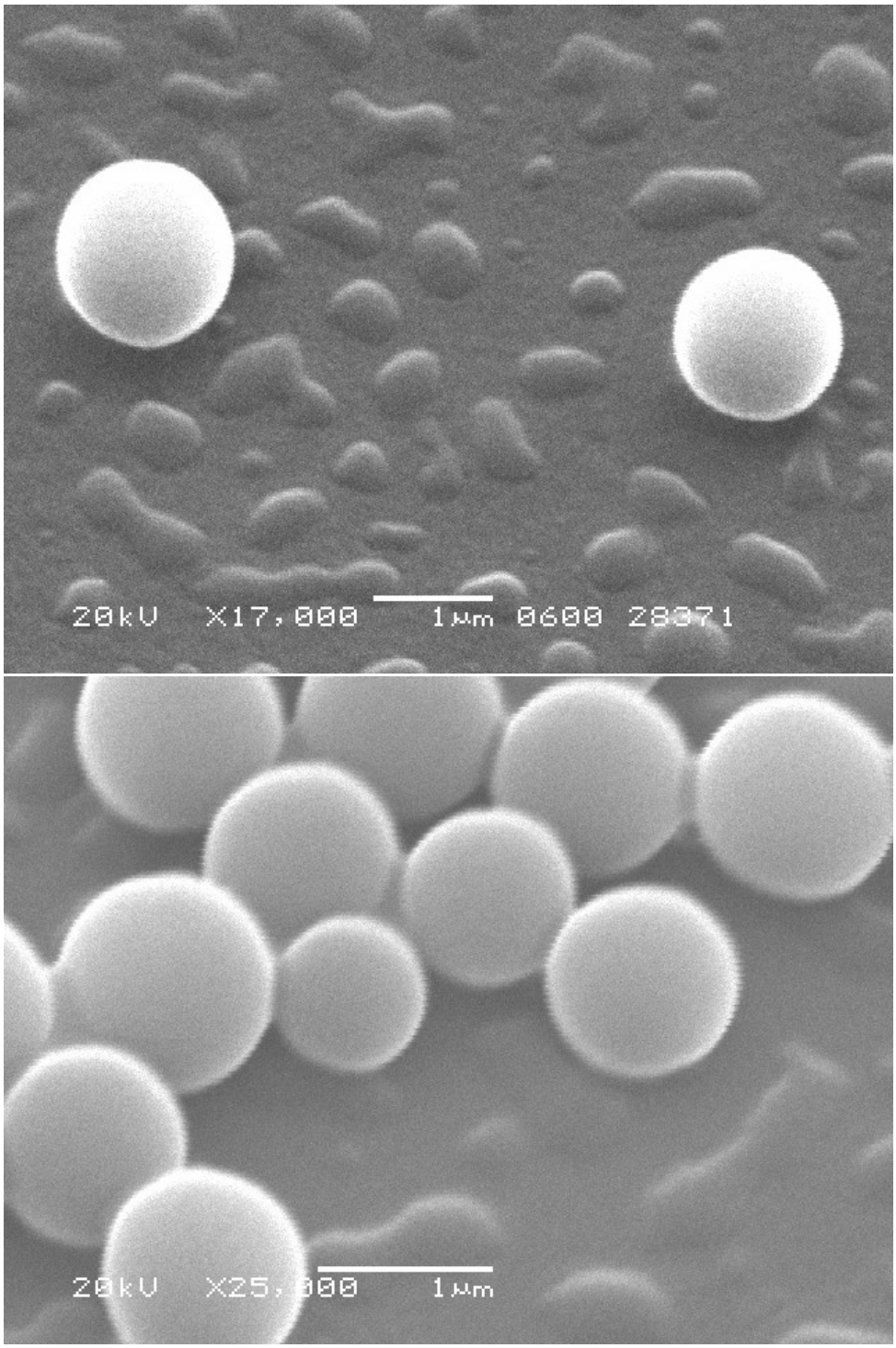

Figure 6: Detail view of a cyclosporin A suspension containing 2.3 mg/ml of β-cyclodextrin and 8.86 mg/ml Pluronic F68® in an aqueous system. Magnified x17000 and x25000.

After confirming the feasibility of producing β-cyclosporin A/cyclodextrin nanospheres, a new suspension was prepared (see Figure 7 and Figure 8). The composition of the formulation is described in Table 3.

Table 3: Formulation composition of a β-cyclodextrin /cyclosporin A nanospheres suspension.

Formulation composition	Conc. (mg/ml)
Cyclosporin A	1.7
β-Cyclodextrin	1.13
Pluronic F68®	1.02

The particle size of this suspension was also determined. The results are shown in table 4.

Table 4: Particle size of a cyclosporin A/β-cyclodextrin suspension.

Formulation composition	Size (nm)	PI
1.7 mg/ml of cyclosporin A + 1.13 mg/ml of β-cyclodextrin + 1.02 mg/ml Pluronic F68®	341	0.102

(PI: Polydispersity index)

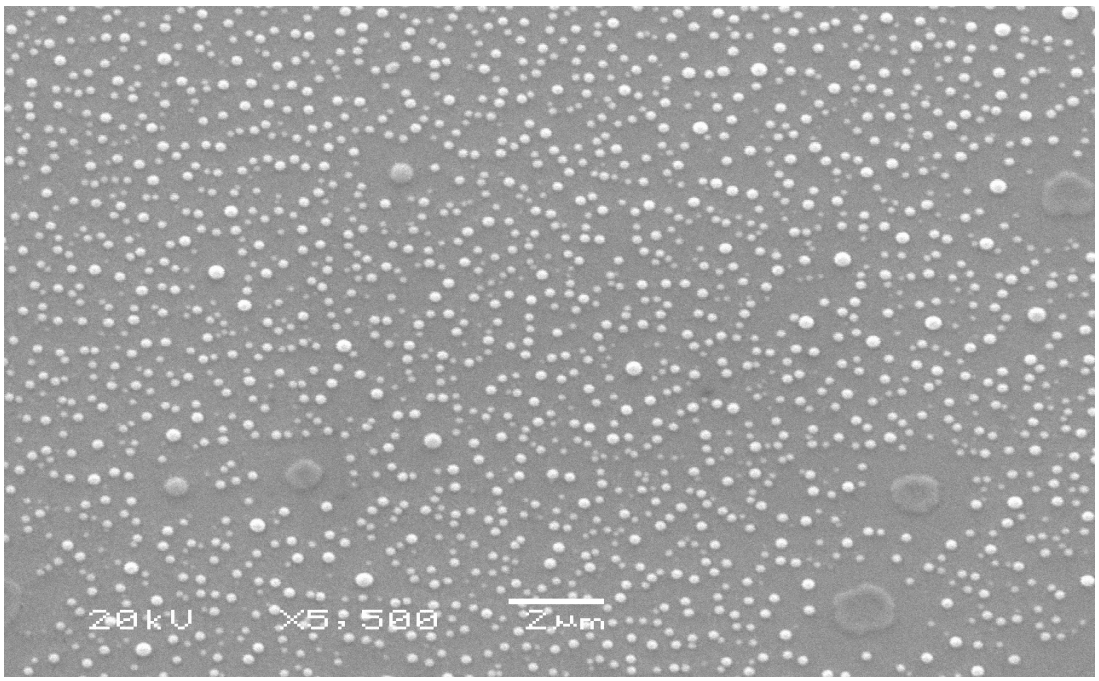

Figure 7: Overall view of a cyclosporin A suspension containing 1.7 mg/ml of cyclosporin A, 1.13 mg/ml of β-cyclodextrin and 1.02 mg/ml Pluronic F68® in an aqueous system. Magnified x5500.

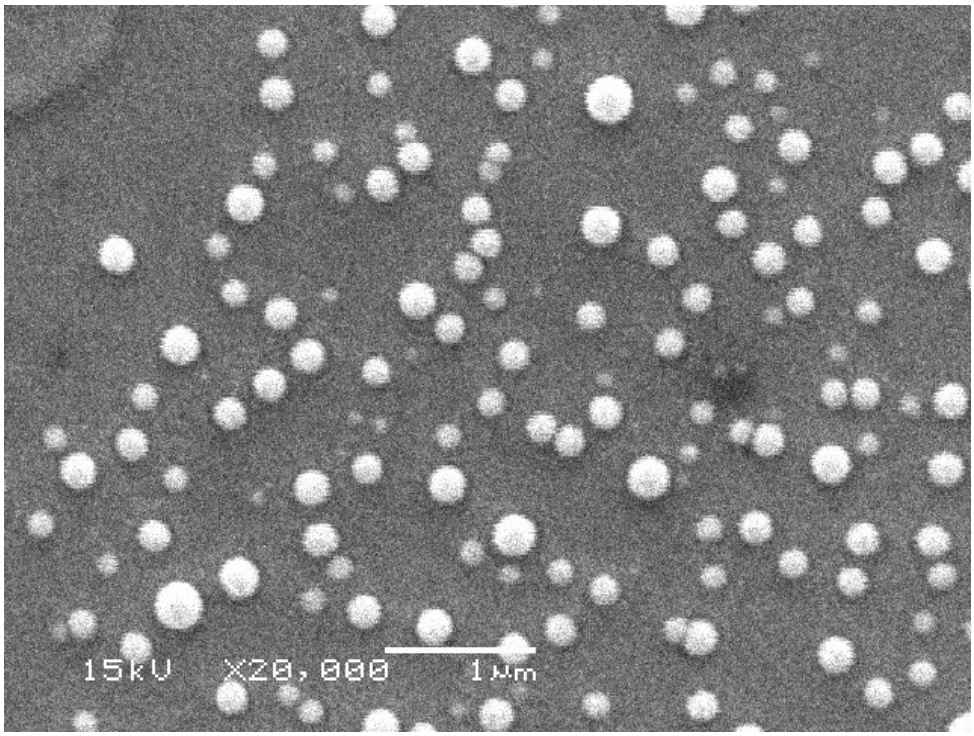

Figure 8: Magnified view of a cyclosporin A suspension containing 1.7 mg/ml of cyclosporin A, 1.13 mg/ml of β-cyclodextrin and 1.02 mg/ml Pluronic F68® in an aqueous system. Magnified x20000.

A new β-cyclosporin A/cyclodextrin suspension was prepared with the purpose of investigate its nebulisation characteristics and also to ascertain the feasibility of increasing the cyclosporin A concentration by increasing the amount of β-cyclodextrin used (see Figure 9 to Figure 12).

Table 5: Nebulization properties of a β-cyclosporin A/cyclodextrin nanospheres suspension containing 4 mg/ml of cyclosporin A, 2.08mg/ml of β-cyclodextrin, 5.3 mg/ml Pluronic F68® and 3.08 mg/ml of Tween 80®.[38]

Formulation Composition	MMD (µm)	GSD	TOR (mg/ml)	Percent. Particles <5 µm	Percent. Particles <2 µm
4 mg/ml of cyclosporin A + 2.08 mg/ml of β-cyclodextrin + 5.3 mg/ml Pluronic F68® + 3.08 mg/ml of Tween 80®	2.81	1.49	281	91.5	18.3

The particle size of this suspension was also determined. The results are shown in Table 6.

[38] MMD (NaCl) = 4.36 µm; TOR (NaCl) = 319 mg/ ml. Measured at a Malvern Zetasizer (Model Zetasizer 3000 HAS, Germany). The nebulisation trial was performed using an electronic nebulizer, eflow® (Pari GmbH, Germany).

Table 6: Characteristics of the β-cyclosporin A/cyclodextrin nanospheres in a suspension containing 4 mg/ml of cyclosporin A, 2.08mg/ml of β-cyclodextrin, 5.3 mg/ml Pluronic F68® and 3.08 mg/ml of Tween 80®.

Formulation composition	Size (nm)	PI	Zeta Potential (mV)
4 mg/ml of cyclosporin A + 2.08 mg/ml of β-cyclodextrin + 5.3 mg/ml Pluronic F68® + 3.08 mg/ml Tween 80®	249	0.33	- 2,8

(PI: Polydispersity index)

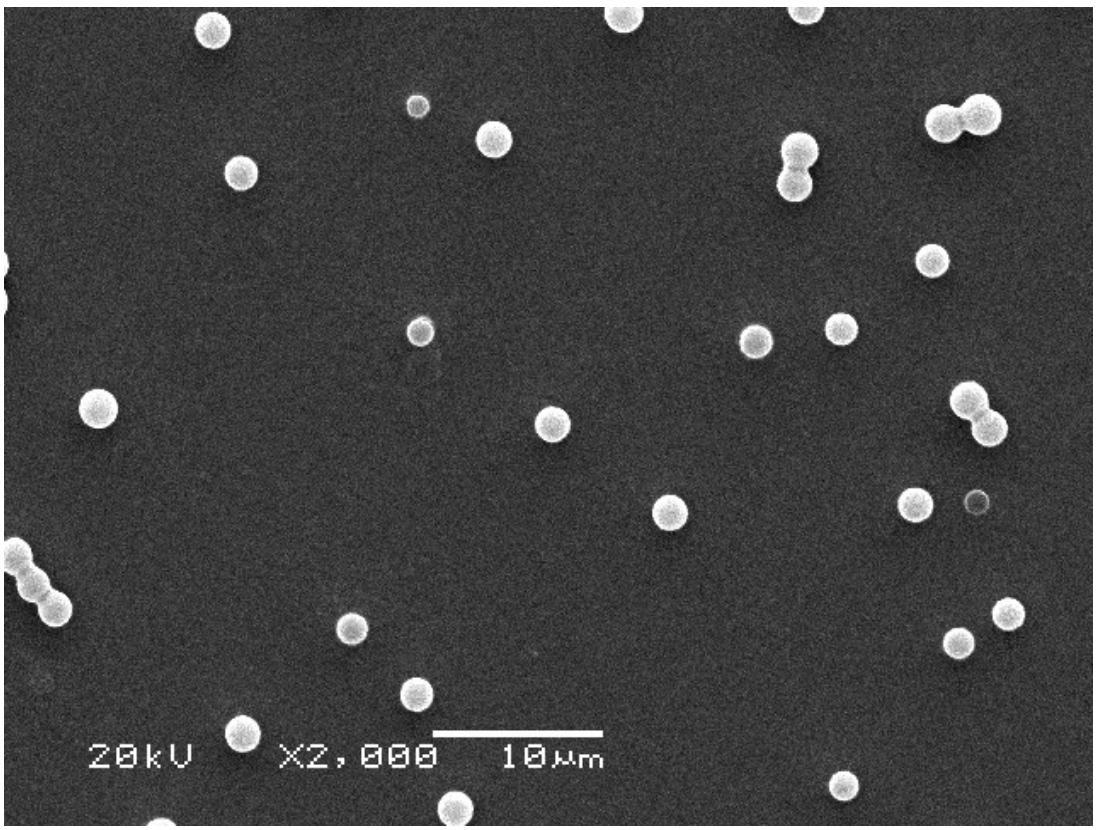

Figure 9: Overall view of a cyclosporin A suspension containing 4 mg/ml of cyclosporin A, 2.08 mg/ml of β-cyclodextrin, 5.3 mg/ml Pluronic F68 and 3.08 mg/ml of Tween 80® in an aqueous system. Magnified x2000.

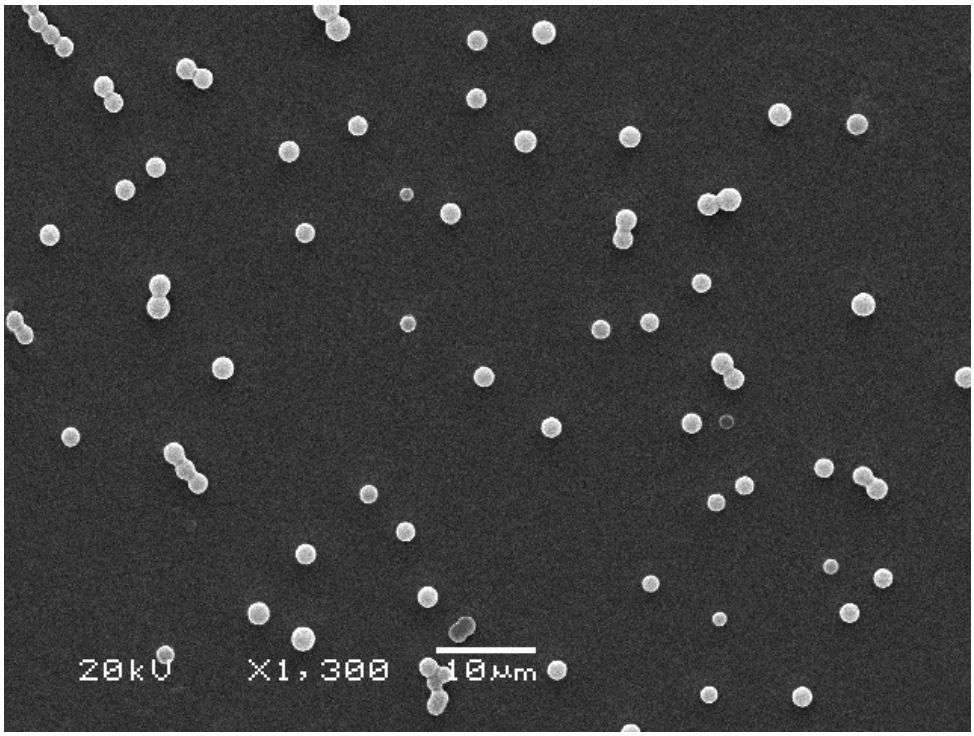

Figure 10: Overall view of a cyclosporin A suspension containing 4 mg/ml of cyclosporin A, 2.08 mg/ml of β-cyclodextrin, 5.3 mg/ml Pluronic F68® and 3.08 mg/ml of Tween 80® in an aqueous system Magnified x1300.

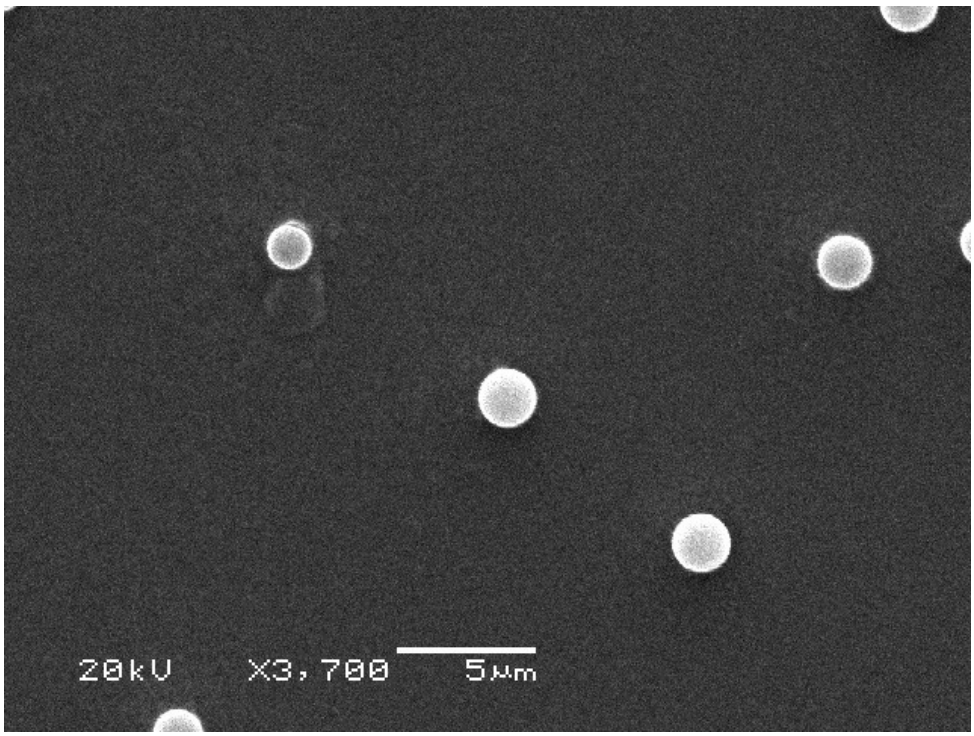

Figure 11: Magnified view of a cyclosporin A suspension containing 4 mg/ml of cyclosporin A, 2.08 mg/ml of β-cyclodextrin, 5.3 mg/ml Pluronic F68® and 3.08 mg/ml of Tween 80® in an aqueous system. Magnified x3700.

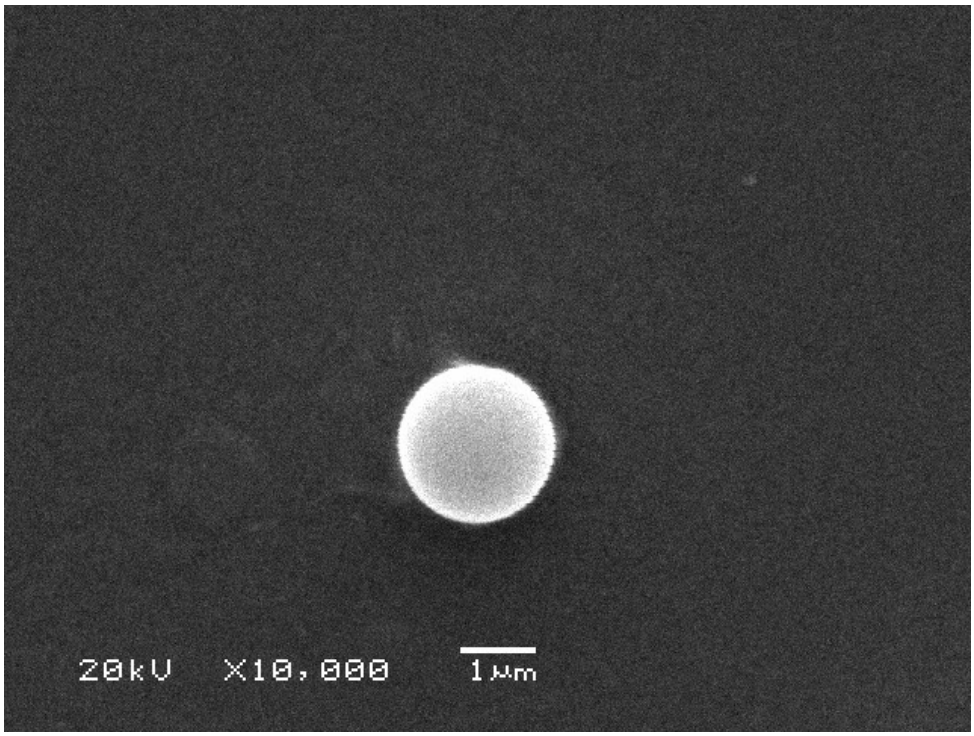

Figure 12: Magnified view of a cyclosporin A suspension containing 4 mg/ml of cyclosporin A, 2.08 mg/ml of β-cyclodextrin, 5.3 mg/ml Pluronic F68® and 3.08 mg/ml of Tween 80® in an aqueous system. Magnified x10000.

From the previous experiments, it was noticed that the solubility of β-CD in ethanol may have an important influence on the particle size of the formulation. The presence of unsolubilized β-CD can affect the particle size due to the formation of uncomplexed β-CD aggregates.
For this reason, in the following experiment, an ethanolic solution containing β-CD was filtrated using a 0.22 µm filter in order to assure that all the β-CD present in the ethanolic solution is solubilized.

The method of preparation was:

- The β-CD was added to 25 ml flask. Afterwards, the ethanol was added until the mark was reached. Sonification was applied for 10 min;
- After that operation was concluded, the excess of β-CD was removed by using a 0.22µm filter;
- To the last solution, 98.38 mg of cyclosporin A were added. After volume correction, the flask was sonificated until a clear solution was obtained;
- To this last solution, 25 ml of an aqueous solution containing 10 mg/ml of Pluronics F68® were added;
- After evaporation of the ethanol, c.a. 18 ml of the β-CD/cyclosporin A were obtained[39];
- Then, the suspension was diluted to 50 ml using a volumetric flask. As NaCl was used as a solvent.

At end, the particle size of the formulation was 207.2 nm with a polydispersity index of 0.24.

[39] In addition to the evaporation of the ethanol, also a part of the water was removed.

Figure 13: View of a cyclosporin A suspension containing 1.4 mg/ml of cyclosporin A and 10 mg/ml Pluronic F68® in an aqueous system.

Table 7: Physicochemical properties of cyclosporin A suspension.

Parameter	Unit	Value
pH value (22.0°C)	-	6.09
Osmolarity	mOsmol/kg	432 ± 3
Dyn. viscosity	mPas	1.18 ± 0.03
Surface tension	mN/m	38.41 ± 0.520

Table 8: Nebulization properties of a cyclosporin A/cyclodextrin nanospheres suspension containing 1.4 mg/ml of cyclosporin A and 10 mg/ml Pluronic F68® in an aqueous system. [40]

MD (µm)	GSD	TOR (mg/ml)	Percent. Particles <5 µm	Percent. Particles <2 µm
3.86	1.58	587	71.44	8.13

[40] MMD (NaCl) = 4.13 µm; TOR (NaCl) = 754 mg/ ml. Measured at a Malvern Zetasizer (Model Zetasizer 3000 HAS, Germany). The nebulisation trial was performed using a electronic nebulizer, eflow® (Pari GmbH, Germany)

6.3.1.3 Azithromycin Suspensions

The main purpose of this experiment was to investigate the feasibility of producing azithromycin/ β-cyclodextrin nanospheres in comparison with the cyclosporin A suspensions. From the results obtained (see Figures 14 through 18), we could confirm that it is also feasible to manufacture azithromycin/β-cyclodextrin nanospheres. The formulation composition is described in table 7.

Table 9: Formulation composition of a β-cyclodextrin/azithromycin nanospheres suspension.

Formulation composition	Conc. (mg/ml)
Azithromycin	7.0
β-cyclodextrin	1.11
Pluronic F68®	7.94

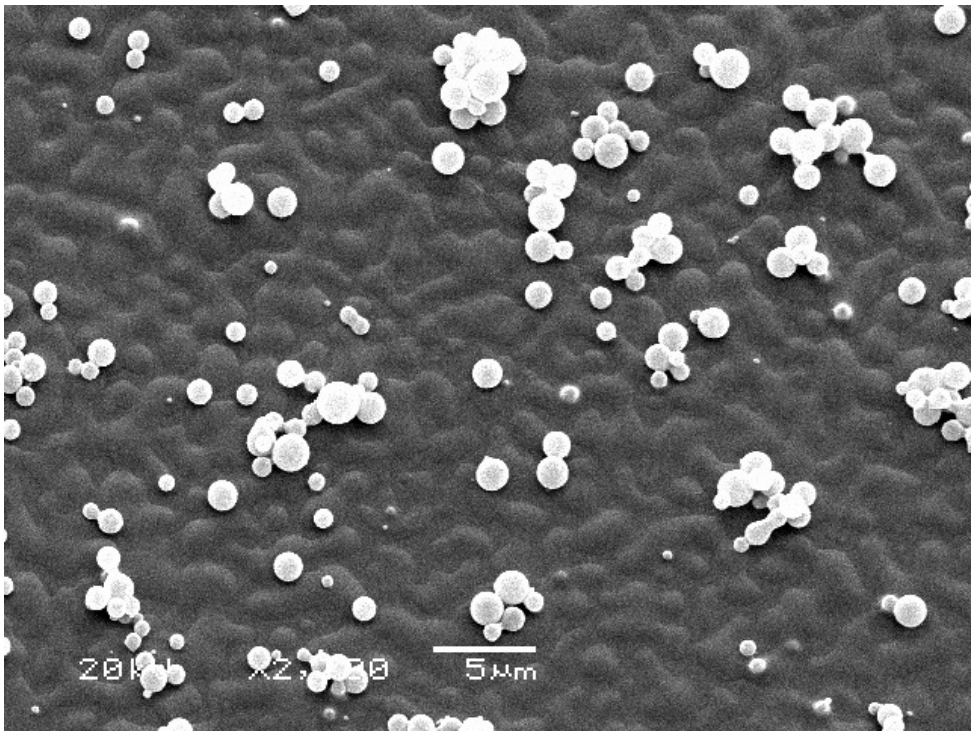

Figure 14: Overall view of an azithromycin suspension containing 7.0 mg/ml of azithromycin, 1.11 mg/ml of β-cyclodextrin and 7.94 mg/ml Pluronic F68® in an aqueous system. Magnified x2000.

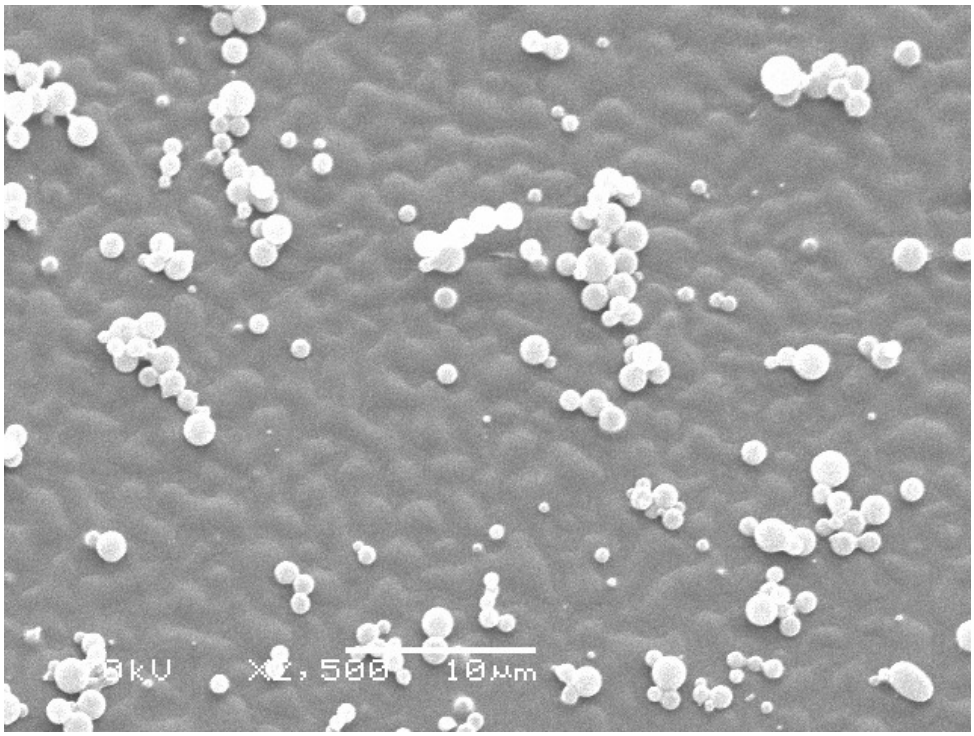

Figure 15: Overall view of an azithromycin suspension containing 7.0 mg/ml of azithromycin, 1.11 mg/ml of β-cyclodextrin and 7.94 mg/ml Pluronic F68® in an aqueous system. Magnified x2500.

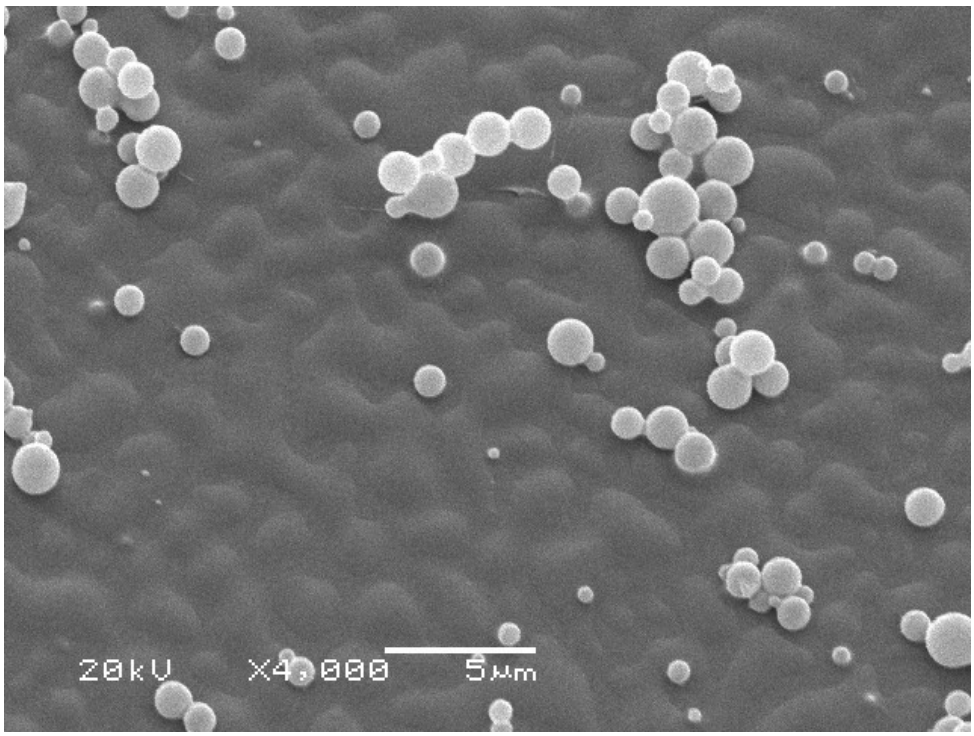

Figure 16: Magnified view of an azithromycin suspension containing 7.0 mg/ml of azithromycin, 1.11 mg/ml of β-cyclodextrin and 7.94 mg/ml Pluronic F68® in an aqueous system. Magnified x4000.

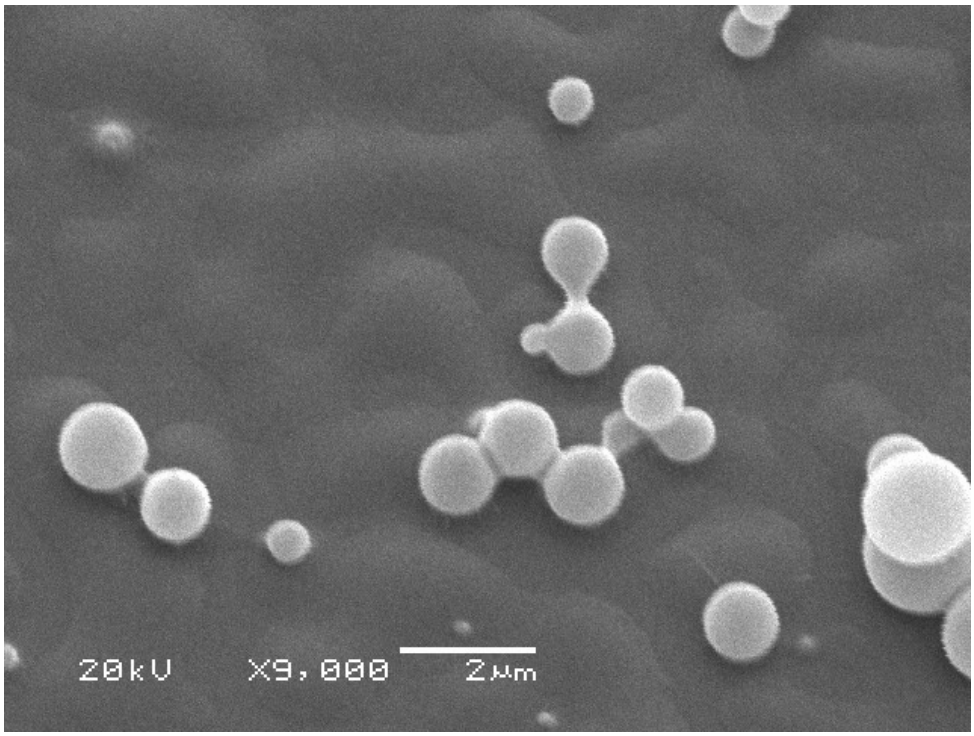

Figure 17: Magnified view of an azithromycin suspension containing 7.0 mg/ml of azithromycin, 1.11 mg/ml of β-cyclodextrin and 7.94 mg/ml Pluronic F68® in an aqueous system. Magnified x9000.

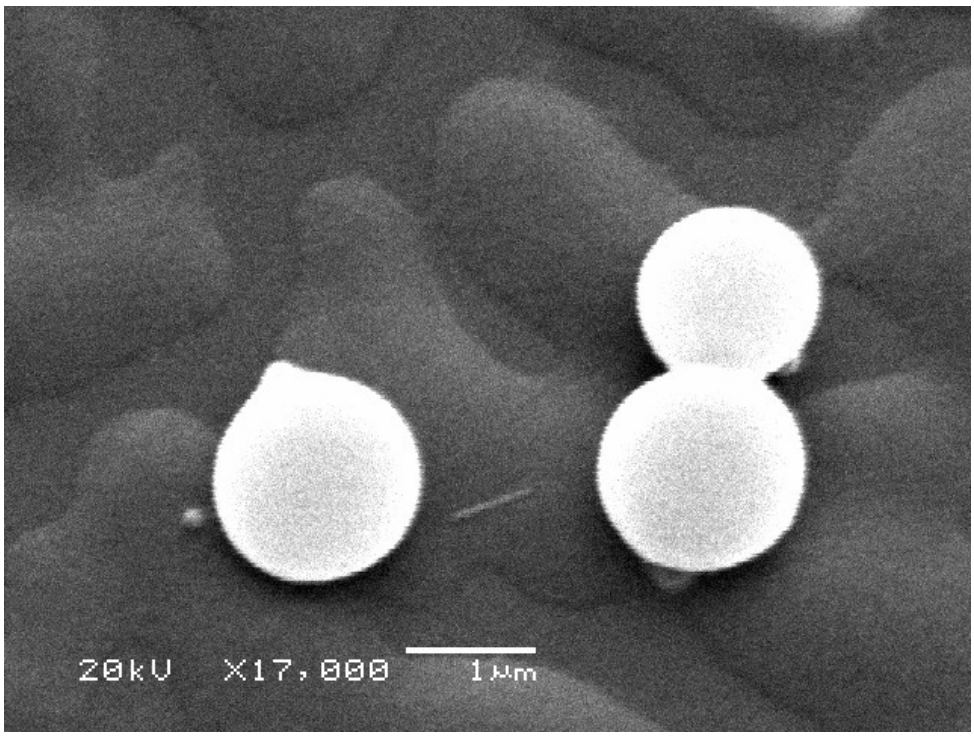

Figure 18: Detail view of a particle in of an azithromycin suspension containing 7.0 mg/ml of azithromycin, 1.11 mg/ml of β-cyclodextrin and 7.94 mg/ml Pluronic F68® in an aqueous system. Magnified x9000.

Chapter conclusion

The main purpose of this part of the study was to investigate the possibility of preparing suspensions of water insoluble drugs through the use of cyclodextrins. The results show that it is possible to prepare nanosuspensions of water insoluble drugs such as cyclosporin A and azithromycin using the nanoprecititation method.
In addition, the results also show that the nanosuspensions are capable of being efficiently nebulized through the use of the eflow®. The droplet size permits an efficient delivery of the active substances into the deep lung. Nevertheless, the stability of the nanosuspensions also needs to be taken into consideration due to the high surface area of the nanospheres. For this reason, the use of surfactants and also of substances that alter the zeta potential of the nanosuspensions, such as chitosan, needs to be studied in more detail in order to obtain a more stable suspension[41]. Furthermore, it would be advisable to use substances that provide a positive zeta potential such as chitosan opposing to substances that can generate negative charges such as sodium glycolcholate. This is due to the higher bioavailability of the CSA/CD nanospheres.
Although the preparation of stable nanosuspensions of insoluble drugs was always an objective, a detail study of the use of such substances on the stability of the nanosuspensions was not a fucral part of this work. However, the stability of this cyclodextrin nanospheres can be further improved by the use more suitable surfactants (or combination of surfactants) and also through the use of substances that can alter the zeta potential of the particles with the purpose of obtaining a more stable nanosuspensions.
As a conclusion, the use cyclodextrins through the application of the nanoprecititation method allows the preparation of nanosuspensions that are able to be efficiently nebulized into the deep lungs.

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[41] In terms of the stability of the nanosuspensions, the use of lyophilization also constitutes a very good option in order to obtain more stable nanosuspensions.

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7 SYSTEMATIC FORMULATION DEVELOPMENT

7.1 Theoretical approach

In the development process, it is necessary to take into account various topics: from the API's properties, over excipient interactions, application device to the desired product; just to mention a few. Figure 1 describes a standard formulation development process divided into its tasks and subtasks.

However, the development of pharmaceutical products suffers lack of well-established theories that describe interactions and relationships between excipients and drug formulation. The formulator may routinely access databases on previous formulations as well as make use of mathematical models. During the formulation process, specific tests may need to be performed to evaluate the properties of the proposed formulation and an analysis of unexpected results may result in an adjustment of the excipients and/or their levels.
This is the reason why human experience is still very important for the optimal formulation search, but in order to speed up the formulation process and to increase efficacy, computer-aiding systems can be applied. They provide assistance in understanding the complicated and non-linear relationships influenced by many key-factors in the pharmaceutical formulation development. These systems would operate under human supervision and their role would be associated to the decision supporting rather than taking over the whole process.
Consequently, empirical modelling seems to be a logical alternative or addition. Among various tools for empirical modelling, expert systems are can be regarded to be one of the most common and effective.

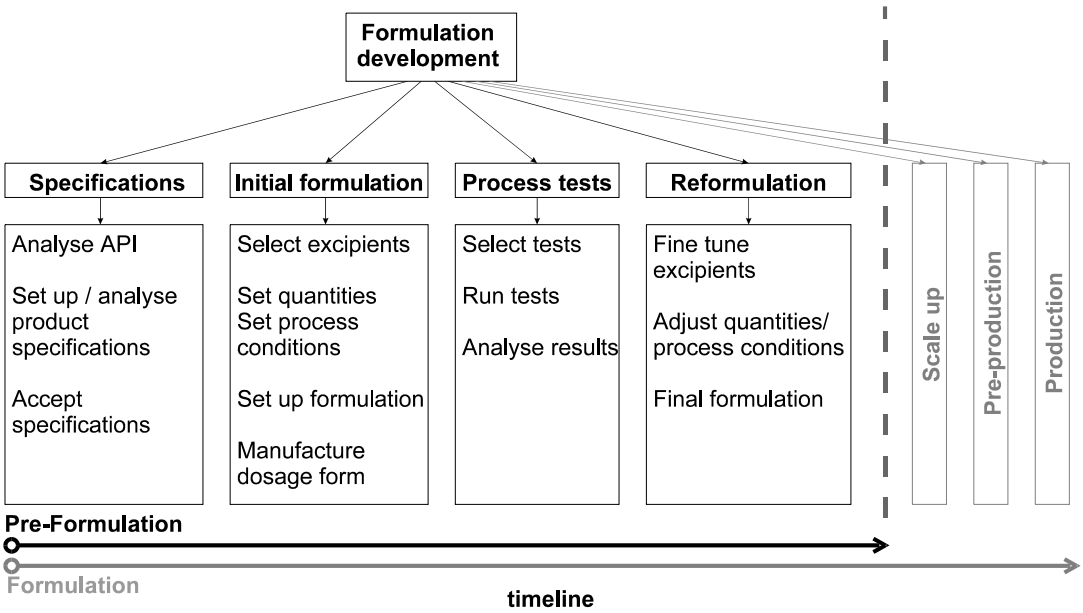


Figure 1: General operations usually performed during the (pre)formulation development process in the order from left to right.

An expert system is a knowledge-based system that emulates the knowledge of human experts to solve significant problems in a particular area of expertise, such as pharmaceutical formulation development.
Expert systems are basically computer programs that either recommend or make decisions for people, based on knowledge collected from experts in the field. They are mostly rule-based systems.
At its basic configuration, an expert system comprises three main components:

- A user interface system that allows a two-way communication between the user and the system.
- A knowledge base where all the knowledge related to the subject is stored.
- An inference engine where the knowledge is extracted and applied to solve a specific problem.

In a rule-based system, the knowledge needs to be highly structured[42]. It is often represented as production rules, expressing the relationship between several pieces of information. These are conditional statements that specify actions to be taken or advice to be followed, and have the form If (condition), Then (action), [43]Unless (exception) and Because (reason). More elements can be added by having several clauses joined by operators such as And or Or or by nesting conditional clauses. Mathematical formulae and algorithms can also be included.

The use of expert systems as an aid tool for the pharmaceutical formulation development process has several advantages. For example:

1	**Knowledge protection and availability**	The existence of a coherent and constant knowledge base which is not affected by staff turnover.
2	**Consistency**	It introduces robust formulations with an increased level of certainty and consistency. This is a particularly important benefit in terms of regulatory issues.
3	**Training aid**	It can be used to provide training for both novice and experienced formulators.
4	**Speed of development**	It allows the reduction of time in the development formulation process.
5	**Cost savings**	Cost savings can be achieved not only by reducing the development time but also by a more effective use of materials.
6	**Freeing experts from trivial tasks**	The implementation of expert systems in the product formulation process allows expert formulators to dedicate more for other tasks.
7	**Improved communication**	Provides a common platform from which to discuss and manage changes in the working practice and also to identify those critical areas requiring research.

[42] The InSilico-MAX software system does necessarily require that the knowledge is presented in a highly structured manner.

[43] The Unless (exception) and Because (reason) terms are not so often used Seite: 3 in the development process.

In this work, the InSilico-MAX software system was used to build up the expert system. It is tree like based structure that permits not only the processing of commands but also calculations at each step.

In order for the software system to give an possible formulation, it is necessary to describe in detail the API properties and also the desired product profile. In addition, the device specific properties also have to be taken in consideration.
As an example, the following tables show the typical API properties and product profile.

- Sample API properties

Table 1: Sample API properties

Property	**Value and unit**
Name	Cyclosporine A
Solubility (pH7)	Soluble in methanol, ethanol, ether, chloroform and methylene chloride. It is practically insoluble in water and saturated hydrocarbons.
m_r	148-151°C
Incompatibilities	The plasticiser diethylhexyl phthalate, which is a possible carcinogen, was leached from PVC containers by cyclosporin preparations containing polyethoxylated castor oil. Such preparations should not be given through PVC tubing nor stored in PVC containers. Polysorbate 80, which is an excipient in other cyclosporin preparations, also leached plasticiser from PVC, and similar precautions would apply to preparations so formulated.
Requires light protection	Yes
Hygroscopic	No
Stable in aqueous solution	No
Stable in buffered solution	No

- Sample product profile

Table 2: Sample product profile

Properties (limited by customer)	Value and unit
Name	eflow®
Type of device	Nebuliser
Max. Volume for formulation	4 ml
Viscosity	1 to 2.5 mPa s
Surface tension	< 73 mN/m
pH	4.5-8.0
Suitable for suspensions	Yes

By choosing a device in the previous list, a number of device specific properties (requirements) are attached to the product profile list. A sample list listed:

Table 3: Sample product profile list.

Properties (limited by device)	Value and unit
Device Name	Eflow
Max. applicable volume	4 ml
Surface tension	< 73 mNm
Viscosity	< 2.5 mPa
PH	4.5-8.0

7.1.1 Measures (Excipients and Procedures)

In the database, several excipients and procedures can be employed in order to resolve the possible formulation development problems. A description of the formulation development problems is showed on table 1. The excipients and also the procedures required to solve the development problems are presented in table 2 to table 16. However, the substances and the procedures described serve as reference. Nevertheless, further substances and procedures can be added to the system.

Table 4: Description of the possible development problems.

Development Problem	Development Step	Measures
Solubility	Solubility enhancement	Yes
Viscosity	Viscosity correction	Yes
Surface Tension	Surface tension correction	Yes
Osmolarity	Osmolarity correction	Yes
Total Output Rate	Total Output Rate correction	Yes
Particle size	Particle size correction	Yes
pH	pH correction	Yes
Volume	Volume correction	Yes
Taste	Taste Masking	Yes
Self Life Stability	Stability	Yes

Table 5: Hydrophilic Cyclodextrins

Name	Function	Company	Group Number
Acetyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Acetyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Acetyl-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
2,3,6-Tri-O-methyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
2,3,6-Tri-O-methyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
2,3,6-Tri-O-methyl-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
2,6-Di-O-methyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Randomly methylated-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Randomly methylated-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Randomly methylated-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
2-Hydroxyproply-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
2-Hydroxyproply-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
2-Hydroxyproply-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
sulfobutylether-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
6-O-maltosyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
6-O-maltosyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Carboxymethyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1

Carboxymethyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Carboxymethyl-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Succinyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Succinyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Succinyl-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Succinyl-β-Hydroxypropyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Succinyl-2-Hydroxypropyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1
Succinyl-2-Hydroxypropyl-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	1

Table 6: Hydrophobic Cyclodextrins

Name	Function	Company	Group Number
2,3,6-Tri-O-acetyl-1-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	2
2,3,6-Tri-O-acetyl-2-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	2
2,3,6-Tri-O-acetyl-3-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	2
Ethyl-1-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	2
Ethyl-2-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	2
Ethyl-3-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	2
2,6-Di-O-ethyl-2-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	2
2,6-Di-O-ethyl-3-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	2
2,3,6-Tri-O-ethyl-2-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	2

Table 7: List of the solubility enhancement additives.

Name	Function	Company	Group Number
HPMC	Solubilisation Enhancement	Fluka, Germany	3
PVP K30	Solubilisation Enhancement	Fluka, Germany	3
Ascorbic Acid	Solubilisation Enhancement	Fluka, Germany	3
Sodium Ascorbate	Solubilisation Enhancement	Fluka, Germany	3
Nicotine amide	Solubilisation Enhancement	Fluka, Germany	3
CMC	Solubilisation Enhancement	Fluka, Germany	3
Lysine Monohydrate	Solubilisation Enhancement	Fluka, Germany	3
Sodium acetate	Solubilisation Enhancement	Fluka, Germany	3
Sodium citrate	Solubilisation Enhancement	Fluka, Germany	3
Lactic acid	Solubilisation Enhancement	Fluka, Germany	3
Citric Acid	Solubilisation Enhancement	Fluka, Germany	3

MgSO4.6H20	Solubilisation Enhancement	Fluka, Germany	3
CaCl2.2H2O	Solubilisation Enhancement	Fluka, Germany	3
Vitamin E	Solubilisation Enhancement	Fluka, Germany	3
Vitamin E acetate	Solubilisation Enhancement	Fluka, Germany	3

Table 7: Alpha Type Hydrophilic Cyclodextrins

Name	Function	Company	Group Number
Acetyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	4
2,3,6-Tri-O-methyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	4
Randomly methylated-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	4
2-Hydroxyproply-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	4
6-O-maltosyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	4
Carboxymethyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	4
Succinyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	4

Table 8: Beta Type Hydrophilic Cyclodextrins

Name	Function	Company	Group Number
Acetyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	5
2,3,6-Tri-O-methyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	5
2,6-Di-O-methyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	5
Randomly methylated-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	5
2-Hydroxyproply-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	5
Sulfobutylether-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	5
6-O-maltosyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	5
Carboxymethyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	5
Succinyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	5
Succinyl-2-Hydroxypropyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	5

Table 9: Gamma Type Hydrophilic Cyclodextrins

Name	Function	Company	Group Number
Acetyl-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	6
2,3,6-Tri-O-methyl-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	6
Randomly methylated-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	6
2-Hydroxyproply-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	6
Carboxymethyl-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	6
Succinyl-2-Hydroxypropyl-γ-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	6

Table 10: Alpha Type Hydrophobic Cyclodextrins

Name	Function	Company	Group Number
2,3,6-Tri-O-acetyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	7
Ethyl-α-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	7

Table 11: Beta Type Hydrophobic Cyclodextrins

Name	Function	Company	Group Number
2,3,6-Tri-O-acetyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	8
Ethyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	8
2,6-Di-O-ethyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	8
2,3,6-Tri-O-ethyl-β-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	8

Table 12: Gamma Type Hydrophobic Cyclodextrins

Name	Function	Company	Group Number
2,3,6-Tri-O-acetyl-3-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	9
2,6-Di-O-ethyl-3-cyclodextrin	Solubilisation	Sigma Aldrich, Germany	9

Table 13: List of excipients for the surface tension correctors.

Name	Function	Company	Group Number
Tyloxapol	Surface tension adjustment	Sigma Aldrich, Germany	10
Polysorbate	Surface tension adjustment	Merck, Germany	10
Sorbitan monooleate	Surface tension adjustment	Merck, Germany	10

Table 14: List of excipients for the taste masking.

Name	Function	Company	Group Number
Macrogolglycerol-hydroxystearate	Taste Masking	Sigma Aldrich, Germany	11
Sodium Citrate	Taste Masking	Caelo, Germany	11
Lysine monohydrate	Taste Masking	Sigma-Aldrich	11
Sodium Chloride	Taste Masking	Caelo, Germany	11

Table 15: List of excipients for the taste masking.

Name	Function	Company	Group Number
Glucose	Taste Masking	Sigma Aldrich, Germany	12
Fructose	Taste Masking	Sigma Aldrich, Germany	12
Saccharin	Taste Masking	Sigma Aldrich, Germany	12

7.1.2 Monographs

See appendix 3.

7.1.3 Rules

7.1.3.1.1 Rules for the “Solubility” problem

The solubility of the water insoluble drugs needs to be increased as much as possible. For this reason, several excipients can be used.
However, if the solubility can not be increased through the use of the several different actions, other approaches can be investigated. As an example, the increase of the intrinsic solubility of the drug through the use of specific solvent or solvents combinations.

Rule 1:

IF Desired Release Profile: Immediate Release

THEN System Decision: Choose from Hydrophilic CD (Group 1)

Rule 2:

IF Desired Release Profile: Sustained Release

THEN System Decision: Choose from Hydrophobic CD (Group 2)

Rule 3:

IF Result Test A[44]=α Type CD AND Desired Release Profile: Immediate Release

THEN System Decision: System Decision: Choose from Hydrophilic-α-CD (Group 4)

Rule 4:

IF Result Test A=β Type CD AND Desired Release Profile: Immediate Release

THEN System Decision: Choose from Hydrophilic-β-CD (Group 5)

[44] Test A: see appendix 3 for a detailed description.

Rule 5:

IF Result Test A=γ Type CD AND Desired Release Profile: Immediate Release

THEN System Decision: Choose from Hydrophilic-γ-CD (Group 6)

Rule 6:

IF Result Test A=α Type CD AND Desired Release Profile: Sustained Release

THEN System Decision: Choose from Hydrophobic-α-CD (Group 7)

Rule 7:

IF Result Test A=β Type CD AND Desired Release Profile: Sustained Release

THEN System Decision: Choose from Hydrophobic-β-CD (Group 8)

Rule 8:

IF Result Test A=γ Type CD AND Desired Release Profile: Sustained Release

THEN System Decision: Choose from Hydrophobic-γ-CD (Group 9)

Rule 9:

IF Solubility increase <5% AND Cyclodextrin concentration >100 mg/ml

THEN System Decision: Choose from Additives from Group 3

Rule 10:

IF Solubility increase <5% AND Cyclodextrin concentration >100 mg/ml OR Additives concentration >5%

THEN System Decision: Change Cyclodextrin Derivative

7.1.3.1.2 Rules for the “Viscosity” problem

The viscosity of the formulation plays a very important role in the in the drug delivery performance. It has not only an important influence on the droplet size but also on the total output rate of the nebulizer. For this reason, the viscosity value must be located between a determined range. In order to achieve this goal, the possible actions that can be taken are described in the following rules.

Rule 1:

IF Viscosity of formulation <2.5 mPa s

THEN System Decision: No Action required.

Rule 2:

IF Viscosity of formulation > 2.5 mPa s

THEN System Decision: decrease the concentration of the cyclodextrin derivative

Rule 3:

IF Viscosity of formulation > 2.5 mPa s AND concentration of the cyclodextrin derivative <1%

THEN System Decision: decrease the concentration of the additive

7.1.3.1.3 Rules for the “Surface Tension” problem

The surface tension of the formulation has influence on the droplet size and consequently, in the drug delivery performance. For this reason, the surface tension of the formulations must be below a specific value. In order to achieve this goal, the possible actions that can be taken are described in the following rules.

Rule 1:

IF Surface Tension of formulation <73 mN/m

THEN System Decision: No Action required.

Rule 2:

IF Surface Tension of formulation >73 mN/m

THEN System Decision: Choose Additive from Group 10

7.1.3.1.4 Rules for the “Osmolarity” problem

The osmolarity value of the formulations has to be located within the physiological values that can be tolerated by the lungs cells. Consequently, several actions have to be taken if the osmolarity value of the formulation is not located within the allowed value range.

Rule 1:

IF Osmolarity of formulation 286<Ohm<600 mosmol/kg

THEN System Decision: No Action required.

Rule 2:

IF Osmolarity of formulation <286 mosmol/kg

THEN System Decision: Add NaCl to the formulation

Rule 3:

IF Osmolarity of formulation >600 mosmol/kg

THEN System Decision: decrease concentration of additives

7.1.3.1.5 Rules for the “Total Output Rate” problem

The total output rate of the nebuliser is closely related to the patient compliance. Longer nebulisation time usually exert a negative effect on the treatment compliance by the patients, in particular with younger patients. For this reason, it is important to have a high total output rate in order improve the patient compliance.

Rule 1:

IF Total Output Rate of formulation ≥ 200 mg/min

THEN System Decision: No Action required.

Rule 2:

IF Total Output Rate of formulation <200 mg/min

THEN System Decision: decrease viscosity of formulation

7.1.3.1.6 Rules for the “Particle Size” problem

For optimal lung delivery, it is important to have the correct aerosol particle size. Consequently, particles should range from 1 to 3.5 µm in diameter for optimal deposition efficiency. The following rules can be applied in order to obtain the correct particle size.

Rule 1:

IF Particle Size of Formulation 1 µm<PS<3.5 µm

THEN System Decision: No Action required.

Rule 2:

IF Particle Size of Formulation >3.5 µm

THEN System Decision: Decrease size of nebulising head

Rule 3:

IF Particle Size of Formulation <1 µm

THEN System Decision: Increase size of nebulising head

7.1.3.1.7 Rules for the “pH” problem

The pH value of the formulations has to be located within the physiological values that can be tolerated by the lungs cells. The pH adjustment of the formulations can be obtained by performing the following actions.

Rule 1:

IF pH of formulation 4.5>pH<8.0

THEN System Decision: No Action required.

Rule 2:

IF pH of formulation 4.5<pH> 8.0

THEN System Decision: Add acid or Base

7.1.3.1.8 Rules for the “Volume” problem

In order to have an uniform drug dose delivery, the volume that can be used has to be located between specific values. If not, the following actions have to be taken.

Rule 1:

IF Volume of formulation Product-profile.volume-lowlimit >Vol. <4.0 ml

THEN System Decision: No Action required.

Rule 2:

IF Volume of formulation Vol.>4 ml

THEN System Decision: Doses reduction

Rule 3:

IF Volume of formulation Vol. <0.5 ml

THEN System Decision: Add isotonic solution

7.1.3.1.9 Rules for the “Taste” problem

The taste of the formulations has a very important influence in patient compliance, especially in the case of young patients. Consequently, the following actions have to be taken in order to obtain a formulations that is suitable for inhalation in terms of its taste.

Rule 1:

IF Taste of Formulation is Neutral OR Tolerable

THEN System Decision: No Action required.

Rule 2:

IF NOT Taste of Formulation is Neutral OR Tolerable

THEN System Decision: Increase Cyclodextrin Concentration

Rule 3:

IF NOT Taste of Formulation is Neutral OR Tolerable AND Cyclodextrin concentration > 100 mg/ml

THEN System Decision: Decrease Drug Concentration

Rule 4:

IF NOT Taste of Formulation is Neutral OR Tolerable AND Cyclodextrin concentration > 100 mg/ml AND Drug concentration too Low

THEN System Decision: Choose Additives from Group 11 and 12

7.1.3.1.10 Rules for the “Stability” problem

To have a stable formulation it is also an important objective to achieve. Consequently, the following approaches can be taken.

Rule 1:

IF Stability of Formulation >4 weeks

THEN System Decision: No Action required.

Rule 2:

IF Stability of Formulation <4 weeks

THEN System Decision: Lyophilization

Rule 3:

IF Stability of Formulation <4 weeks

THEN System Decision: N_2 Degassing

7.1.3.1.11 Rules for the back steps

The return to a previous development step occurs when the system has no pre-determined action available for a specific problem in the development process. Consequently, a system should offer specific back steps prepared for these situations. The back steps that can be applied by the system are described in table 15.
However, with the InSilico-MAX software system, it is possible to step back to any specific development step when wanted. No special structuring is required. The back steps described in table 15 serve only as guidance.

Table 16: Description of the back steps present in the system.

From Development Step	To Development Step
Viscosity	Solubility
Surface Tension	Solubility
Osmolarity	Solubility
Taste Masking	Solubility
Stability	Solubility

7.1.3.1.12 Rules for the prognosis of formulation properties

The rules applied by system for the formulation prognosis are described on table 17.

Table 17: Rules for the formulation prognosis

Property	Action Step	Formula
Particle size (MMD)	Nebulisation	MMD=4.25-0.72*Viscosity
Particle size (MMD)	Nebulisation	MMD=10,67-0.013*Surface Tension
Total Output Rate (TOR)	Nebulisation	TOR=748.2-204*Viscosity
Surface tension	Nebulisation	Predicted by Neural Network

Surface tension is predicted by applying a neural network with 3 nodes in a hidden layer. Viscosity is included in the network for comparison only since the expert system will use a conventional formula for the predictions.

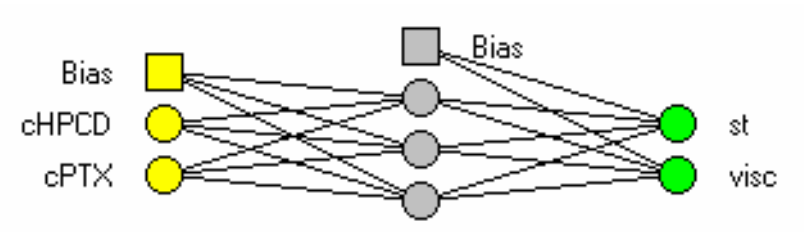


Figure 2: Structure of the applied neural network

The quality of the predicted values is shown in the following figures:

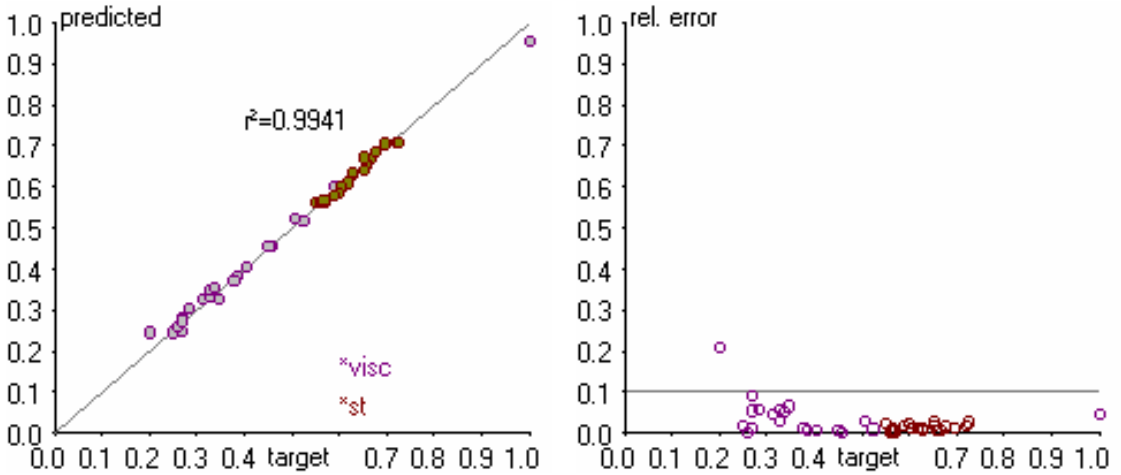


Figure 4: Correlation and relative deviation between real and predicted values of surface tension and viscosity

7.1.3.1.13 Rules for the quantity of excipients

Concentrations or amounts will be calculated based on previous knowledge. However, the quantity of each excipient that can be added will also depend on its toxicology.
The amount of excipients added to the formulation will be determined based on the following formulas:

Table 18: Rules for the quantification of the amount of excipients

Action Group	**Formula/Amount (per ml)[45]**
Solubility improvement with CD/ immediate release	$m_{CD}= (100-m_{DS})/S_0$ [mg]
Solubility improvement with CD/ sustained release	$m_{CD}= (10-m_{DS})/S_0$ [mg]
Solubility enhancement additives	$m_{EA}=m_{CD}/10$ [mg]
Surface tension improvement	Rule of thumb ($m_{ST}=m_{CD}/100$ [mg]) or based on functional connection /Neural Net

7.2 Implementation

The development of a new formulation starts with an API and should result in a formulation, which has to meet a certain profile (several requirements like dose, applicable volume etc.). API as well as the profile can be characterised by properties and their values. These are stored in variables. The assigned variable names for API and profile are listed in the following two tables.

Notes how to read the tables:

- variables, which characterise an excipient, device etc. but not relevant for program execution are printed on grey background for these and the following tables in this chapter.
- The last column contains information about where this data comes from:
  D: stored in a knowledge base data file
  S: generated during system execution

Table 19: Properties of an API represented by variables

Variable	Remark	origin
Name		D
Shortname		D
Mass	Quantity of substance expressed in g	S

[45] CD: Cyclodextrins; TM: taste masking;ST: Surface tension;EA: Enhancement additives: S_0: Intrinsic solubility

Solubility		D
Taste		D
Conc	Concentration expressed in g/ml	S
Fvisc		D
Ovisc		D
Liso	L_{Iso} value required for calculation of osmolarity	D
DT	Freezing point depression of a 1% solution [°C]	D
Size	Geometrical molecule size [nm]. Used to see whether molecule fits into CD cavity	D
MW	Molecular weight	D
Mol	Quantity of substance expressed in mol	S
PH	PH value of 5% dispersion in water at RT	D
CDequivalents	The number of CD molecules needed to complex one API molecule (=1 for smal molecules, 2 or more for larger API molecules)	S
Hydrolip$	Whether API is hydrophilic or lipophilic	D
Oxprob	Is API sensitive to oxidation?	D
Lightprob	Does the API require light protection?	D
Hygroscopic	Wheter the API is hygroscopic	D
Aqstable	Whether the API is stable in aqueous solution	D
Buffstable	Whether the API is stable in a buffered solution	D
Incomp$	The API's known incompatibilitites	D

Table 20: Properties of a product profile

Variable	Remark	Origin
Name		D
Shortname		D
Type$	immediate	
Dose		D
Mintaste		D
Device$		D
Release$	Immediate or sustained release	D
MinpH	Minimally tolerable pH	D
MaxpH	Maximally tolerable pH	D
OptpH	Optimum pH	D

Some important properties regarding volume, the type of dosage form (suspension/solution), viscosity etc. seem to be missing in the above table. Instead the device is a part of the product profile and the device's properties are stored in a separate file. It makes sense to split the

formulation into two parts, because device specific properties stay constant for a certain device while dosing or the acceptance criteria for taste might differ from product to product. Nevertheless, the device specific properties must be regarded as a part of the formulation. They are listed in the next table.

Table 21: Properties of a device

Variable	Remark	Origin
Name		D
Shortname		D
Suspsol$	Whether the device can handle suspensions, solutions or both	D
Maxviscosity	The maximum viscosity for a solution to be nebulised by the device	D
Optvolume	optimum volume that the device should be filled with	D
Maxvolume	maximum capacity of formulation that the device can hold	D
Maxsurf	Maximum surface tension	D
holediameter	The diameter of the nebuliser membrane's holes	D
Maxpartsize	Maximum size of particles	D

Based on the API data, the profile and the device characteristics, the system is supposed to guide the user in the selection of appropriate ecxipients in order to generate a formulation.
Ihis formulation again exhibits some characteristics; mainly regarding actual volume, actual taste, pH, viscosity, surface tension etc. The formuletion's properties are listed in the following table:

Table 22: Formulation properties

Variable	Remark	Origin
Name$		(S)
Shortname$		(S)
Type$	Whether it is a solution or a suspension	S
Volume	The total volume of the final formulation	S
Viscosity	The actual viscosity	S
PH	The formulation's pH	S
Surftens	The surface tension of the formulation	S
Osmol	Its osmolariry	S
MMD	The size of the drops generated by the nebuliser when the formulation is nebulised	S
TOR	Total output ratio	S

The excipients needed to transfer the API into the final formulation can be grouped into the following categories:

Table 23: Excipient categories

Category	Names used by the system	Comment
Solubility enhancers	Solenhancers	For poorly soluble drugs, solubility enhancers can be used to keep the drug in solution. The main solubility enhancers used and investigated in this thesis are cyclodextrins (CD), especially β-cyclodextrinderivates
Additional solubility enhancers	Additional	To incorporate a drug in the center of the CD and to fill out the CD-cavity perfectly, some additional excipients might be used.
Surfactants	Surfactant	Used to decrease surface tension
PH agents	Phcorrect	Used to correct the pH of the formulation to tolerable levels. PH agents are split into two groups: acids and bases
Isotonants	Isotonant	Used to correct the osmolarity of the formulation to tolerable levels

Each group of excipients can be characterised by specific properties as listed in the following tables:

Table 24: properties of solubility enhancer

Variable	Remark	Origin
Name		D
Shortname		D
MW	The molecular mass	D
Glucunits	The number of glucose units	D
PKa	PKa at 25°C	D
Enthalpy of solution		D
Entropy of solution		D
Hydrolysis	Semiquantitative evaluation of hydrolysisby a. oryzea α-amylase	D
Type	Whether it's a Cyclodextrin (CD) or other	D
Hydrolip	Whether CD is hydrophilic or lipophilic	D
Cavdiam	Diameter of the cavity	D
Torusheight	Height of the torus	D
Solubility	The enhancer's solubility in Water at 25°C (not to be messed up with the solubilising potential of the solubility enhancer)	D
Fvisc		D

Ovisc		D
Liso	L_{Iso} value required for calculation of osmolarity	D
Mass	Quantity of substance expressed in g	S
Conc	Concentration expressed in g/ml	S
DT	Freezing point depression of a 1% solution [°C]	D
Mol	Quantity of substance expressed in mol	S
Surftens		D

Table 25: properties of additional solubility anhancers

Variable	Remark	Origin
Name		D
Shortname		D
Fvisc		D
Ovisc		D
Liso	L_{Iso} value required for calculation of osmolarity	D
Mass	Quantity of substance expressed in g	S
Conc	Concentration expressed in g/ml	S
DT	Freezing point depression of a 1% solution [°C]	D

Table 26: properties of taste masking excipients

Variable	Remark	Origin
Name		D
Shortname		D
Liso	L_{Iso} value required for calculation of osmolarity	D
Mass	Quantity of substance expressed in g	S
Conc	Concentration expressed in g/ml	S
DT	Freezing point depression of a 1% solution [°C]	D
Tasteimp	Tasteimprovement in ranks	D
Usualconc	Usual concentration in g/ml	D

Table 27: surfactant properties

Variable	Remark	Origin
Name		D
Shortname		D
Fsurf		D
Liso	L_{Iso} value required for calculation of osmolarity	D
Mass	Quantity of substance expressed in g	S

Conc	Concentration expressed in g/ml	S
DT	Freezing point depression of a 1% solution [°C]	S

Table 28: properties of pH agents (acids, bases, buffers)

Variable	Remark	Origin
Name		D
Shortname		D
Type	Whether the pH-agent is a buffer, acid or base	D
Subtype	"Strong" or "weak" (applies only to acids and bases and is used to calculate the resulting pH correctly)	D
PH	The resulting pH (applies only to buffers since resulting pH is mainly independent of the buffer concentration)	D
PKa	The pka is necessary to calculate the	D
Liso	L_{Iso} value required for calculation of osmolarity	D
Volume		S
Mass	Quantity of substance expressed in g	S
Conc	Concentration expressed in g/ml	S
MW	Molecular weight	D
DT	Freezing point depression of a 1% solution [°C]	D
Usualconc	The usual concentration in which a buffer is applied	D
Val		D

Table 29: isotonant properties

Variable	Remark	Origin
Name		D
Shortname		D
Liso	L_{Iso} value required for calculation of osmolarity	D
Mass	Quantity of substance expressed in g	S
Conc	Concentration expressed in g/ml	S
DT	Freezing point depression of a 1% solution [°C]	D

Table 30: medium properties

Variable	Remark	Origin
Name		D
Shortname		D
mass	Quantity of substance expressed in g	S

Table 31: Specific non-continuous, attributive values for some variables

Variable	Values
aqstable	0: no 1: yes = is stable in aqueous solution
buffstable	0: no 1: yes = is stable in buffered solution
hydrolipo$	Hydrophilic Lipophilic
Hydrolysis	0: none 1: negligible 2: low 3: rapid
hygroscopic	0: no 1: yes = is hygroscopic
lightprob	0: no 1: yes = shows sensitivity to light
oxprob	0: no 1: yes = shows oxidative problems
release$	Immediate Sustained
subtype	Weak Strong
suspsol$	Suspension (which means suitable for suspensions and solutions) Solution (for solutions only; device not suitable for suspensions)
taste	1: excellent / no taste 2: slightly noticable taste 3: noticable bad taste (slightly bitter) 4: just tolerable (bitter) 5: intolerable (very bitter) 6: disgusting
Type$	CD for cyclodextrins Acid for acids Base for bases Buffer for buffers

Provided all necessary data concerning API, excipients and devices are given and a profile is specified, the system can start formulation development. The following problems are handled:

Solubility / dispersion type (cell 20)
Based on the API's solubility, the program checks whether a true solution can be accomplished.

If this is not the case, either solubility enhancers must be used or a suspension can be formulated.
For solutions (cell 11), the necessary amount of CD is calculated on molar basis while the API size is also taken into account.
If the device can't handle suspensions, the solubility enhancer pathway (cell 18) must be used.

Taste masking (cell 11)
If the profile requires a taste better than the API's taste, taste masking has to take place.
If a CD is already used for solubility issues, the CD's taste masking properties can be exploited. Otherwise one of the taste masking agents ("masker") has to be used.

Additional solubility enhancers (cell 11)
In most of the cases when a CD is used as the main excipient, the API won't perfectly fit into the cavity. Therefore additional excipients, the so called additional solubility enhancers ("additional") are used. They are selected in dependence of the mismatch between CD cavity size and API molecule size.
The amount of additional is set to 1/10th of the solubility enhancer's mass.

PH (cell 14)
The pH is influenced by the API first. All APIs are regarded to be weak acids/bases[46] and their strength is represented by their pKa values.
PH calculation is based on the formula

$$\mathrm{pH} = \frac{1}{2}\left(\mathrm{pKa} - \log \mathrm{c}\right)$$

with c being the API concentration.

A pH deviation from the profile's optimum pH has to be corrected using acidic or basic pH agents (pHcorrect). They cause a change in osmolarity in return, however.
The necessary amount or pHcorrect is calculated by applying the titration formula for weak acids with strong bases:

$$\mathrm{pH} = \mathrm{pKa} - \log\frac{1-\tau}{\tau}$$

in its delogarithmed form:

$$\mathrm{c}(\mathrm{H}^+) = \mathrm{K_a}\,\frac{\mathrm{c} - \mathrm{c_{base}}}{\mathrm{c_{base}}}$$

which results in

$$\mathrm{c_{base}} = \frac{\mathrm{c}}{10^{\mathrm{pKa}-\mathrm{pH}} + 1}$$

c_{base} represents the concentration of a onebasic (val = 1) base. This is transformed to the bases mass by

$$m_{base} = c_{base} \cdot M \cdot V \cdot val$$

where M is the bases molecular weight and V the formulation's volume. Val contains the number of OH^- equivalents per mole of base.

Surface tension (cell 13)
Surfactants may be used to correct the formulation's surface tension. The use of surfactants also influences osmolarity.

Osmolarity (cell 15)
Since osmolarity should be within the range specified in the product profile, either isotonants have to be added or the formulation must be diluted

Viscosity (cell 12)
The viscosity of the final formulation is not changed by any means but must be calculated since it's a necessary parameter for the calculation of derived formulation properties as MMD and TOR.

When using the suspension pathway (cell 18), the program performs the same actions except for unnecessary calculations concerning the API like pH, surface tension etc. Also a different output template is used since manufacturing of a suspension differs from the manufacturing procedure of a solution, of course. Therefore the following excerpt of the knowledge base concentrates on the formulation development of solutions.

7.2.1 Knowledge base

The complete knowledge base and the appropriate datafiles are available via internet and will be updated from time to time. The latest version is accessible via http://www.jmbnet.de/thesis/raposo/index.html. An excerpt of the knowledge based is presented here:

No: **1**
Root: 0

Question:
Would you like to start a formulation or view the instructions?

Comment:
keytext "start formulation\view instructions"

Branches:
Key: **s** branch to cell nr. **3**
Key: **i** branch to cell nr. **2**

No: **2**
Root: 1

Question:
Would you like to read the system manual or instructions how to add new APIs, devices and excipients?

Comment:
keytext "system manual", "instructions"

Branches:
Key: **s** branch to cell nr. **4**
Key: **i** branch to cell nr. **5**

No: **3**
Root: 1

Question:
choose: existing or new api

Commandfile:
```
tablechoosefrom "API", "api.txt", "api", a$, b$, n
'loadincomps "incomp.txt", "known"
'clearincomps

if b$ = "newdrug" then
 gotocell 9
else
```

```
        concat sect$, b$, ":api"
        tablereadvariables "api.txt", sect$
        'addtoincomps api.excipient$, api.backbone$, api.incomp$
        gotocell 10
       endif
```

No: **4**
Root: 2

Question:
system manual

Branches:
Key: **n** branch to cell nr. **6**

Commandfile:

```
rtfload "system_manual.rtf"
rtfview
gotocell 6
```

No: **5**
Root: 2

Question:
Would you like to add new properties to existing APIs/Devices/Excipients or add completely new API/Device/Excipient entities?

Comment:

```
keytext "new properties\new entities"
```

Branches:
Key: **p** branch to cell nr. **7**
Key: **e** branch to cell nr. **8**

No: **6**
Root: 4

Answer:
Resume from start

Commandfile:

```
gotocell 1
```

No: **7**
Root: 5

Answer:
Instructions: enter new properties

Commandfile:

```
rtfload "new_properties.rtf"
rtfview
```

gotocell 6

No: **8**
Root: 5

Answer:
instructions: enter new entities

Commandfile:
rtfload "new_entities.rtf"
rtfview
gotocell 6

No: **9**
Root: 3

Answer:
Ask for relevant API data

Commandfile:
askfor "API Name", "Name of API", api.name$
askfor "API shortname", "Name of API", api.short$
askfor "API", "molecular mass", api.mw
askfor "API solubility", "solubility", api.solubility
askfor "API taste", "API taste", api.taste
askfor "API", "viscosity slope", api.fvisc
askfor "API", "viscosity offset", api.ovisc
askfor "API", "L Iso", api.Liso
askfor "API", "oxidation", api.oxproblem
askfor "API", "sensitivity to light", api.lightproblem

askfor "API", "hygroscopicity", api.hygroscopic
askfor "API", "stability in water", api.aqstable
askfor "API", "stability in buffer", api.buffstable
askfor "API", "Name of API", api.name$
askfor "API", "known incompatibilities", api.incomp$
askfor "API", "alkaline / acid reaction", api.acidbase$
askfor "API", "pKa", api.pKa

gotocell 10

No: **10**
Root: 3

Question:
choose or enter product profile

Comment:
branch to solutions or suspension afterwards

Commandfile:
'***** Product profile *****
tablechoosefrom "Product profile", "profile.txt", "profile", a$, b$, n

```
        concat b$, b$, ":profile"
        tablereadvariables "profile.txt", b$

        if api.dose = 0 then
         askfor "Dose", "Dose in mg", profile.dose
        endif

        if profil.sname$ = "newprofile" then
         'not implemented yet
         'ask for all the relevant properties here
         gotocell 19
        endif

        gotocell 20
```

No: **11**
Root: 20

Question:
solutions

Branches:
Key: **n** branch to cell nr. **12**

Commandfile:

```
        '*** solutions ***
        chooseenhancer = 0
        solenhancer.name$ = "(not required)"
        solenhancer.mass = 0
        masker.mass = 0
        masker.name$ = "(not required)"
        isotonant.mass = 0
        isotonant.name$ = "(not required)"
        additional.mass = 0
        additional.name$= "(not required)"
        surfactant.mass = 0
        surfactant.name$ = "(not required)"
        explanation$ = ""
        formulation.volume = device.optvolume
        formulation.name$ = profile.name$
        api.mass = profile.dose
        formulation.taste = api.taste
        required.solubility = api.mass / formulation.volume

        'use water as medium
         tablereadvariables "medium.txt", "H2O:medium"


        'if solubility or taste is too bad, CD complexation has to be chosen
        if api.solubility < required.solubility then
         Message "Solubility", "API solubility is too poor. A solubility enhancer is needed."
         concat explanation$, explanation$, "API solubility is too poor; therefore a solubility
        enhancer is used. "
         chooseenhancer = 1
```

```
endif

if api.taste > profile.mintaste then
 if chooseenhancer = 0 then
  'no extra taste masking needed when CD is used
  message "Taste masking", "API taste is inacceptable. Tastemasking is required."
  tablechoosefrom "taste masker", "masker.txt", "masker", a$, b$, n
  concat b$, b$, ":masker"
  tablereadvariables "masker.txt", b$
  formulation.taste = formulation.taste - masker.tasteimp
  concat explanation$, explanation$, "Poor API taste requires tastemasking
([masker.name$]). "
  masker.mass = masker.usualconc * formulation.volume
  masker.conc = masker.mass / formulation.volume
  showvariables
 endif
endif

'there's no better taste than "excellent"
if formulation.taste < 1 then
 formulation.taste = 1
endif

if chooseenhancer = 1 then
 'choose a solubility enhancer here
 if profile.release$ = "immediate" then
  tablechoosefrom "hydrophilic solubility enhancer", "solenhancer.txt", "solenhancer",
a$, b$, n
  concat explanation$, explanation$, "For immediate release a hydrophilic solubility
enhancer ([solenhancer.name$]) is chosen. "

 else
  tablechoosefrom "lipophilic solubility enhancer", "solenhancer.txt", "solenhancer", a$,
b$, n
   concat explanation$, explanation$, "for sustained release a hydrophobic solubility
enhancer  ([solenhancer.name$]) is chosen. "

 endif
 concat b$, b$, ":solenhancer"
 tablereadvariables "solenhancer.txt", b$
 api.conc = api.mass / formulation.volume
 api.mol = api.mass / api.mw
 'use an excess of 50% CD to complex API
 concat explanation$, explanation$, "A 50% (mol/mol) excess of solubility enhancer is
used to complex [api.name$]. "
 solenhancer.mol = api.mol * 1.5
 solenhancer.mass = solenhancer.mw * solenhancer.mol
 solenhancer.conc = solenhancer.mass / formulation.volume

 if solenhancer.type$ = "CD" then
  concat explanation$, explanation$, "The use of a cyclodextrin also improves taste for
up to [solenhancer.tasteimp] units. "
  formulation.taste = api.taste - solenhancer.tasteimp
  'check for cavity size
  cavsizemismatch = solenhancer.cavsize / api.size
```

```
  if ( cavsizemismatch < 0.9 )
   message "Cavity size", "CD cavity size doesn't mach api size"
   'choose an additional excipient
   tablechoosefrom "Additional enhancer", "additional.txt", "additional", a$, b$, n
   concat b$, b$, ":additional"
   tablereadvariables "additional.txt", b$
   concat explanation$, explanation$, "Since API size ([api.size] pm) doesn't match CD
cavity ([solenhancer.cavdiam] pm), an additional enhancer ([additional.name$]) is
required. It's mass is set to one tenth of the CD's mass. "

   additional.mass = solenhancer.mass / 10
   additional.conc = additional.mass / formulation.volume
  endif
 endif
endif


'calculate those properties here, which are needed afterwards
formulation.surftens = medium.surftens
additional.conc = additional.mass / formulation.volume
solenhancer.conc = solenhancer.mass / formulation.volume
api.conc = api.mass / formulation.volume
formulation.viscosity = solenhancer.fvisc * solenhancer.conc + solenhancer.ovisc


formulation.pH = api.pH


'surface tension is calculated by a neural net
'formulation.surftens = 53.386 + solenhancer.ksurf * solenhancer.conc ^ .33
nnload "surftensNET.ann"
nnclearinputs
sec = solenhancer.conc * 100
apic = api.conc * 100
nnsetinput "cHPCD", sec
nnsetinput "cAPI", apic
nngetoutput "surftens", formulation.surftens
message "surface tension", formulation.surftens
formulation.osmol = solenhancer.fosm * solenhancer.conc + additional.fosm *
additional.conc
```

No: **12**
Root: 11

Question:

viscosity

Branches:

Key: **n** branch to cell nr. **13**

Commandfile:

```
'viscosity
if formulation.viscosity > device.maxviscosity then
 'decrease CD concentration
```

```
 solenhancer.conc
 solenhancer.mass = solenhancer.conc * form.volume

 'eventually decrease concentration of additive
 additional.mass = solenhancer.mass / 10
 additional.conc = additional.mass / form.volume
endif
```

No: **13**
Root: 12

Question:
surface tension

Branches:
Key: **n** branch to cell nr. **14**

Commandfile:

```
'surface tension
if formulation.surftens > device.maxsurf then
 tablechoosefrom "Surfactant", "surfactant.txt", "surfactant", a$, b$, n
 concat b$, b$, ":surfactant"
 tablereadvariables "surfactant.txt", b$
 surfactant.conc = ( formulation.surftens - device.maxsurf )  / surfactant.fsurf * 10
 surfactant.mass = surfactant.conc * formulation.volume
 formulation.surftens = medium.surftens - sqr( solenhancer.ksurf * solenhancer.conc ) -
( surfactant.mass * surfactant.fsurf * 10 )
 showvariables
endif
```

No: **14**
Root: 13

Question:
pH

Branches:
Key: **n** branch to cell nr. **15**

Commandfile:

```
'until now, formulation's pH depends mainly on the API's acidity

api.mconc = api.conc / api.mw
message "api.mconc", api.mconc
if api.acidbase$ = "neutral" then
 formulation.pH = 7
endif
if api.acidbase$ = "acid" then
 formulation.pH = 0.5 * ( api.pKa - log( api.mconc ) )
endif
if api.acidbase$ = "base" then
 formulation.pH = 14 - 0.5 * ( log( api.mconc ) + 14 - api.pKa )
endif
```

```
'adjust pH

if formulation.pH < profile.minpH then
 'add base
 tablechoosefrom "pH Adjustment", "bases.txt", "pHcorrect", a$, b$, n
 concat b$, b$, ":phcorrect"
 tablereadvariables "bases.txt", b$
 'calculate necessary amount. Distinguish between buffers and bases
 if bases.type$ = "buffer" then
  phcorrect.mass = phcorrect.usualconc * device.optvol
 else
  'for strong bases
  'ca is concentration of weak acid (api)
  ca = api.mconc
  valconc = ca / ( 10 ^ ( api.pKa - profile.optpH ) + 1 )
  phcorrect.mass = valconc * phcorrect.mw * formulation.volume / phcorrect.val

  formulation.pH = api.pKa - log( ( ca - valconc ) / valconc )
 endif
endif

if formulation.pH > profile.maxph then
 'add acid
 tablechoosefrom "pH Adjustment", "acids.txt", "pHcorrect", a$, b$, n
 concat b$, b$, ":phcorrect"
 tablereadvariables "bases.txt", b$
 'calculate necessary amount. Distinguish between buffers and acids
 if bases.type$ = "buffer" then
  phcorrect.mass = phcorrect.usualconc * device.optvol
 else
  'for strong acids
  conc = 10 ^ profile.optpH
  phcorrect.mass = conc * device.optvolume
 endif


endif
```

No: **15**
Root: 14

Question:

osmolarity

Branches:

Key: **n** branch to cell nr. **16**

Commandfile:

```
'osmolarity
osmoldtfactor = 1000 / medium.cryoconst
profile.optdt = profile.optosmol / osmoldtfactor
profile.maxdt = profile.maxosmol / osmoldtfactor
```

```
formulation.dT = ( api.mass * api.dT + solenhancer.mass * solenhancer.dT +
additional.mass * additional.dT + pHcorrect.mass * pHcorrect.dT + surfactant.mass *
surfactant.dT ) / formulation.volume

if formulation.dT > profile.optdT then
 message "Dilution required", "solution is hyperosmotic and needs to be diluted"
 isotonant.mass = 0
 'cf is correction factor
 cf = formulation.dt / profile.maxdT
 api.mass = api.mass / cf
 solenhancer.mass = solenhancer.mass / cf
 additional.mass = additional.mass / cf
 pHcorrect.mass = pHcorrect.mass / cf
 surfactant.mass = surfactant.mass / cf

else
 'add isotonant
 tablechoosefrom "Osmolarity", "isotonant.txt", "isotonant", a$, b$, n
 concat b$, b$, ":isotonant"
 tablereadvariables "isotonant.txt", b$
 isotonant.mass = ( profile.optdT - formulation.dT ) * formulation.volume / isotonant.dT

endif

formulation.dT = ( api.mass * api.dT + solenhancer.mass * solenhancer.dT +
additional.mass * additional.dT + pHcorrect.mass * pHcorrect.dT + surfactant.mass *
surfactant.dT + isotonant.mass * isotonant.dT ) / formulation.volume
formulation.osmol = formulation.dt * osmoldtfactor
```

No: **16**
Root: 15

Question:

TOR, volume, droplet size

Branches:

Key: **n** branch to cell nr. **17**

Commandfile:

```
'final calculations
formulation.ingredientmass = api.mass + solenhancer.mass + additional.mass +
pHcorrect.mass + surfactant.mass + isotonant.mass
'medium mass is calculated assuming a density of 1g/cm³
medium.mass = formulation.volume - form.ingredients
formulation.mass = formulation.ingredientmass + medium.mass

api.conc = api.mass / formulation.volume
solenhancer.conc = solenhancer.mass / formulation.volume
additional.conc = additional.mass / formulation.volume
pHcorrect.conc = pHcorrect.mass / formulation.volume
surfactant.conc = surfactant.mass / formulation.volume
isotonant.conc = isotonant.mass / formulation.volume
```

```
'showvariables

'**********
'formulation.surftens = medium.surftens (step 11)
if chooseenhancer = 1 then
 formulation.viscosity = solenhancer.fvisc * solenhancer.conc + solenhancer.ovisc
else
 formulation.viscosity = medium.viscosity
endif



formulation.MMD = 4.25 - 0.7217 * formulation.viscosity
formulation.TOR = 748.27 - 203.74 * formulation.viscosity

'TOR (Total output rate)
if formulation.tor < 200 then
 'decrease viscosity
 reductionfactor = 1
 'reductionfactor = put_formula_here

 api.mass = api.mass / reductionfactor
 isotonant.mass = isotonant.mass / reductionfactor
 'formulation.tor = recalculate_tor_here
endif

if api.stabilityproblem = 1 then
 concat comment$ , comment$, "Due to stability issues during preformulation tests,
Nitrogen gassing or lyophilisation are suggested. "
endif

if api.lightproblem = 1 then
 concat comment$, comment$, "API's light sensitivity requires light protection during
prosessing and for primary packaging. "
endif

if ( api.aqstable = 0 ) and ( api.hygroscopic = 0 ) then
 concat comment$, comment$, "Since the API exhibits a poor stability in aqueous
solution, it is recommended to produce a lyophilised dosage form that has to be
reconstituted prior usage. "
endif

if ( api.aqstable = 0 ) and ( api.hygroscopic = 1 ) then
 concat comment$, comment$, "API shows poor stability in aqeuous solution AND
hygroscopicity. Either pH optimisation, reduction of shelf life or lyophilisation with
terminal nitrogen flooding is suggested. "
endif

if api.oxproblem = 1 then
 concat comment$, comment$, "Terminal nitrogen flooding is required due to oxidation
issues. "
endif


'MMD (mean mass diameter = droplet size)
if formulation.MMD > device.holediameter  then
```

```
 'change size of nebulising head
 message "Droplet size too large", "Please change nebulising head's holesize"
endif

if formulation.MMD < 1.0 then
 'change size of nebulising head
 message "Droplet size too small", "Please change nebulizing head's holesize"
endif
```

No: **17**
Root: 16

Answer:
output

Commandfile:

```
rtfload "formulation_solution.rtf"
' first of all, replace all variables
rtfvarreplace
' second: replace variablenames within the replaces variable values (especially
required for explanation$)
rtfvarreplace
rtfview
```

No: **18**
Root: 10

Answer:
suspension

Commandfile:
(not shown here)

No: **19**
Root: 10

Question:
New profile

Commandfile:
(not shown here)

No: **20**
Root: 10

Question:
Choose dispersion type

Branches:
Key: **n** branch to cell nr. **11**

Commandfile:

```
'***** Dispersion type *****


'If no device is specified in the profile, then choose one from list
if profile.device$ = "" then
 tablechoosefrom "Inhalation device", "device.txt", "device", device.name$,
device.shortname$, n
else
 device.shortname$ = profile.device$
endif
'Now load the data that describes the device precisely
concat sect$, device.shortname$, ":device"
tablereadvariables "device.txt", sect$

if device.suspsol$ = "solution" then
 message "Dispersion type", "Device requires the formulation of a solution"
 gotocell 18
else
 'usual volume is 4ml
 formulation.estvolume = 4
 required.totalsolubility = api.mass / formulation.estvolume
 required.partialsolubility = required.totalsolubility / 10
 if api.solubility > required.totalsolubility then
  message "Solubility", "API solubility is sufficient for solution development."
 else
  if api.solubility > partialsolubility then
   message "Solubility", "Poor API solubility might be enhanced in order to develop a
solution."
  else
   message "Solubility", "API solubility is too low. The development of a suspension is
suggested."
  endif
 endif
 tablechoosefrom "Dispersion type", "extra.txt", "suspsol", disp.type$, disp.shortype$, n

 if disp.type$ = "solution" then
  gotocell 11
 else
  gotocell 18
 endif
endif
```

7.2.2 Output

The system will provide a possible formulation in terms of the excipients and the amounts that can be used. The layout as well as the contents can be freely composed by means of a template file meet personal preferences or company requirements. A sample output looks like this:

Spezialisten für effektive Inhalation PARI

May 4th 2006

Testformulation 1

Composition

#	*Function*	*name*	*quantity*	
1	active ingredient	**API P-034025-2006**	7.22	mg
2	solubility enhancer	alphaCD	23.08	mg
3	taste masking	(not required)	0	mg
4	additional excipient	citric acid	2.308	mg
5	surfactant	(not required)	0	mg
6	pH adjustment	sodiumhydroxide	.609	mg
7	isotonant	(not required)	0	mg
	Total		33.22	mg
	Medium	Water	ad 3	ml

Device

Device	PARI eFlow
optimal volume	3 ml
maximum volume	4 ml
holesize	3.5 µm
maximum viscosity	2.5 mPas
max. surface tension	70 mN/m

Manufacturing

Disperse CD in approx. 90% of water, add API, stirr at RT, add additional excipient, wait 3-5 days, filtrate, remove excess, add taste masking agent and surfactant, correct pH and add isotonant. Then fill up with rest of water

1 of 2

May 4th 2006

Testformulation 1

Properties

Property	*predicted*	*demanded*	*unit*
TOR	748	-	mg/min
Freezing p. depress.	.73	0.558 (<=0.558)	K
Osmolarity	390	300 (<=390)	mosmol/kg
pH	7.4	6.2 - 8.2	-
surface tension	54.8	<=70	mN/m
final volume	3	3 (<=4)	ml
taste ranking	1	<= 3	-
viscosity	2	<=2.5	mPas
MMD	4.3	-	µm

Footnotes

Taste ranking
1 = excellent / no taste
2 = slight bad taste
3 = noticable bad taste
4 = just tolerable
5 = intolerable
6 = disgusting

Notes

The formulation doesn't meet the desired dose of 25mg in the given volume without being hyperosmotic. Therefore the developer reduced the dose to obtain a isotonic solution.
API's light sensitivity requires light protection during prosessing and for primary packaging. Terminal nitrogen flooding is required due to oxidation issues.

Incompatibilities

No incompatibilities were detected between the ingredients of this formulation.

Explanations

API solubility is too poor; therefore a solubility enhancer is used. For immediate release a hydrophilic solubility enhancer (alphaCD) is chosen. A 50% (mol/mol) excess of solubility enhancer is used to complex Test API. The use of a cyclodextrin also improves taste for up to 4 units. Since API size (432 pm) doesn't match CD cavity (500 pm), an additional enhancer (citric acid) is required. Its mass is set to one tenth of the CD's mass.

2 of 2

Subchapter conclusion

The ability to rapidly assess the potential for a new molecule in a manner consistent with speed of the market is a key step in the development of new drugs.
The elaboration of a database system for the development of formulations constitutes a very important aid tool. It allows not only the reduction of time during the development formulation process but also the decrease costs by a more effective management of time and materials.

8 DISCUSSION

This work had as its main objective the development of systematic approach for water insoluble and water soluble drug formulations suitable for nebulisation using a specific nebulisation device - the eflow.
It constituted a new and interesting approach which, however, presented additional (and difficult) challenges. Apart from the common difficulties present in the development of a water insoluble drug formulation, such as the physicochemical properties of the drugs itself, the operation requirements of the nebulisation device (e.g., viscosity limits) also had to be taken into account. This meant that the link between the drug formulation and the nebulisation device was an ever present concern during the entire development process and constituted one of the pillars of this work.
In order to achieve the above-mentioned objectives, the solubility/stability enhancement of three water insoluble drugs was investigated by using cyclodextrins in two different forms: solutions and suspensions.

One of the drugs studied was cyclosporin A, a water insoluble drug used as an immunosuppressive to prevent transplant rejection and to treat autoimmune diseases.
Cyclodextrins were investigated as a means of improving the water solubility and stability of cyclosporin A.
With respect to solutions, the first results obtained showed that the cavity size of the α-CDs was the most suitable for the complex formation between the cyclosporin A and the CD. However, due to toxicological/cost issues and also the project objectives previously set out (e.g. required concentration of CSA in the deep lung), the amount and also the type of cyclodextrin that could be used to enhance the water solubility/stability of CSA was limited. Amongst the group of possible CD available for use in the work, the 2-HP-β-CD was chosen because it is the most widely accepted representative of the hydroxyalkylated β-cyclodextrin derivatives, due to its high water solubility, solubilizing power, low cost and low toxicology.
Nevertheless, results did not show the same increase in the solubility when compared with the α-CDs. For this reason, it was decided to add auxiliary substances, such as amino acids, in order to allow for the formation of non-inclusion complexes between cyclosporin A and the cyclodextrin complexes.
From the results obtained, it could be ascertained that the use of auxiliary substances did not increase the solubility aptitude of the cyclodextrin molecules. This was probably due to the fact that the CSA molecule was not able to interact with the molecules of the several auxiliary substances added to the complexing system.
Consequently, the critical factor in the CSA/CD complexation formation was the cavity size of the cyclodextrin.
Taking in consideration the physical chemical properties of the CSA molecule, it was decided to investigate the possibility of “inserting” the CSA in another molecule as a means of overtaking the natural properties of the CSA molecule.
Thus, lecithin was used as the “carrier” molecule for cyclosporin A, which is made from biological phospholipids that are biodegradable, lacking imunogenicity and exhibit low

intrinsic toxicity. In addition, the presence of suitable functional groups in the molecular structure of the lecithin molecules permits the interaction between the several auxiliary substances and the cyclodextrin, allowing this way the formation and stabilization of non-inclusion complexes.
From the several auxiliary substances investigated in combination with the lecithin/CSA system, ascorbic acid proved to be the best in terms of the improvement of the water solubility of CSA. However, due to stability problems of the ascorbic acid in aqueous solutions, it was decided to use sodium ascorbate instead.
An investigation of the influence of temperature, pH and equilibrium time on the solubility and stability of CSA was also performed with the purpose of determining the values of these parameters, which allow for a maximum of solubility/stability of CSA.
Finally, in order to obtain a stable formulation, it was prepared a lyophilizate of the CSA/CD formulation. This was mainly due to the hydrolysis of the lecithin molecules and also the oxidation of unsaturated fatty acid residues in the lecithin molecules.
The results of the stability tests show that the lyophilised cyclosporin A/cyclodextrin formulation kept at 25°C and 60% relative humidity is stable for at least 5 months.
In parallel, it was also analyzed the nebulisation properties of each of the prepared formulations aimed at achieving not only the best formulations in terms of solubility/stability but also the most suitable formulation to be efficiently nebulised by the eflow. The results showed that it was possible to develop a CSA formulation that can be efficiently delivered to the deep lungs using the eflow.
A new approach aimed at improving the water solubility of CSA through the nanoprecipitation method was also attempted using hydrophobic cyclodextrins. This, in turn, posed different problems and challenges in the development process in comparison with the solution approach such as the influence of the amount and type of the cyclodextrin used on the particle size. Nevertheless, with this approach, it was also possible to improve the water solubility of CSA and the developed formulations allowed for an efficient nebulisation with the eflow.
Azithromycin was one of the other model drugs investigated. Although, it is also a water insoluble drug, like CSA, the issue of the taste of the formulation was pivotal.
By means of a factorial design, it was possible to ascertain which CD and auxiliary substances could be used to improve the solubility and stability of azithromycin Consequently, the formulation prepared using this new method had not only improved the water solubility of azithromycin but it had also produced a suitable taste for inhalation.
Based on the stability tests, we could ascertain that the azithromycin was not stable in aqueous solutions, which led us to lyophilizate the azithromycin/CD formulations. The results show that the lyophilizate presented no significant degradation after 6 weeks, even at 40°C / 75% r.h. storage conditions. Only after 16 weeks under 40°C / 75% r.h. storage conditions could we witness some degradation although not worth mentioning.
The use of the nanoprecipitation method (like in the case of CSA), improved the solubility of azithromycin.

Budesonide was another water insoluble drug studied in this work. The objectives were not only to improve its water solubility/stability but also to obtain a formulation that posed no problems in term of its taste. In fact, budesonide represented the common ground between cyclosporin A and azithromycin, as it shared the same development objectives as cyclosporin A and azithromycin.

Based on previous experiences with CSA and azithromycin, it was possible to prepare, through the use of CD, a sterile and stable aqueous solution of budesonide that could be efficiently delivered into the deep lung.

On the other hand, pentoxifylline presented a different challenge in comparison with the previously described drugs. It is a water soluble drug, which meant that the taste masking of the formulation constituted the main objective in the development of the formulation. In the end, it was possible to obtain a formulation that could be efficiently delivered into the deep lung and presented a suitable taste for inhalation.

The elaboration of a knowledge based system for the development of formulations suitable for nebulisation constitutes a very important aid tool.
This system is able not only to reduce the time in the development formulation process but also decrease costs by reducing development time as well as by a more effective use of materials.

The interdependency link between the development process of the drug formulation and the nebulisation device was at the centre of the entire development process and represented the cornerstone upon which this work was build.
It constituted an interesting but difficult challenge which, nevertheless, yield the systematic preparation of formulations that can be efficiently nebulised and delivered into the deep lungs.

9 CONCLUSION

Despite the long-term use of inhalation technologies for therapeutic purposes, there still is room for improvement in a number of areas such as deposition efficiency and targeting. The inhalation route, primarily employed for drugs acting in the respiratory tract, is now being extended for systemic macromolecule delivery. These challenges can be achieved in part by formulation development and device engineering for inhalation delivery.

This work had as main objectives not only the development of formulations suitable for nebulisation but it also investigated and customised the formulations for a specific nebulisation device, the eflow. This new approach represented an interesting challenge during the formulation development due the physicochemical properties of the drugs itself but also to the required operating parameters of the device. It was a valid approach because having a suitable formulation nebulisation does not necessary mean that it will be efficiently delivered into the deep lungs. In fact, the device has to have also the ability to nebulise the formulation in an efficient way, for example in terms of droplet size and delivery rate and breath pattern.

This work demonstrated that the use of CD can contribute new possibilities and advantages to the preparation of new nebulised formulations for both: water insoluble and water soluble drugs. The use of CD permitted an increase in stability and solubility of water insoluble dugs by the formation of Drug:CD complexes. In addition, the taste or local irritation of a drug can also be reduced through the use of CD. Furthermore, this work also showed that the development of formulations in association with the inhalation device allows the drug to be more efficiently delivered into the lungs.

10 OUTLOOK

As further steps of research, the influence of other auxiliary substances on the solubility and stability enhancement provided by the cyclodextrins could be investigated in closer detail.
The type and amount of auxiliary substances used can be studied in order to maximize the solubility and stability of the water insoluble drugs. As an example, the use of different amino acids or metals ions can be investigated in order to ascertain the possibility of increasing even more the solubility and stability of the water insoluble drugs.

Furthermore, the type and amount of CD used can also be closer investigated as a way of improving the solubility/stability of the model drugs. This can be applied for both the solutions and suspensions. Nevertheless, in the case of the suspensions, the type and the amount of surfactants used and also the use of substances that can alter the zeta potential of the nanospheres could be subject to further investigations in order to obtain more stable suspensions.

11 GLOSSARY

BPD (Bronchopulmonary Dysplasia)

Bronchopulmonary dysplasia (BPD) is a chronic lung disease of babies, which develops most commonly in the first 4 weeks after birth. It mostly occurs in babies who are born more than 4 weeks before their due dates, though sometimes the babies are full term.

Bronchiolitis

Is a common illness of the respiratory tract caused by a respiratory infection that affects the tiny airways, called the bronchioles, which lead to the lungs. As these airways become inflamed, they swell and fill with mucus, making it difficult for a child to breathe.

CF (Cystic fibrosis)

Cystic fibrosis is a genetic disease affecting approximately 30,000 children and adults in the United States. A defective gene causes the body to produce abnormally thick, sticky mucus that clogs the lungs and leads to life-threatening lung infections. These thick secretions also obstruct the pancreas, preventing digestive enzymes from reaching the intestines to help break down and absorb food. The mucus also can block the bile duct in the liver, eventually causing permanent liver damage in approximately six percent of people with CF.

COPD (Chronic Obstructive Pulmonary Disease)

Chronic obstructive pulmonary disease (COPD) is a lung disease in which the lung is damaged, making it hard to breathe. In COPD, the airways-the tubes that carry air in and out of your lungs-are partly obstructed, making it difficult to get air in and out.

DD (Delivered Dose)

Dose that reaches the patient and it is not eliminated during exhalation.

DPI (Dry Powder Inhaler)

A dry powder inhaler (DPI) is similar to a metered dose inhaler. Both are handheld devices that deliver a precisely measured dose of drug. into the lungs. In a dry powder inhaler, drug comes in a dry powder form - inside a small capsule, a disk or a compartment inside the inhaler.

GI (Gastrointestinal)

The digestive or gastrointestinal (GI) tract includes the esophagus, stomach, small intestine, large intestine or colon, rectum and anus.

GSD (Geometric Standard Deviation)

Corresponds to the ratio of the diameters of particles from aerosols corresponding to 84% and 50% on the cumulative distribution curve of the weights of particles. The use of a geometric standard deviation to describe the particle size distribution requires that particle sizes are log-normally distributed. If, as is frequently the case, particles are not log-normally distributed, the geometrical standard deviation is meaningless and a misleading representation of the distribution. Heterogeneous aerosols have, by definition, a GSD of greater than or equal to 1.22.

MDI (Metered Dose Inhaler)

Metered dose inhalers (MDI) are pharmaceutical delivery systems designed for oral or nasal use, which deliver discrete doses of aerosolized medicament to the respiratory tract.

MMAD (Mass Median Aerodynamic Diameter)

Makes it possible to define the granulometry of aerosol particles by taking into account their geometrical diameter, shape, and density: MMAD= MMD × Density.

MMD (Mass Median Diameter)

Corresponds to the diameter of the particles for which 50% w/w of particles have a lower diameter and 50% w/w have a higher diameter.

Parkinson Disease

Parkinson's disease is a slowly progressive neurodegenerative illness characterized by: tremor, stiffness, (rigidity), slowness of movement (bradykinesia) and difficulty with balance (postural instability). The symptoms appear when there is not enough dopamine in the brain.

RDS (Respiratory Distress Syndrome):

Acute Respiratory Distress Syndrome is inflammatory disease of the lung characterized by the sudden onset of pulmonary edema and respiratory failure, usually in the setting of other acute medical conditions resulting from local (e.g. pneumonia) or distant (e.g. multiple trauma) injury.

TOR (Total Output Rate)

Corresponds to the rate of aerosol delivery (mg/ml).

12 SYMBOLS AND ABBREVIATION (ALPHABETIC ORDER)

Å: Ångstrøm (1 Å = 0.0001 µm)
AM: Alveolar Macrophages
BDP: Bronchopulmonary Dysplasia
CD: Cyclodextrin
CF: Cystic Fibrosis
COPD: Chronic Obstructive Pulmonary Disease
CSA: Cyclosporin A
DPI: Dry Powder Inhaler
DPPC: Dipalmitoyl phosphatidylcholine
***e.g.*:** Exempli gratia or for example
Eq.: Equation
***et al.* :** Et alii or and others
FDA: U.S. Food and Drug Administration
G.I.: Gastro intestinal tract
HP: Hydroxypropyl
HPLC: High Pressure Liquid Chromatography
***i.e.*:** Id est or that is
i.m.: Intramusculous
i.v.: Intravenous
IL-2: Interleukin-2
K: Inclusion complex stability constant
MDI: Metered-Dose Inhaler
MW: Molecular weight
M-β-CD: Maltosyl-β-Cyclodextrin
OH : Hydroxyl
PI: Polydispersity index
PTX: Pentoxifylline
r.h.: Relative humidity
RDS: Respiratory Distress Syndrome
s.c.: Subcutaneous
SOP: Standart operation procedure
T: Temperature
TNF-α: Tumor Necrosis Factor alpha
UV: Ultra violet
α-CD: Alpha-Cyclodextrin (six glucose units)
β-CD: Beta-Cyclodextrin (seven glucose units)
γ-CD: Gamma-Cyclodextrin (Eight glucose units)

13 APPENDIX

13.1 Appendix 1 (The problem with propellants)

It is known that CFCs could lead to the depletion of stratospheric ozone. For this reason, the pharmaceutical industry has been working since 1987 to find alternative propellants with which to replace the CFCs used in MDIs.

The research for propellants with low or zero ozone depletion potential has contributed to the identification of a number of potential compounds. Several chemical industry research groups were created to investigate the acute toxicity of the most promising candidates under The Programme for Alternative Fluorocarbon Toxicity Testing (PAFTT).

The use of hydrofluorocarbons (HCFC) has been taken in consideration, although they still have a significant effect on the ozone depletion. For example, the HCFC 22 could be a suitable replacement for CFC 12. However, revisions to the Montreal Protocol in 1990 require the phase-out of HCFCs by the year 2020.

The odour and flammability of hydrocarbons such as isobutane has prevented their use in medical aerosols although purer grades, which are odourless, are now available.

Dimethylether (DME) is also a possibility because it combines low ozone depletion with a superior solvency for various active components and appreciable miscibility with water. Nevertheless, the high flammability of both the hydrocarbons and DME would require expensive modifications of facilities for the manufacture, storage, and transportation of MDIs.

In addition, the use of both hydrocarbons and DME as propellants in a suspension MDI formulation, is their low density, compared with most drug substances. This could generate a poor suspension stability and, consequently, to the potential for inconsistent dose delivery.

Another alternative identified, was the hydrofluoroalkanes (HFAs) or HFCs. Within this class, 134a and 227ea were adopted for inhalation toxicity testing by two consortia of pharmaceutical companies: IPACT 1 for 134a and IPACT 2 for 227ea (IPACT: International Pharmaceutical Aerosol Consortium for Toxicity Testing).

13.2 Appendix 2: Lecithin (properties and experimental data)

13.2.1 Physical and Chemical Properties of Lecithin

Lecithin is usually used as synonym for phosphatidylcholine (PC), which is the major component of a phosphatide fraction which is frequently isolated from either egg yolk or soya beans and is commercially available in high purity. PC is a mixture of differently substituted sn-glycerol-3-phosphatidylcholine backbones.

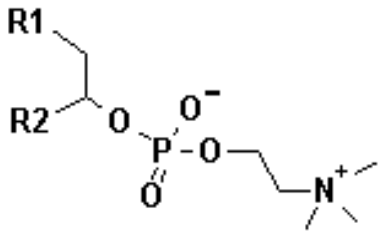
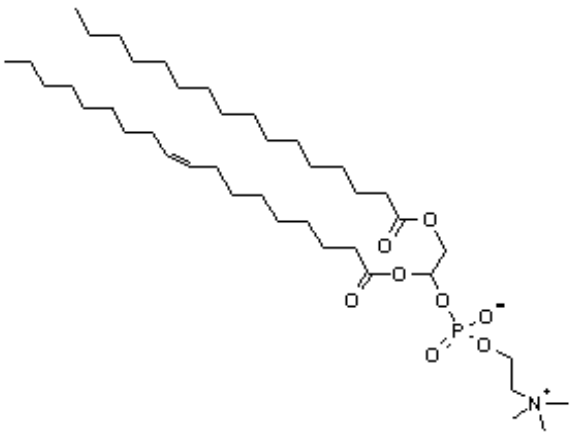
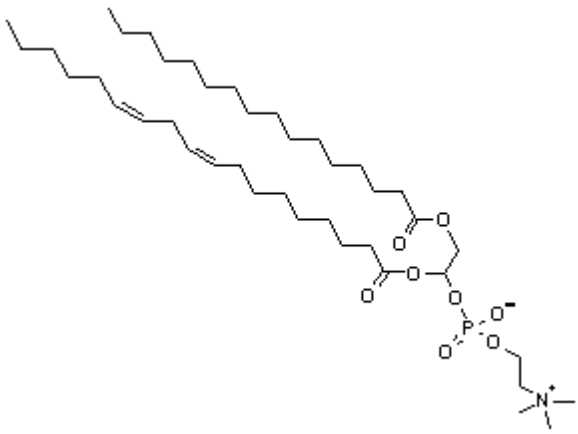


Figure 1: Molecular structure of PC and their natural occurrence in egg yolk lecithin (mol/mol). Values according to (Lit. [1]).

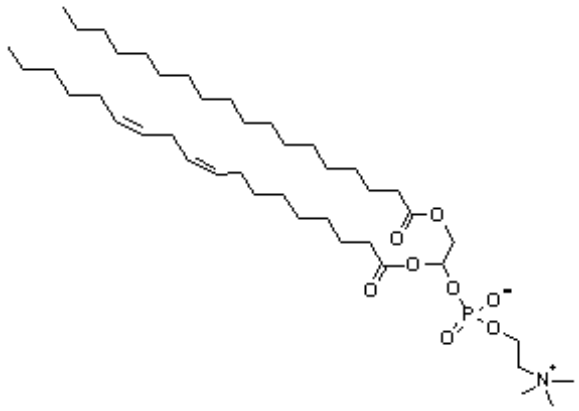
From Figure 35, the structure of PC is variable and dependent on fatty acid substitution. In the sn-1-position (R_1), saturated acyl-groups, and in sn-2-position (R_2), unsaturated species are more common (Lit. [1]). By dietetic means the fatty acid substitution of egg phospholipids can be altered in the sn-2-position (Lit [2]). Fatty acids of mainly 16-20 C in chain length dominate in egg PC. The sn-1-chain typically shows an average of 16 C, whereas the sn-2-chain shows an average of 18 C. Naturally occurring unsaturated fatty acids are almost entirely of all-cis-conformation (Lit [3]).



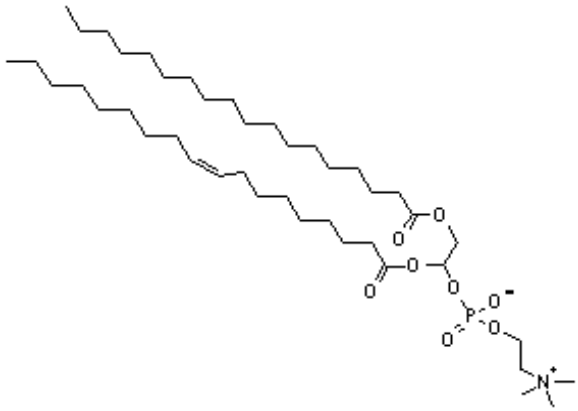
Palmitoyl/Oleoyl [16:0/18:1]-PC **(POPC)** 38.2% in Egg PC



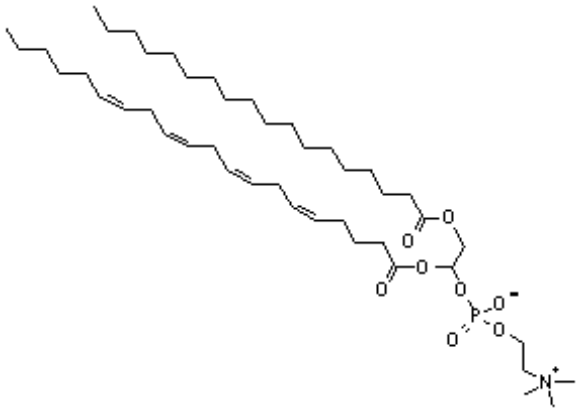
Palmitoyl/Linoleoyl [16:0/18:2]-PC **(PLPC)** 21.8% in Egg PC



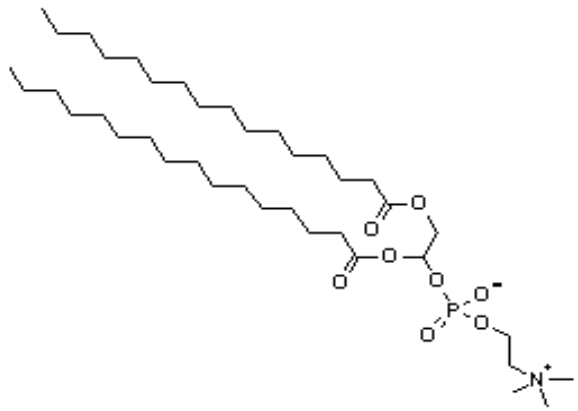
Stearoyl/Linoleoyl [18:0/18:2]-PC **(SLPC)** 11.22% in Egg PC



Stearoyl/Oleoyl [18:0/18:1]-PC **(SOPC)** 9.3% in Egg PC



Stearoyl/Arachidoyl [18:0/20:4]-PC **(SAPC)** 3.36% in Egg PC



Di-Palmitoyl [16:0/16:0]-PC **(DPPC)** 0.72% in Egg PC

Figure 2: Different species of PC and their natural occurrence in egg yolk lecithin (mol/mol). Values according to Lit.1.

Lecithin is commonly regarded as safe to use for parenteral administration because lecithin can be totally biodegraded and metabolised, since it is an integral part of biological membranes, making it virtually non-toxic.
For this reason, lecithin is regarded as a well tolerated and non-toxic compound (which is also expressed by its *GRAS-status ['Generally Recognised As Safe']* approved by the FDA), making it suitable for long-term and large-dose infusion, for example.

13.2.2 Stability and Degradation of Lecithin

Possible chemical degradation includes oxidation of unsaturated fatty acid residues in the lecithin molecules (Lit [4]) and also hydrolysis (Lit. [5]).
According to (Lit. [6]), a possible mechanism for the oxidation of unsaturated fatty acid residues in the lecithin involves the oxidation of the double bonds within the lecithin.

On the other hand, the hydrolysis of phospholipids leads to formation of free fatty acids, lyso-phospholipids and glycerophosphorylic compounds (please see Figure 3).
Hydrolysis products need to be minimised since lyso-phospholipids exhibit haemolytic effects in vitro and have, therefore, to be regarded as toxic Lit [7]. Based on the Lit [8] and Lit [9], several hydrolysis rates of PC and PE in aqueous media at various temperatures and at pH 6.5 were determined. The degradation products were more water-soluble than the original phospholipids, which promote further degradation as they cause lowering of pH (Lit. [10] and Lit [11]).

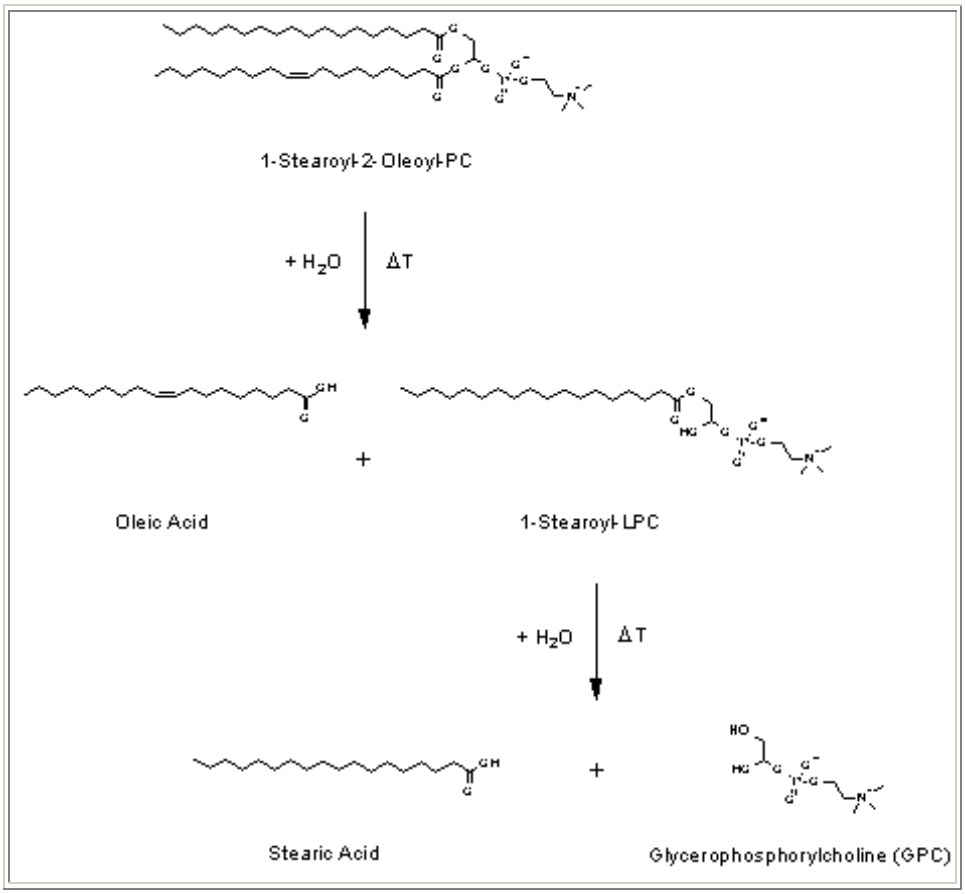


Figure 3: Pathways of phospholipids hydrolysis.

With the purpose of avoiding hydrolysis reactions of lecithin phosphatide groups, lyophilization can be used.
As an example, a microemulsion of amphotericin B containing 10% (w/w) of lecithin was lyophilized in order to prevent hydrolysis reactions of lecithin (Lit. [13]).

Table 1: Composition (% w/w) of an amphotericin B microemulsion

Composition	Percentage (w/w)
Amphotericin B	0.2
Isopropyl myristate	10
Dist. Water	59.8
Polysorbate 80	20
Soybean lecithin	10

Due to the fluid nature of most the excipients, 5 % (w/v) of mannitol was added to the external phase of the formulation as a bulky agent. Conditions of the lyophilization process are described in Table 1.

Table 2: Lyophilization conditions of an amphotericin B microemulsion. Values according to (Lit. [12]).

Step in process	Duration	Product: Final temperature	Condenser: Final temperature	Pressure in chamber
Freezing	2 hours	Approx. -40°C	Approx. 19°C	Atmospheric
1^{ry} Dessication	24 hours	Approx. -36°C	Approx. -50°C	30 microns
2^{ry} Dessication	40 hours	Approx. 30°C	Approx. -50°C	30 microns

The lyophilized amphotericin B microemulsion was characterised in terms of macroscopically appearance, water content and reconstitution. It was also compared with a non lyophilized microemulsion. The results are presented table 3 and table 4.

Table 3: Results from the stability evaluation of a non lyophilized amphotericin B microemulsion. Values according to (Lit. [12]).

Time (days)	0	7	14	21
Reconstituted microemulsion appearance	As specified[a]	Amphotericin B precipitated	Phase separation[b]	Phase separation[b]
pH	8.97±0.03	8.20±0.11	---	---
Amphotericin B content (%)	8.97±0.03	8.97±0.9	---	---

a Stable amphotericin B microemulsion macroscopical characteristics.

b After phase separation systems were no longer consider as microemulsions.

Table 4: Results from the stability evaluation of a lyophilized amphotericin B microemulsion. Values according to (Lit. [12]).

Time (months)	0	1	2	6
Lyophilized products appearance	As specified[a]	As specified[a]	As specified[a]	As specified[a]
Humidity content (%)	0.75±0.08	0.52±0.01	0.57±0.04	0.47±0.02
Reconstituted microemulsions appearance	As specified[b]	As specified[b]	As specified[b]	As specified[b]
pH	9.12±0.08	9.2±0.02	8.96±0.07	8.98±0.06
Amphotericin B content (%)	95.6±1.5	99.2±2.2	96.5±1.4	97.6±1.0

a Stable lyophilized product amphotericin B microemulsion macroscopical characteristics.

b Stable reconstituted amphotericin B microemulsion macroscopical characteristics.

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13.3 Appendix 3: Description of Test A

13.3.1 Description of Test A

The Test A is used to determine which type of CDs (α, β or γ) is more suitable for the complex formation between the drug substance and the CDs. It consists of:

- Preparation of aqueous solutions of cyclodextrin containing a specific concentration (for example, between 10%);
- To each of the vials containing the cyclodextrin aqueous solutions, an excess of the drug substance is added;
- Afterwards, the vials are stirred at a specific temperature and for defined period of time;
- Then, the solutions are filtered, using 0.45 μm filters, or centrifuged;
- Finally, the drug content is determined.

The type of CD in which the most solubility increase of the drug substance was obtained will be chosen for the further formulation development process.

13.4 Appendix 4: Properties of used substances

Ascorbic Acid

Nonproprietary Names

BP: Ascorbic acid
JP: Ascorbic acid
PhEur: Acidum ascorbicum
USP: Ascorbic acid

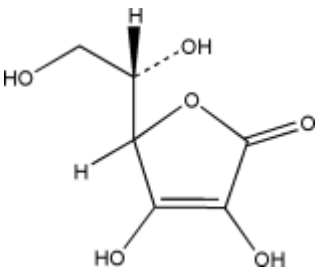
Synonyms

C-97; cevitamic acid; 2,3-didehydro-L-threo-hexono-1,4-lactone; E300; 3-oxo-L-gulofuranolactone, enol form; vitamin C.

Molecular Formula and Molecular Weight

$C_6H_8O_6$
Mw=176.13 g/mol

Structural Formula



Functional Category

Antioxidant; therapeutic agent.

Description

Ascorbic acid occurs as a white to light yellow colored, nonhygroscopic, odorless, crystalline powder or colourless crystals with a sharp, acidic taste. It gradually darkens in colour upon.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for ascorbic acid.

Test	JP 2001	PhEur 2002 (suppl. 4.3)	USP 26
Identification	+	+	+
Characters	—	+	—
Specific rotation	+ 20.5° to	+ 20.5° to	+ 20.5° to
(10% w/v solution)	+ 21.5°	+ 21.5°	+ 21.5°
Residue on ignition	≤0.1 %	—	≤0.1 %
pH	2.2–2.5	2.1–2.6	—
Sulfated ash	—	≤0.1%	—
Copper	—	≤5 ppm	—
Heavy metals	≤20 ppm	≤10 ppm	≤0.002%
Loss on drying	≤0.2%	—	—
Iron	—	≤2 ppm	—
Appearance of Solution	+	+	—
Oxalic acid	—	+	—
Organic volatile impurities	—	—	+
Assay	≥99.0%	99.0–100.5%	99.0–100.5%

Typical Properties

Acidity/alkalinity
pH = 2.1–2.6 (5% w/v aqueous solution)

Density (bulk)

0.7–0.9 g/cm3 for crystalline material
0.5–0.7 g/cm3 for powder
Density (particle)

1.65 g/cm3

Density (tapped)

1.0–1.2 g/cm3 for crystalline material
0.9–1.1 g/cm3 for powder

Density (true)

1.688 g/cm3

Dissociation constant

pKa1 = 4.17
pKa2 = 11.57

Melting point

190°C (with decomposition)

Moisture content

0.1% w/w

Solubility

Table 2: Solubility of ascorbic acid.

Solvent	**Solubility at 20°C**
Chloroform	Practically insoluble
Ethanol	1 in 50
Ethanol (95%)	1 in 25
Ether	Practically insoluble
Fixed oils	Practically insoluble
Glycerin	1 in 1000
Propylene glycol	1 in 20
Water	1 in 3.5

Stability and Storage Conditions

In powder form, ascorbic acid is relatively stable in air. In the absence of oxygen and other oxidizing agents it is also heat stable. Ascorbic acid is unstable in solution, especially alkaline solution, readily undergoing oxidation on exposure to the air. The oxidation process is accelerated by light and heat and is catalyzed by traces of copper and iron. Ascorbic acid solutions exhibit maximum stability at about pH 5.4. Solutions may be sterilized by filtration.
The bulk material should be stored in a well-closed non-metallic container, protected from light, in a cool, dry place.

Incompatibilities

Incompatible with alkalis, heavy metal ions, especially copper and iron, oxidizing materials, methenamine, phenylephrine hydrochloride, pyrilamine maleate, salicylamide, sodium nitrite, sodium salicylate, the obromine salicylate, and picotamide. Additionally, ascorbic acid has been found to interfere with certain colorimetric assays by reducing the intensity of the colour produced.

Safety

Ascorbic acid is an essential part of the human diet, with 40 mg being the recommended daily dose in the UK and 60 mg in the US. However, these figures are controversial, with some advocating doses of 150 or 250 mg daily. Mega doses of 10 g daily have also been suggested to prevent illness.
The body can absorb about 500 mg of ascorbic acid daily with any excess immediately excreted by the kidneys. Large doses may cause diarrhea or other gastrointestinal disturbances. Damage to the teeth has also been reported. However, no adverse effects have been reported at the levels employed as an antioxidant in foods and pharmaceuticals. The WHO has set an acceptable daily intake of ascorbic acid, potassium ascorbate, and sodium ascorbate, as antioxidants in food, at up to 15 mg/kg body-weight in addition to that naturally present in food.

LD50 (mouse, IV): 0.52 g/kg
LD50 (mouse, oral): 3.37 g/kg
LD50 (rat, oral): 11.9 g/kg

Handling Precautions

Ascorbic acid may be harmful if ingested in large quantities and may be irritating to the eyes. Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and rubber or plastic gloves are recommended.

Regulatory Status

GRAS listed. Accepted as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (inhalations, injections, oral capsules, suspensions, tablets, topical preparations, and suppositories). Included in medicines licensed in the UK.

Related Substances
Ascorbyl palmitate; sodium ascorbate.

Sodium Ascorbate

Non-proprietary Names

PhEur: Natrii ascorbas
USP: Sodium ascorbate

Synonyms

L-Ascorbic acid monosodium salt; E301; 3-oxo-L-gulofuranolactone sodium enolate; SA-99; vitamin C sodium.

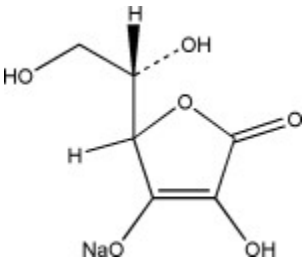
Chemical Name

Monosodium L-(+)-ascorbate

Molecular Formula and Molecular Weight

$C_6H_7NaO_6$
Mw= 198.11 g/mol

Structural Formula



Functional Category

Antioxidant; therapeutic agent.

Description

Sodium ascorbate occurs as a white or slightly yellow-colored, practically odorless, crystalline powder with a pleasant saline taste.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for sodium ascorbate.

Test	PhEur 2002	USP 26
Identification	+	+
Characters	+	—
Appearance of solution	+	—
pH	7.0–8.0	7.0–8.0
Specific optical rotation (10% w/v aqueous solution)	+103° to +108°	+103° to +108°
Oxalic acid	≤0.30%	—
Benzene	≤2 ppm	—
Sulfates	≤150 ppm	—
Copper	≤5 ppm	—
Iron	≤2 ppm	—
Nickel	≤1 ppm	—
Heavy metals	≤10 ppm	≤0.002%
Loss on drying	≤0.25%	≤0.25%
Organic volatile impurities	—	+
Assay (dried basis)	99.0–101.0%	99.0–101.0%

Typical Properties

Acidity/alkalinity

pH = 7–8 (10% w/v aqueous solution)

Density (tapped)

0.6–1.1 g/cm3 for fine powder
0.8–1.1 g/cm3 for fine granular grade

Density (true)

1.826 g/cm3

Hygroscopicity

Not hygroscopic. Sodium ascorbate adsorbs practically no water up to 80% relative humidity at 20°C and less than 1% w/w of water at 90% relative humidity.

Melting point

218°C (with decomposition)

Particle size distribution

Various grades of sodium ascorbate with different particle-size distributions are commercially available, e.g., approximately 98% passes through a 149 μm mesh for a fine powder grade (Takeda Europe GmbH), and approximately 95% passes through a 840 μm mesh for a standard grade (Takeda).

Solubility

Table 2: Solubility of sodium ascorbate.

Solvent	**Solubility at 20°C**
Chloroform	Practically insoluble
Ethanol (95%)	Very slightly soluble
Ether	Practically insoluble
Water	1 in 1.6
	1 in 1.3 at 75°C

Stability and Storage Conditions

Sodium ascorbate is relatively stable in air, although it gradually darkens on exposure to light. Aqueous solutions are unstable and subject to rapid oxidation in air at pH > 6.0.
The bulk material should be stored in a well-closed non-metallic container, protected from light, in a cool, dry place.

Incompatibilities

Incompatible with oxidizing agents, heavy metal ions, especially copper and iron, methenamine, sodium nitrite, sodium salicylate, and the obromine salicylate. The aqueous solution is reported to be incompatible with stainless steel filters.

Safety

The parenteral administration of between 0.25 and 1.00 g of sodium ascorbate, given daily in divided doses, is recommended in the treatment of vitamin C deficiencies. Various adverse reactions have been reported following the administration of 1 g or more of sodium ascorbate, although ascorbic acid and sodium ascorbate are usually well tolerated. There have been no reports of adverse effects associated with the much lower concentrations of sodium ascorbate and ascorbic acid, which are employed as antioxidants.
The WHO has set an acceptable daily intake of ascorbic acid, potassium ascorbate, and sodium ascorbate, as antioxidants in food, at up to 15 mg/kg body-weight in addition to that naturally present in food.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Sodium ascorbate may be irritant to the eyes. Eye protection and rubber or plastic gloves are recommended.

Regulatory Status

GRAS listed. Accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (IV preparations; oral tablets). Included in nonparenteral and parenteral medicines licensed in the UK.

Related Substances

Ascorbic acid; ascorbyl palmitate; calcium ascorbate.

Sodium citrate

Non-proprietary Names

BP: Sodium citrate
JP: Sodium citrate
PhEur: Natrii citras
USP: Sodium citrate

Synonyms

Citric acid trisodium salt; E331; sodium citrate tertiary; trisodium citrate.

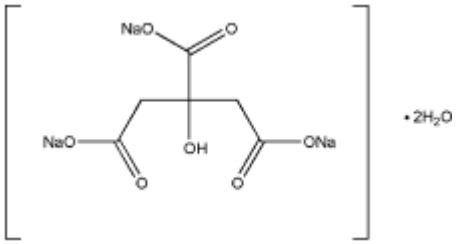
Chemical Name

Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate

Molecular Formula and Molecular Weight

$C_6H_5Na3O_7 \cdot 2H_2O$
Mw = 294.10 g/mol

Structural Formula



Functional Category

Alkalizing agent; buffering agent; emulsifier; sequestering agent.

Description

Sodium citrate dihydrate consists of odorless, colourless, monoclinic crystals, or a white crystalline powder with a cooling, saline taste. It is slightly deliquescent in moist air, and in warm dry air it is efflorescent. Although most pharmacopoeias specify that sodium citrate is the dihydrate, the USP 26 states that sodium citrate may be either the dihydrate or anhydrous material.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for sodium citrate dihydrate.

Test	JP 2001	PhEur 2002	USP 26
Identification	+	+	+
Characters	—	+	—
pH	7.5–8.5	—	—
Appearance of solution	+	+	—
Acidity or alkalinity	+	+	+
Loss on drying	10.0–13.0%	—	—
Water	—	11.0–13.0%	10.0–13.0%
Oxalate	+	≤300 ppm	—
Sulphate	≤0.048%	≤150 ppm	—
Heavy metals	≤10 ppm	≤10 ppm	≤0.001%

Arsenic	≤2 ppm	—	—
Chloride	≤0.015%	≤50 ppm	—
Tartrate	+	—	+
Readily carbonizable substances	+	+	—
Pyrogens	—	+(a)	—
Assay (anhydrous basis)	≥99.0%	99.0–101.0%	99.0–100.5%

Typical Properties

Acidity/alkalinity

pH = 7.0–9.0 (5% w/v aqueous solution)

Density (bulk)

1.12 g/cm3

Density (tapped)

0.99 g/cm3

Density (true)

1.19 g/cm3

Melting point

Converts to the anhydrous form at 150°C.

Osmolarity

A 3.02% w/v aqueous solution is iso-osmotic with serum.

Particle size distribution

Various grades of sodium citrate dihydrate with different particle sizes are commercially available.

Solubility

Soluble 1 in 1.5 of water, 1 in 0.6 of boiling water; practically insoluble in ethanol (95%).
Stability and Storage Conditions

Incompatibilities
Aqueous solutions are slightly alkaline and will react with acidic substances. Alkaloidal salts may be precipitated from their aqueous or hydro-alcohol solutions. Calcium and strontium salts will cause precipitation of the corresponding citrates. Other incompatibilities include bases, reducing agents, and oxidizing agents.

Safety

After ingestion, sodium citrate is absorbed and metabolized to bicarbonate. Although it is generally regarded as a non-toxic and non-irritant excipient, excessive consumption may cause gastrointestinal discomfort or diarrhea. Therapeutically, in adults, up to 15 g daily of sodium citrate dihydrate may be administered orally, in divided doses, as an aqueous solution to relieve the painful irritation caused by cystitis.
Citrates and citric acid enhance intestinal aluminium absorption in renal patients, which may lead to increased, harmful serum aluminium levels. It has therefore been suggested that patients with renal failure taking aluminium compounds to control phosphate absorption should not be prescribed citrate- or citric acid-containing products.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Sodium citrate dihydrate dust may be irritant to the eyes and respiratory tract. Eye protection and gloves are recommended. Sodium citrate should be handled in a well-ventilated environment or a dust mask should be worn.

Regulatory Status

GRAS listed. Accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (inhalations, injections, ophthalmic products, oral solutions, suspensions, syrups and tablets, nasal, otic, rectal, topical, transdermal, and vaginal preparations). Included in nonparenteral and parenteral medicines licensed in the UK.

Related Substances

Anhydrous sodium citrate; citric acid monohydrate.

Sodium Acetate

Non-proprietary Names

BP: Sodium acetate
JP: Sodium acetate
PhEur: Natrii acetas trihydricus
USP: Sodium acetate

Synonyms

E262; acetic acid, sodium acetate trihydrate; sodium ethanoate; sodium ethanoate trihydrate; sodium salt.

Chemical Name

Sodium acetate anhydrous [127-09-3]
Sodium acetate trihydrate [6131-90-4]

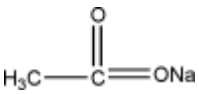
Molecular Formula and Molecular Weight

$C_2H_3NaO_2$
Mw= 82.0 (for anhydrous)

$C_2H_3NaO_2 \cdot 3H_2O$
Mw= 136.1 (for trihydrate)

The PhEur 2002 contains a monograph for sodium acetate; however, in Supplement 4.3 this was replaced with a monograph for sodium acetate trihydrate, where it is defined as sodium ethanoate trihydrate.

Structural Formula



Functional Category

Antimicrobial preservative; buffering agent; flavouring agent, stabilizing agent.

Description

Sodium acetate occurs as colourless, transparent crystals or a granular crystalline powder with a slight acetic acid door.

Pharmacopeial Specifications

The PhEur 2002 (Suppl. 4.3) and JP 2001 refer to sodium acetate trihydrate, whereas the USP 26 refers to the anhydrous material.

Table 1: Pharmacopeial specifications for sodium acetate.

Test	JP 2001	PhEur 2002 (Suppl. 4.3)	USP 26
Identification	+	+	+
Description	+	—	—
Characters	—	+	—
Appearance of solution	+	+	—
Acid or alkali	+	—	—
pH	—	7.5–9.0	7.5–9.2
Insoluble matter	—	—	≤0.05%

Chloride	≤0.011%	≤200 ppm	≤0.035%
Sulphate	≤0.017%	≤200 ppm	≤0.005%
Heavy metals	≤10 ppm	≤10 ppm	≤10 ppm
Calcium and magnesium	+	≤50 ppm	+
Potassium	—	—	+
Arsenic	≤2 ppm	≤2 ppm	—
Iron	—	≤10 ppm	—
Reducing substances	+	+	—
Aluminium	—	≤0.2 ppm	≤0.2 μg/g
Loss on drying	39.0–40.5%	39.0–40.5%	≤1.0%
Organic volatile impurities	—	—	+
Assay	≤99.5%	≤99.0–101.0%	≤99.0–101.0%

Typical Properties

Acidity/alkalinity

pH = 7.5–9.0 (5% w/v aqueous solution)

Hygroscopicity

The anhydrous and trihydrate sodium acetate are hygroscopic.

Melting point

58°C for trihydrate; 324°C for anhydrous

Solubility

Soluble 1 in 0.8 in water, 1 in 20 in ethanol.

Stability and Storage Conditions

Sodium acetate should be stored in airtight containers.

Incompatibilities

Sodium acetate reacts with acidic and basic components. It will react violently with fluorine, potassium nitrate, and diketene.

Safety

Sodium acetate is widely used in cosmetics, foods, and pharmaceutical formulations and is generally regarded as a non-toxic and non-irritant material.
A short-term feeding study in chickens with a diet supplemented with 5.44% sodium acetate showed reduced growth rates that were attributed to the sodium content. Sodium acetate is poisonous if injected intravenously, is moderately toxic by ingestion, and is an irritant to the skin and eyes.
LD50 (rat, oral): 3.530 g/kg
LD50 (mouse, IV): 0.380 g/kg
LD50 (mouse, SC): 8.0 g/kg

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Sodium acetate is a mild skin and eye irritant; gloves and eye protection are recommended. On exposure, wash eyes and skin with large amounts of water. Inhalation of dust may cause pulmonary tract problems. When heated to decomposition, sodium acetate emits toxic fumes of NaO_2.

Regulatory Status

GRAS listed. Accepted as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (injections, nasal, otic, ophthalmic, and oral preparations).

Related Substances
Acetic acid, glacial.

Lactic Acid

Non-proprietary Names

BP: Lactic acid
JP: Lactic acid
PhEur: Acidum lacticum
USP: Lactic acid

Synonyms

E270; Eco-Lac; 2-hydroxypropanoic acid; α-hydroxypropionic acid; L18; DL-lactic acid; milk acid; racemic lactic acid.

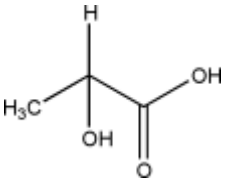
Chemical Name

2-Hydroxypropionic acid
(R)-(–)-2-Hydroxypropionic acid
(S)-(+)-2-Hydroxypropionic acid
(RS)-(±)-2-Hydroxypropionic acid

Molecular Formula and Molecular Weight

$C_3H_6O_3$
Mw= 90.08 g/mol

Structural Formula



Functional Category

Acidifying agent; acidulant.

Description

Lactic acid consists of a mixture of 2-hydroxypropionic acid, its condensation products, such as lactoyllactic acid and other polylactic acids, and water. It is usually in the form of the racemate, (RS)-lactic acid, but in some cases the (S)-(+)-isomer is predominant.
Lactic acid is a practically odorless, colourless or slightly yellow-colored, viscous, hygroscopic, nonvolatile liquid.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for lactic acid.

Test	JP 2001	PhEur 2002 (Suppl 4.1)	USP 26
Identification	+	+	+
Appearance of solution	—	+	—
Specific rotation	—	—	−0.05° to +0.05°
Calcium	—	≤200 ppm	—
Heavy metals	≤10 ppm	≤10 ppm	≤0.001%
Iron	≤5 ppm	—	—
Sulphate	≤0.01%	≤200 ppm	+
Chloride	≤0.036%	—	+
Citric, oxalic, phosphoric, and tartaric acids	+	+	+
Ether-insoluble substances	—	+	—
Cyanide	+	—	—
Sugars and other reducing substances	+	+	+
Glycerin and mannitol	+	—	—

Methanol and methyl esters	—	≤50 ppm	—
Reducing substances	—	+	—
Readily carbonizable substances	+	—	+
Bacterial endotoxins	—	≤5 IU/g	—
Volatile fatty acids	+	+	—
Residue on ignition	≤0.1%	≤0.1%	≤3.0 mg
Sulfated ash	—	≤0.1%	≤0.05%
Assay	+	88.0–92.0%	88.0–92.0%

Typical Properties

Boiling point

122°C at 2 kPa (15 mmHg)

Dissociation constant

pKa = 4.14 at 22.5°C

Melting point

17°C

Osmolarity

a 2.3% w/v aqueous solution is isoosmotic with serum.

Specific gravit

1.21

Viscosity (dynamic)

28.5 mPa s (28.5 cP) for 85% aqueous solution at 25°C.

Solubility

Miscible with ethanol (95%), ether, and water; practically insoluble in chloroform.

Stability and Storage Conditions

Lactic acid is hygroscopic and will form condensation products such as polylactic acids on contact with water. The equilibrium between the polylactic acids and lactic acid is dependent on concentration and temperature. At elevated temperatures lactic acid will form lactide, which is readily hydrolyzed back to lactic acid.
Lactic acid should be stored in a well-closed container in a cool, dry place.

Incompatibilities

Incompatible with oxidizing agents, iodides, and albumin. Reacts violently with hydrofluoric acid and nitric acid.

Safety

Lactic acid occurs in appreciable quantities in the body as an end product of the anaerobic metabolism of carbohydrates and, while harmful in the concentrated form, can be considered non-toxic at the levels at which it is used as an excipient. A 1% v/v solution, for example, is harmless when applied to the skin.
There is evidence that neonates have difficulty in metabolizing (R)-lactic acid and this isomer and the racemate should therefore not be used in foods intended for infants aged less than 3 months old.
There is no evidence that lactic acid is carcinogenic, teratogenic, or mutagenic.
LD50 (guinea pig, oral): 1.81 g/kg
LD50 (mouse, oral): 4.88 g/kg
LD50 (mouse, SC): 4.5 g/kg
LD50 (rat, oral): 3.73 g/kg

Handling Precautions

Lactic acid is caustic in concentrated form and can cause burns on contact with the skin and eyes. It is harmful if swallowed, inhaled, or absorbed through the skin. Observe precautions appropriate to the circumstances and quantity of material handled. Eye protection, rubber gloves, and respirator are recommended. It is advisable to handle the compound in a chemical fume hood and to avoid repeated or prolonged exposure. Spillages should be diluted with copious quantities of water. In case of excessive inhalation, remove the patient to a well-ventilated environment and seek medical attention. Lactic acid presents no fire or explosion hazard but emits acrid smoke and fumes when heated to decomposition.

Regulatory Status

GRAS listed. Accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (IM, IV, and SC injections, oral syrups and tablets, topical and vaginal preparations). Included in medicines licensed in the UK.

Related Substances

Aliphatic polyesters.

Citric Acid

Non-proprietary Names

BP: Citric acid monohydrate
JP: Citric acid
PhEur: Acidum citricum monohydricum
USP: Citric acid

Synonyms

E330; 2-hydroxypropane-1,2,3-tricarboxylic acid monohydrate.

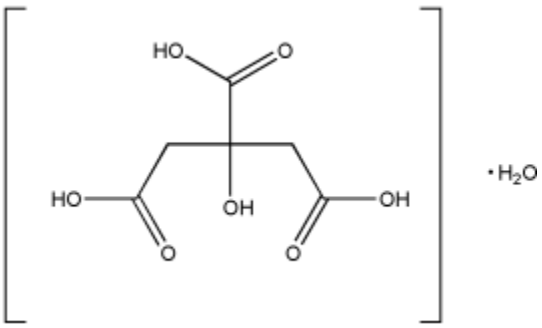
Chemical Name

2-Hydroxy-1,2,3-propanetricarboxylic acid monohydrate

Molecular Formula and Molecular Weight

C6H8O7˙H2O
Mw= 210.14 g/mol

Structural Formula



Functional Category

Acidifying agent; antioxidant; buffering agent; chelating agent; flavour enhancer.

Description

Citric acid monohydrate occurs as colourless or translucent crystals, or as a white crystalline, efflorescent powder. It is odorless and has a strong acidic taste. The crystal structure is orthorhombic.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for citric acid monohydrate (and anhydrous).

Test	JP 2001	PhEur 2002	USP 26(a)
Identification	+	+	+
Characters	—	+	—
Appearance of solution	—	+	—

Water			
(hydrous form)	—	7.5–9.0%	≤8.8%
(anhydrous form)	≤0.5%	≤1.0%	≤0.5%
Bacterial endotoxins	—	+	—
Residue on ignition	≤0.1%	—	≤0.05%
Sulfated ash	—	≤0.1%	—
Calcium	+	—	—
Aluminium	—	≤0.2 ppm	—
Oxalate	+	—	+
Oxalic acid	—	≤350 ppm	—
Sulphate	≤0.048%	≤150 ppm	+
Arsenic	≤1 ppm	—	≤3 ppm
Heavy metals	≤10 ppm	≤10 ppm	≤0.001%
Related substances	+	—	—
Readily carbonizable substances	+	+	+
Polycyclic aromatic hydrocarbon	+	—	—
Organic volatile impurities	—	—	+
Assay (anhydrous basis)	≥99.5%	99.5–101.0%	99.5–100.5%

Typical Properties

Acidity/alkalinity

pH = 2.2 (1% w/v aqueous solution)

Density

1.542 g/cm3

Hygroscopicity

At relative humidities less than about 65%, citric acid monohydrate effloresces at 25°C, the anhydrous acid being formed at relative humidities less than about 40%. At relative humidities between about 65% and 75%, citric acid monohydrate absorbs insignificant amounts of moisture, but under more humid conditions substantial amounts of water are absorbed.

Melting point

≈100°C (softens at 75°C)
Particle size distribution

Various grades of citric acid monohydrate with different particle sizes are commercially available.

Solubility

Soluble 1 in 1.5 parts of ethanol (95%) and 1 in less than 1 part of water; sparingly soluble in ether.

Viscosity (dynamic)

6.5 mPa s (6.5 cP) for a 50% w/v aqueous solution at 25°C.

Stability and Storage Conditions

Citric acid monohydrate loses water of crystallization in dry air or when heated to about 40°C. It is slightly deliquescent in moist air. Dilute aqueous solutions of citric acid may ferment on standing.
The bulk monohydrate or anhydrous material should be stored in airtight containers in a cool, dry place.
Incompatibilities
Citric acid is incompatible with potassium tartrate, alkali and alkaline earth carbonates and bicarbonates, acetates, and sulfides. Incompatibilities also include oxidizing agents, bases, reducing agents, and nitrates. It is potentially explosive in combination with metal nitrates. On storage, sucrose may crystallize from syrups in the presence of citric acid.

Safety

Citric acid is found naturally in the body, mainly in the bones, and is commonly consumed as part of a normal diet. Orally ingested citric acid is absorbed and is generally regarded as a non-toxic material when used as an excipient. However, excessive or frequent consumption of citric acid has been associated with erosion of the teeth.
Citric acid and citrates also enhance intestinal aluminium absorption in renal patients, which may lead to increased, harmful serum aluminium levels. It has therefore been suggested that patients with renal failure taking aluminium compounds to control phosphate absorption should not be prescribed citric acid or citrate-containing products.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and gloves are recommended. Direct contact with eyes can cause serious damage. Citric acid should be handled in a well-ventilated environment or a dust mask should be worn.

Regulatory Status

GRAS listed. The anhydrous form is accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (inhalations; IM, IV, and other injections; ophthalmic preparations; oral capsules, solutions, suspensions and tablets; topical and vaginal preparations). Included in nonparenteral and parenteral medicines licensed in Japan and the UK.

Related Substances

Anhydrous citric acid; fumaric acid; malic acid; sodium citrate dihydrate; tartaric acid.

Vitamin E

Non-proprietary Names

BP: Alpha tocopherol
JP: Tocopherol
PhEur: α-Tocopherolum
USP: Vitamin E

Synonyms

Copherol F1300; (±)-3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-ol; E307; synthetic alpha tocopherol; all-rac-α-tocopherol; dl-α-tocopherol; 5,7,8-trimethyltocol.

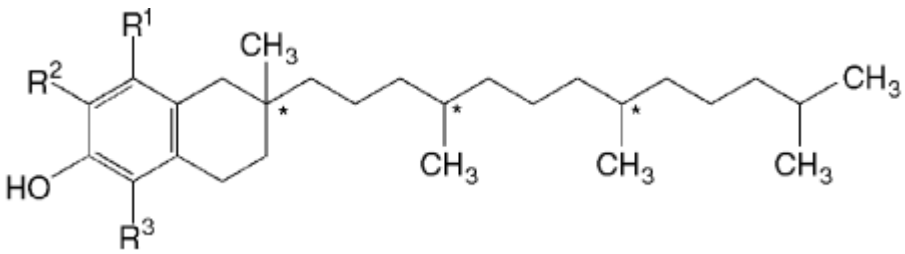
Chemical Name

(±)-(2RS,4′RS,8′RS)-2,5,7,8-Tetramethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol
Note that alpha tocopherol has three chiral centres, giving rise to eight isomeric forms. The naturally occurring form is known as d-alpha tocopherol or (2R,4′R,8′R)-alpha-tocopherol. The synthetic form, dl-alpha tocopherol or simply alpha tocopherol, occurs as a racemic mixture containing equimolar quantities of all the isomers.
Similar considerations apply to beta, delta, and gamma tocopherol and tocopherol esters.

Molecular Formula and Molecular Weight

$C_{29}H_{5}0O_{2}$
Mw= 430.72 g/mol

Structural Formula



Alpha tocopherol: R1 = R2 = R3 = CH3
Beta tocopherol: R1 = R3 = CH3; R2 = H
Delta tocopherol: R1 = CH3; R2 = R3 = H
Gamma tocopherol: R1 = R2 = CH3; R3 = H
Indicates chiral centers.

Functional Category

Antioxidant; therapeutic agent.

Description

Alpha tocopherol is a natural product. Therefore, it is available either as a practically odorless, clear, colourless, yellow, yellowish-brown, or greenish-yellow viscous oil.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for alpha tocopherol.

Test	JP 2001	PhEur 2002	USP 26
Identification	+	+	+
Acidity	—	—	+
Acid value	—	≤2	—
Optical rotation	—	–0.01° to +0.01°	+
Heavy metals	≤20 ppm	≤20 ppm	—
Sulfated ash	—	≤0.1%	—
Organic volatile impurities	—	—	+
Absorbance	+	+	—
at 255 nm	—	6.0–8.0	—
at 292 nm	—	72.0–76.0	—
Refractive index	1.503–1.507	—	—
Specific gravity	0.947–0.955	—	—
Clarity and colour	+	—	—
of solution			
Assay	96.0–102.0%	96.0–102.0%	96.0–102.0%

Note that the USP 26 describes vitamin E as comprising d- or dl-alpha tocopherol, d- or dl-alpha tocopheryl acetate, or d- or dl-alpha tocopheryl acid succinate. However, the PhEur 2002 describes alpha tocopherol and alpha tocopheryl acetate in separate monographs.
The diversity of the tocopherols described in the various pharmacopeial monographs makes the comparison of specifications more complicated..

Typical Properties

Boiling point

235°C

Density
0.947–0.951 g/cm3

Refractive index

n20D = 1.503–1.507

Solubility

Practically insoluble in water; freely soluble in acetone, ethanol, ether, and vegetable oils.
Stability and Storage Conditions
Tocopherols are oxidized slowly by atmospheric oxygen and rapidly by ferric and silver salts. Oxidation products include tocopheroxide, tocopherylquinone, and tocopherylhydroquinone, as well as dimers and trimers. Tocopherol esters are more stable to oxidation than the free tocopherols but are in consequence less effective antioxidants.
Tocopherols should be stored under an inert gas, in an airtight container in a cool, dry place and protected from light.

Incompatibilities

Tocopherols are incompatible with peroxides and metal ions, especially iron, copper, and silver. Tocopherols may be absorbed into plastic.

Safety

Tocopherols (vitamin E) occur in many food substances that are consumed as part of the normal diet. The daily nutritional requirement has not been clearly defined but is estimated to be 3.0–20.0 mg. Absorption from the gastrointestinal tract is dependent upon normal pancreatic function and the presence of bile. Tocopherols are widely distributed throughout the body, with some ingested tocopherol metabolized in the liver; excretion of metabolites is via the urine or bile. Individuals with vitamin E deficiency are usually treated by oral administration of tocopherols, although intramuscular and intravenous administration may sometimes be used.
Tocopherols are well tolerated, although excessive oral intake may cause headache, fatigue, weakness, digestive disturbance, and nausea. Prolonged and intensive skin contact may lead to erythema and contact dermatitis.
The use of tocopherols as antioxidants in pharmaceuticals and food products is unlikely to pose any hazard to human health since the daily intake from such uses is small compared to the intake of naturally occurring tocopherols in the diet.
The WHO has set an acceptable daily intake of tocopherol used as an antioxidant at 0.15–2.0 mg/kg body-weight.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Gloves and eye protection are recommended.

Regulatory Status

GRAS listed. Accepted in Europe as a food additive. Included in the FDA Inactive Ingredients Guide (oral capsules, tablets, and topical preparations). Included in nonparenteral medicines licensed in the UK.

Related Substances

d-Alpha tocopherol; d-alpha tocopheryl acetate; dl-alpha tocopheryl acetate; d-alpha tocopheryl acid succinate; dl-alpha tocopheryl acid succinate; beta tocopherol; delta tocopherol; gamma tocopherol; tocopherols excipient.

Calcium Carbonate

Non-proprietary Names

BP: Calcium carbonate
JP: Precipitated calcium carbonate
PhEur: Calcii carbonas
USP: Calcium carbonate

Synonyms

Barcroft; Cal-Carb; CalciPure; calcium carbonate (1 : 1); creta preparada; Destab; E170; MagGran CC; Millicarb; Pharma-Carb; Precarb; precipitated carbonate of lime; precipitated chalk; Sturcal; Vivapress Ca.

Chemical Name

Carbonic acid, calcium salt (1 : 1)

Molecular Formula and Molecular Weight

$CaCO_3$
Mw= 100.09 g/mol

Functional Category

Tablet and capsule diluents; therapeutic agent.

Description

Calcium carbonate occurs as an odorless and tasteless white powder or crystals.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for calcium carbonate.

Test	JP 2001	PhEur 2002	USP 26
Identification	+	+	+
Characters	—	+	—
Loss on drying	≤1.0%	≤2.0%	≤2.0%
Substances insoluble in acetic acid	≤0.2%	≤0.2%	≤0.2%
Fluoride	—	—	≤0.005%
Arsenic	≤5 ppm	≤4 ppm	≤3 ppm
Barium	+	+	+
Chlorides	—	≤330 ppm	—
Lead	—	—	≤3 ppm
Iron	—	≤200 ppm	≤0.1%

Heavy metals	≤20 ppm	≤20 ppm	≤0.002%
Magnesium and alkali (metals) salts	≤0.5%	≤1.5%	≤1.0%
Sulfates	—	≤0.25%	—
Mercury	—	—	≤0.5 μg/g
Organic volatile impurities	—	—	+
Assay (dried basis)	≥ 98.5%	98.5%–100.5%	98.0%–100.5%

Typical Properties

Acidity/alkalinity

pH = 9.0 (10% w/v aqueous dispersion)

Density (bulk)

0.8 g/cm3

Density (tapped)

1.2 g/cm3

Melting point

Decomposes at 825°C.

Solubility

Practically insoluble in ethanol (95%) and water. Solubility in water is increased by the presence of ammonium salts or carbon dioxide. The presence of alkali hydroxides reduces solubility.

Stability and Storage Conditions

Calcium carbonate is stable and should be stored in a well-closed container in a cool, dry place.

Incompatibilities

Incompatible with acids and ammonium salts.

Safety

Calcium carbonate is mainly used in oral pharmaceutical formulations and is generally regarded as a non-toxic material. However, calcium carbonate administered orally may cause constipation and flatulence. Consumption of large quantities (4–60 g daily) may also result in hypercalcemia or renal impairment. Therapeutically, oral doses of up to about 1.5 g are employed as an antacid. In the treatment of hyperphosphatemia in patients with chronic renal failure, oral daily doses of 2.5–17 g

have been used. Calcium carbonate may interfere with the absorption of other drugs from the gastrointestinal tract if administered concomitantly.
LD50 (rat: oral) 6.45 g/kg

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Calcium carbonate may be irritant to the eyes and on inhalation. Eye protection, gloves, and a dust mask are recommended. Calcium carbonate should be handled in a well-ventilated environment. In the UK, the long-term (8-hour TWA) occupational exposure limit for calcium carbonate is 10 mg/m3 for total inhalable dust and 4 mg/m3 for respirable dust.

Regulatory Status

GRAS listed. Accepted for use as a food additive in Europe. Included in FDA Inactive Ingredients Guide (oral capsules and tablets; otic solutions). Included in nonparenteral medicines licensed in the UK.

Hydroxyethylmethyl Cellulose

Non-proprietary Names

BP: Hydroxyethylmethylcellulose
PhEur: Methylhydroxyethylcellulosum

Synonyms

Cellulose, 2-hydroxyethyl methyl ester; Culminal MHEC; HEMC; hydroxyethyl methylcellulose; hymetellose; MHEC; methylhydroxyethylcellulose; Tylopur MH; Tylopur MHB; Tylose MB; Tylose MH; Tylose MHB.

Chemical Name

Hydroxyethylmethylcellulose

Molecular Formula and Molecular Weight

The PhEur 2002 describes hydroxyethylmethyl cellulose as a partly O-methylated and O-(2-hydroxyethylated) cellulose. Various different grades are available, which are distinguished by appending a number indicative of the apparent viscosity in millipascal seconds (mPa s) of a 2% w/v solution measured at 20°C.

Description

A white, yellowish-white or greyish-white powder or granules, hygroscopic after drying.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for hydroxyethylmethyl cellulose.

Test	PhEur 2002
Identification	+
Appearance of solution	+
pH	5.5–8.0
Apparent viscosity	+
Chlorides	≤0.5%
Heavy metals	≤20 ppm
Loss on drying	≤10.0%
Sulfated ash	≤1.0%

Typical Properties

Acidity/alkalinity

pH = 5.5–8.0 (2% w/v aqueous solution)

Moisture content

≤10%

Solubility

Hydroxyethylmethyl cellulose is practically insoluble in hot water (above 60°C), acetone, ethanol, ether, and toluene. It dissolves in cold water to form a colloidal solution.

Viscosity (dynamic)

22–30 mPa s (22–30 cP) for a 2% w/v aqueous solution at 20°C.

Stability and Storage Conditions

Hydroxyethylmethyl cellulose is hygroscopic and should therefore be stored under dry conditions away from heat.

Incompatibilities

None

Safety

Hydroxyethylmethyl cellulose is used as an excipient in various oral and topical pharmaceutical preparations and is generally regarded as an essentially non-toxic and non-irritant material.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of the material handled. Eye protection and gloves are recommended.

Regulatory Status

GRAS listed. Included in nonparenteral medicines licensed in Europe (oral suspensions, tablets, and topical preparations).

Related Substances

Ethylcellulose; hydroxyethyl cellulose; hypromellose; methylcellulose.

Carboxymethylcellulose Sodium

Non-proprietary Names

BP: Carmellose sodium
JP: Carmellose sodium
PhEur: Carmellosum natricum
USP: Carboxymethylcellulose sodium

Synonyms

Akucell; Aquasorb; Blanose; cellulose gum; CMC sodium; E466; Finnfix; Nymcel; SCMC; sodium carboxymethylcellulose; sodium cellulose glycolate; sodium CMC; Tylose CB.

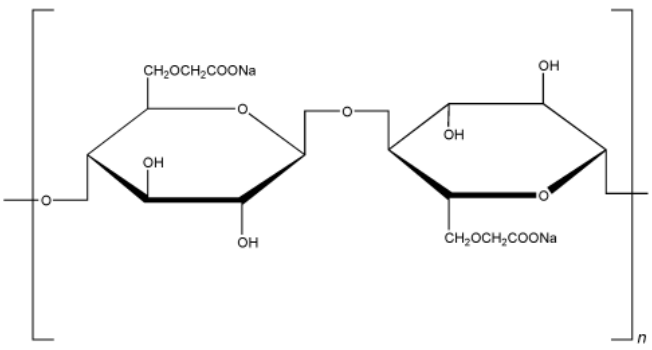
Chemical Name

Cellulose, carboxymethyl ether, sodium salt.

Molecular Weight

The USP 26 describes carboxymethylcellulose sodium as the sodium salt of a polycarboxymethyl ether of cellulose. Typical molecular weight is 90 000–700 000.

Structural Formula



Functional Category

Coating agent; tablet and capsule disintegrate; tablet binder; stabilizing agent; suspending agent; viscosity-increasing agent; water-absorbing agent.

Description

Carboxymethylcellulose sodium occurs as a white to almost white, odorless, granular powder.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for carboxymethylcellulose sodium.

Test	JP 2001	PhEur 2002	USP 26
Identification	+	+	+
Characters	—	+	—
pH (1% w/v solution)	6.0–8.0	6.0–8.0	6.5–8.5
Appearance of solution	+	+	—
Viscosity	+	+	+
Loss on drying	≤10.0%	≤10.0%	≤10.0%
Heavy metals	≤20 ppm	≤20 ppm	≤20 μg/g
Chloride	≤0.64%	≤0.25%	—
Arsenic	≤10 ppm	—	—
Sulphate	≤0.96%	—	—
Silicate	≤0.5%	—	—
Sodium glycolate	—	≤0.4%	—
Starch	+	—	—
Sulfated ash	—	20.0–33.3%	—
Organic volatile impurities	—	—	+
Assay (of sodium)	6.5–8.5%	6.5–10.8%	6.5–9.5%

Typical Properties

Density (bulk)

0.52 g/cm3

Density (tapped)

0.78 g/cm3

Dissociation constant:
pKa = 4.30

Melting point

Browns at approximately 227°C, and chars at approximately 252°C.

Moisture content

Typically contains less than 10% water. However, carboxymethylcellulose sodium is hygroscopic and absorbs significant amounts of water at temperatures up to 37°C at relative humidities of about 80.

Solubility

Practically insoluble in acetone, ethanol, ether, and toluene. Easily dispersed in water at all temperatures, forming clear, colloidal solutions. The aqueous solubility varies with the degree of substitution (DS).

Viscosity

Various grades of carboxymethylcellulose sodium are commercially available that have differing aqueous viscosities. Aqueous 1% w/v solutions with viscosities of 5–13 000 mPa s (5–13 000 cP) may be obtained. An increase in concentration results in an increase in aqueous solution viscosity. Prolonged heating at high temperatures will depolymerise the gum and permanently decrease the viscosity. The viscosity of sodium carboxymethylcellulose solutions is fairly stable over a pH range of 4–10. The optimum pH range is neutral.

Stability and Storage Conditions

Carboxymethylcellulose sodium is a stable, though hygroscopic material. Under high-humidity conditions, carboxymethylcellulose sodium can absorb a large quantity (>50%) of water. In tablets, this has been associated with a decrease in tablet hardness and an increase in disintegration time.
Aqueous solutions are stable at pH 2–10; precipitation can occur below pH 2, and solution viscosity decreases rapidly above pH 10. Generally, solutions exhibit maximum viscosity and stability at pH 7–9.
Carboxymethylcellulose sodium may be sterilized in the dry state by maintaining it at a temperature of 160°C for 1 hour. However, this process results in a significant decrease in viscosity and some deterioration in the properties of solutions prepared from the sterilized material.
Aqueous solutions may similarly be sterilized by heating, although this also results in some reduction in viscosity. After autoclaving, viscosity is reduced by about 25%, but this reduction is less marked than for solutions prepared from material sterilized in the dry state. The extent of the reduction is dependent on the molecular weight and degree of substitution; higher molecular weight grades generally undergo a greater percentage reduction in viscosity. Sterilization of solutions by gamma irradiation also results in a reduction in viscosity.
Aqueous solutions stored for prolonged periods should contain an antimicrobial preservative.
The bulk material should be stored in a well-closed container in a cool, dry place.

Incompatibilities

Carboxymethylcellulose sodium is incompatible with strongly acidic solutions and with the soluble salts of iron and some other metals, such as aluminium, mercury, and zinc. Precipitation may occur at pH <2, and also when it is mixed with ethanol (95%).
Carboxymethylcellulose sodium forms complex coacervates with gelatin and pectin. It also forms a complex with collagen and is capable of precipitating certain positively charged proteins.

Safety

Carboxymethylcellulose sodium is used in oral, topical, and some parenteral formulations. It is also widely used in cosmetics, toiletries, and food products, and is generally regarded as a non-toxic and non-irritant material. However, oral consumption of large amounts of carboxymethylcellulose sodium can have a laxative effect; therapeutically, 4–10 g in daily divided doses of the medium- and high-viscosity grades of carboxymethylcellulose sodium have been used as bulk laxatives.
The WHO has not specified an acceptable daily intake for carboxymethylcellulose sodium as a food additive since the levels necessary to achieve a desired effect were not considered to be a hazard to health. However, in animal studies, subcutaneous administration of carboxymethylcellulose sodium has been found to cause inflammation, and in some cases of repeated injection fibrosarcomas have been found at the site of injection.
Hypersensitivity and anaphylactic reactions have occurred in cattle and horses, which have been attributed to carboxymethylcellulose sodium in parenteral formulations such as vaccines and penicillins.
LD50 (guinea pig, oral): 16 g/kg
LD50 (rat, oral): 27 g/kg

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Carboxymethylcellulose sodium may be irritant to the eyes. Eye protection is recommended.

Regulatory Status

GRAS listed. Accepted as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (dental preparations; inhalations; intra-articular, intrabursal, intradermal, intralesional, IM, intrasynovial and SC injections; oral capsules, drops, solutions, suspensions, syrups and tablets; topical and vaginal preparations). Included in nonparenteral medicines licensed in the UK.

Related Substances

Carboxymethylcellulose calcium.

Sodium Chloride

Non-proprietary Names

BP: Sodium chloride
JP: Sodium chloride
PhEur: Natrii chloridum
USP: Sodium chloride

Synonyms

Alberger; chlorure de sodium; common salt; dendritis; hopper salt; murinate of sada; natural halite; rock salt; saline; salt; sea salt; table salt.

Chemical Name

Sodium chloride

Molecular Formula and Molecular Weight

NaCl
Mw = 58.44 g/mol

Functional Category

Tablet and capsule diluents; tonicity agent.

Description

Sodium chloride occurs as a white crystalline powder or colourless crystals; it has a saline taste. The crystal lattice is a face-centered cubic structure. Solid sodium chloride contains no water of crystallization although, below 0 °C, salt may crystallize as a dihydrate.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for sodium chloride.

Test	JP 2001	PhEur 2002 (Suppl. 4.6)	USP 26
Identification	+	+	+
Appearance of solution	+	+	+
Acidity or alkalinity	pH 4.5–7.0	+	+
Loss on drying	≤0.5%	≤0.5%	0.5%
Arsenic	≤2 ppm	≤1 ppm	1 μg/g
Bromides	+	≤100 ppm	≤0.01%
Barium	+	+	+
Nitrites	—	+	+
Aluminium	—	≤0.2 ppm(a)	≤0.2 μg/g(a)
Calcium and magnesium	+	—	—

Magnesium and alkaline earth metals	—	≤100 ppm	≤0.01%
Iodide	+	+	+
Iron	—	≤2 ppm	≤2 μg/g
Sulphate	—	≤200 ppm	≤0.02%
Ferrocyanides	—	+	+
Heavy metals	≤3 ppm	≤5 ppm	≤5 ppm
Phosphate	—	≤25 ppm	≤0.0025%
Potassium	—	≤500 ppm(a)(b)	≤0.05%(a)(b)
Organic volatile impurities	—	—	—
Bacterial endotoxins	—	≤5 IU/g(b)	—
Assay (dried basis)	≥99.5%	99.0–100.5%	99.5–100.5%

(a) If for use in peritoneal dialysis, hemodialysis or hemofiltration solutions.
(b) If for parenteral use.

Typical Properties

Acidity/alkalinity

pH = 6.7–7.3 (saturated aqueous solution)

Boiling point

1413 °C

Density

2.17 g/cm3
1.20 g/cm3 for saturated aqueous solution

Density (bulk)

0.93 g/cm3

Density (tapped)

1.09 g/cm3

Hygroscopicity

Hygroscopic above 75% relative humidity.

Melting point

804 °C

Osmolarity

A 0.9% w/v aqueous solution is iso-osmotic with serum.

Solubility

Table 2: Solubility of sodium chloride.

Solvent	Solubility at 20 °C unless otherwise stated
Ethanol	Slightly soluble
Ethanol (95%)	1 in 250
Glycerin	1 in 10
Water	1 in 2.8
	1 in 2.6 at 100 °C

Viscosity

A 10% w/v solution has a viscosity of 1.19 mPa s (1.19 cP).

Stability and Storage Conditions

Aqueous sodium chloride solutions are stable but may cause the separation of glass particles from certain types of glass containers. Aqueous solutions may be sterilized by autoclaving or filtration. The solid material is stable and should be stored in a well-closed container, in a cool, dry place.
It has been shown that the compaction characteristics and the mechanical properties of tablets are influenced by the relative humidity of the storage conditions under which sodium chloride was stored.

Incompatibilities

Aqueous sodium chloride solutions are corrosive to iron. They also react to form precipitates with silver, lead, and mercury salts. Strong oxidizing agents liberate chlorine from acidified solutions of sodium chloride. The solubility of the antimicrobial preservative methylparaben is decreased in aqueous sodium chloride solutions and the viscosity of carbomer gels and solutions of hydroxyethyl cellulose or hydroxypropyl cellulose is reduced by the addition of sodium chloride.

Safety

Sodium chloride is the most important salt in the body for maintaining the osmotic tension of blood and tissues. About 5–12 g of sodium chloride is consumed daily, in the normal adult diet, and a corresponding amount is excreted in the urine. As an excipient, sodium chloride may be regarded as an essentially nontoxic and nonirritant material. However, toxic effects following the oral ingestion of 0.5–1.0 g/kg body-weight in adults may occur. The oral ingestion of larger quantities of sodium chloride, e.g., 1000 g in 600 mL of water, is harmful and can induce irritation of the gastrointestinal tract, vomiting, hypernatremia, respiratory distress, convulsions, or death.
In rats, the minimum lethal intravenous dose is 2.5 g/kg body-weight.

LD50 (mouse, IP): 6.61 g/kg
LD50 (mouse, IV): 0.65 g/kg
LD50 (mouse, oral): 4.0 g/kg
LD50 (mouse, SC): 3.0 g/kg
LD50 (rat, oral): 3.0 g/kg

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. If heated to high temperatures, sodium chloride evolves a vapour irritating to the eyes.

Regulatory Status

GRAS listed. Included in the FDA Inactive Ingredients Guide (injections; inhalations; nasal, ophthalmic, oral, otic, rectal, and topical preparations). Included in nonparenteral and parenteral medicines licensed in the UK.

Related Substances

Potassium chloride.

A saturated solution of sodium chloride can be used as a constant-humidity solution; at 25 °C, a relative humidity of 75% is produced. A specification for sodium chloride is contained in the Food Chemicals Codex (FCC).

Saccharin Sodium

Nonproprietary Names

BP: Saccharin sodium
JP: Saccharin sodium
PhEur: Saccharinum natricum
USP: Saccharin sodium

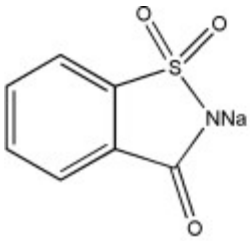
Synonyms

1,2-Benzisothiazolin-3-one 1,1-dioxide, sodium salt; Crystallose; E954; sodium o-benzosulfimide; soluble gluside; soluble saccharin; Sucaryl Sodium.
Chemical Name
1,2-Benzisothiazol-3(2H)-one 1,1-dioxide

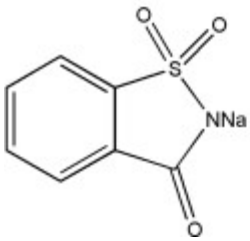
Molecular formula and Molecular Weight

$C_7H_4NNaO_3S$
Mw = 205.16 g/mol
$C_7H_4NNaO_3S \cdot ⅔H2O$
Mw = (84%) 217.24 g/mol
$C_7H_4NNaO_3S \cdot 2H_2O$ (76%)
Mw = 241.19 g/mol

Structural Formula



$\cdot 2H_2O$ 76% saccharin sodium (dihydrate)



$\cdot {}^{2}/_{3}\, H_2O$ 84% saccharin sodium

Functional Category

Sweetening agent.

Description

Saccharin sodium occurs as a white, odorless or faintly aromatic, efflorescent, crystalline powder. It has an intensely sweet taste, with a metallic aftertaste that at normal levels of use can be detected by approximately 25% of the population. Saccharin sodium can contain variable amounts of water.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for saccharin sodium.

Test	JP 2001	PhEur 2002 (Suppl. 4.3)	USP 26
Identification	+	+	+
Characters	+	+	—
Clarity and colour of solution	+	+	—
Acidity or Alkalinity	—	+	+
Water	+	≤15.0%	≤15.0%
Benzoate and salicylate	+	—	+
Arsenic	≤2 ppm	—	—
Selenium	—	—	+

Acidity or alkalinity	+	+	+
Toluenesulfonamides	+	+	+
Heavy metals	≤20 ppm	≤20 ppm	≤0.001%
Readily carbonizable substances	+	—	+
Organic volatile impurities	—	—	+
Assay (anhydrous basis)	≥98.0%	99.0–101.0%	98.0–101.0%

Typical Properties

Unless stated, data refer to either 76% or 84% saccharin sodium.

Acidity/alkalinity

pH = 6.6 (10% w/v aqueous solution)

Density (bulk)

0.8–1.1 g/cm3 (76% saccharin sodium)
0.86 g/cm3 (84% saccharin sodium)
Density (particle)

1.70 g/cm3 (84% saccharin sodium)

Density (tapped)

0.9–1.2 g/cm3 (76% saccharin sodium)
0.96 g/cm3 (84% saccharin sodium)

Melting point

Decomposes upon heating.

Moisture content

Saccharin sodium 76% contains 14.5% w/w water; saccharin sodium 84% contains 5.5% w/w water. During drying, water evolution occurs in two distinct phases. The 76% material dries under ambient conditions to approximately 5.5% moisture (84% saccharin sodium); the remaining moisture is then removed only by heating.

Solubility

Table 2: Solubility of saccharin sodium.

Solvent	Solubility at 20°
Buffer solutions:	
pH 2.2 (phthalate)	1 in 1.15
	1 in 0.66 at 60°C
pH 4.0 (citrate–phosphate)	1 in 1.21
	1 in 0.69 at 60°C
pH 7.0 (citrate–phosphate)	1 in 1.21
	1 in 0.66 at 60°C
pH 9.0 (borate)	1 in 1.21
	1 in 0.69 at 60°C
Ethanol	1 in 102
Ethanol (95%)	1 in 50
Propylene glycol	1 in 3.5
Propan-2-ol	Practically insoluble
Water	1 in 1.2

Stability and Storage Conditions

Saccharin sodium is stable under the normal range of conditions employed in formulations. Only when it is exposed to a high temperature (125°C) at a low pH (pH 2) for over 1 hour does significant decomposition occur. The 84% grade is the most stable form of saccharin sodium since the 76% form will dry further under ambient conditions.

Saccharin sodium should be stored in a well-closed container in a cool, dry place.

Incompatibilities

none

Safety

There has been considerable controversy concerning the safety of saccharin and saccharin sodium in recent years; however, it is now generally regarded as a safe, intense sweetener. The WHO has set a temporary acceptable daily intake of up to 2.5 mg/kg body-weight for saccharin, including its salts. In the UK, the Committee on Toxicity of Chemicals in Food, Consumer Products, and the Environment (COT) has set an acceptable daily intake for saccharin and its salts (expressed as saccharin sodium) at up to 5 mg/kg body-weight.

LD50 (mouse, oral): 17.5 g/kg

LD50 (rat, IP): 7.1 g/kg

LD50 (rat, oral): 14.2 g/kg

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and a dust mask are recommended.

Regulatory Status

Accepted for use as a food additive in Europe ‘E954’ is applied to both saccharin and saccharin salts. Included in the FDA Inactive Ingredients Guide (buccal and dental preparations; IM and IV injections; oral and topical preparations). Included in nonparenteral medicines licensed in the UK.

Related Substances

Alitame, saccharin

Comments

The perceived intensity of sweeteners relative to sucrose depends upon their concentration, temperature of tasting, and pH, and on the flavour and texture of the product concerned.
Intense sweetening agents will not replace bulk, textural, or preservative characteristics of sugar if sugar is removed from a formulation.
Synergistic effects for combinations of sweeteners have been reported. Saccharin sodium is often used in combination with cyclamates and aspartame since the saccharin sodium content may be reduced to minimize any aftertaste.

Glucose, Liquid

Nonproprietary Names

BP: Liquid glucose
PhEur: Glucosum liquidum
USPNF: Liquid glucose

Synonyms

Corn syrup; Flolys; Glucomalt; glucose syrup; Glucosweet; Mylose; Roclys; starch syrup.

Chemical Name

Liquid glucose.

Functional Category

Coating agent; sweetening agent; tablet binder.
Description
Liquid glucose is an aqueous solution of several compounds, principally dextrose, dextrin, fructose, and maltose. It is a colourless, odorless, and viscous sweet-tasting liquid.
Liquid glucose is classified according to its dextrose equivalent (DE) into four types:

Type I: 20–38 DE
Type II: 38–58 DE
Type III: 58–73 DE
Type IV: >73 DE

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for liquid glucose.

Test	PhEur 2002 (Suppl. 4.6)	USPNF 21
Identification	+	+
Characters	+	—
Acidity	—	+
pH	4.0–6.0	—
Water	≤30.0%	≤21.0%
Residue on ignition	≤0.5 %	≤0.5%
Sulphur dioxide	≤20 ppm(a)	—
Dextrose equivalent	+	—
Sulphite	—	+
Heavy metals	≤10 ppm	≤0.001%
Starch	—	+
Organic volatile impurities	—	+
Assay (of dried matter)	≥70.0%	—

(a) Or ≤400 ppm if intended for the production of hard boiled candies, provided the final product contains ≤50 ppm.

Typical Properties

Density

1.43 g/cm3 at 20°C

Solubility

Miscible with water; partially miscible with ethanol (90%).

Viscosity (dynamic)

13.0–14.5 mPa s (13.0–14.5 cP) at 21°C.

Stability and Storage Conditions

Liquid glucose should be stored in a well-closed container in a cool, dry place.

Incompatibilities

Incompatible with strong oxidizing agents.

Safety

Liquid glucose is used in oral pharmaceutical formulations and confectionery products and is generally regarded as a nontoxic and nonirritant material. It may be consumed by diabetics.
LD50 (mouse, IV): 9 g/kg

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled.

Regulatory Status

The PhEur 2002 (Suppl. 4.6) also includes a specification for glucose, liquid, spray dried. Included in the FDA Inactive Ingredients Guide (oral solutions, syrups, and tablets; topical emulsions and gels). Included in nonparenteral medicines licensed in the UK.

Related Substances

Dextrin; dextrose; maltose.

Comments

A specification for glucose syrup is contained in the Food Chemicals Codex (FCC).

Fructose

Nonproprietary Names

BP: Fructose
JP: Fructose
PhEur: Fructosum
USP: Fructose

Synonyms

Advantose FS 95; Fructamyl; Fructofin; D-(–)-fructopyranose; β-D-fructose; fruit sugar; Krystar; laevulose; levulose.

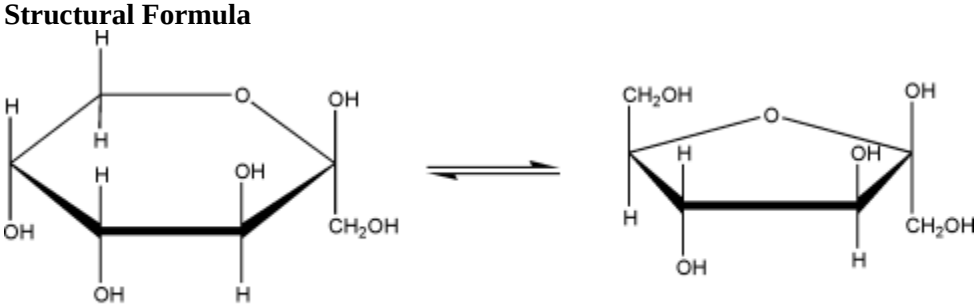
Chemical Name

D-Fructose

Molecular Formula and Molecular Weight

$C_6H_{12}O_6$
Mw = 180.16

Structural Formula



Pyranose form

Furanose form

Functional Category

Dissolution enhancer; flavour enhancer; sweetening agent; tablet diluent.

Description

Fructose occurs as odorless, colourless crystals or a white crystalline powder with a very sweet taste.

Pharmacopeial Specifications

Table 1: Pharmacopeial specifications for fructose.

Test	JP 2001	PhEur 2002	USP 26
Identification	+	+	+
Characters	—	+	—
Colour of solution	+	+	+
Acidity	+	+	+
pH	4.0–6.5	—	—
Specific optical rotation	—	–91.0° to –93.5°	—
Foreign sugars	—	+	—
Loss on drying	≤0.5%	—	≤0.5%
Residue on ignition	≤0.1%	≤0.1%	≤0.5%
Chloride	≤0.018%	—	≤0.018%
Sulphate	≤0.024%	—	≤0.025%
Sulphite	+	—	—
Water	—	≤0.5%	—
Arsenic	≤1.3 ppm	—	≤1 ppm

Barium	—	+	—
Calcium and magnesium (as calcium)	+	—	≤0.005%
Lead	—	≤0.5 ppm	—
Heavy metals	≤4 ppm	—	≤5 ppm
Hydroxymethylfurfural	+	+	+
Assay (dried basis)	≥98.0%	—	98.0–102.0%

Typical Properties

Acidity/alkalinity

pH = 5.35 (9% w/v aqueous solution)

Density

1.58 g/cm3

Hygroscopicity

At 25°C and relative humidities above approximately 60%, fructose absorbs significant amounts of moisture.

Melting point

≈102–105°C (with decomposition)

Osmolarity

a 5.05% w/v aqueous solution is isoosmotic with serum.

Particle size distribution

The average particle size of standard-grade crystalline fructose is 400 μm (Fructofin C, Xyrofin). Other grades are available that have an average particle size of 170 μm (Fructofin CM, Xyrofin). The average particle size of powdered fructose is 25–40 μm (Krystar, AE Staley Mfg Co). Other grades are available: e.g., Krystar 300 and Krystar 450 with average particle sizes of 300 μm and 450 μm, respectively.

Solubility

Table 2: Solubility of fructose.

Solvent	Solubility at 20°C
Ethanol (95%)	1 in 15
Methanol	1 in 14
Water	1 in 0.3

Viscosity (dynamic)

Table 3: Physical properties of aqueous fructose solutions at 20°C.

Concentration of aqueous fructose solution (% w/w)	Density (g/cm3)	Refractive index	Viscosity, dynamic (mPa s)
10	1.04	1.3477	1.35
20	1.08	1.3633	1.80
30	1.13	1.3804	2.90
40	1.18	1.3986	5.60
50	1.23	1.4393	34.0
60	1.29	1.4853	309.2

Stability and Storage Conditions

Fructose is hygroscopic and absorbs significant amounts of moisture at relative humidities greater than 60%. Goods stored in the original sealed packaging at temperatures below 25°C and a relative humidity of less than 60% can be expected to retain stability for at least 12 months.
Aqueous solutions are most stable at pH 3–4 and temperatures of 4–70°C; they may be sterilized by autoclaving.

Incompatibilities

Incompatible with strong acids or alkalis, forming a brown coloration. In the aldehyde form, fructose can react with amines, amino acids, peptides, and proteins. Fructose may cause browning of tablets containing amines.

Safety

Although it is absorbed more slowly than dextrose from the gastrointestinal tract, fructose is metabolized more rapidly. Metabolism of fructose occurs mainly in the liver, where it is converted partially to dextrose and the metabolites lactic acid and pyruvic acid. Entry into the liver and subsequent phosphorylation is insulin-independent. Further metabolism occurs by way of a variety of metabolic pathways. In healthy and well regulated diabetics, glycogenesis (glucose stored as glycogen) predominates.
Excessive oral fructose consumption (>75 g daily) in the absence of dietary dextrose in any form (e.g., sucrose, starch, dextrin, etc.) may cause malabsorption in susceptible individuals, which may result in flatulence, abdominal pain, and diarrhea. Except in patients with hereditary fructose intolerance there is no evidence to indicate that oral fructose intake at current levels is a risk factor in any particular disease, other than dental caries.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Fructose may be irritant to the eyes. Eye protection and gloves are recommended.

Regulatory Status

Included in the FDA Inactive Ingredients Guide (oral solutions and suspensions; rectal preparations).

Related Substances

Dextrose; high-fructose syrup; liquid fructose; milled fructose; powdered fructose; pyrogen-free fructose; sucrose.

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