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HYDROXYPROPYL-β-CYCLODEXTRIN/CICLESONIDE COMPLEXES FOR INHALATION POWDERS

KOMPLEKSI CIKLESONIDA S HIDROKSIPROPIL-BETA-CIKLODEKSTRINI ZA PRAŠKE ZA INHALIRANJE

LJUBLJANA, 2012

I, Tina Oberski, student of pharmacy at the University of Ljubljana, Faculty of Pharmacy performed the thesis research work within Erasmus mobility exchange program at the University of Lisbon, Faculty of Pharmacy, at the department of Pharmaceutical technology. Host supervisor was prof. Dr. Helena Cabral Marques and home supervisor was prof. Dr. Julijana Kristl. Formulations of hydroxypropyl-beta-cyclodextrin/ciclesonide complexes were made at the Faculty of Pharmacy in Lisbon, University of Lisbon. SEM photographs were obtained at the Faculty of Pharmacy in Ljubljana, University of Ljubljana. Molecular modelling was done by prof. Dr. Mire Zloh from London University.

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Statement

I declare that I made the thesis research work under the supervision of Prof. Dr. Julijana Kristl and Prof. Dr. Helena Cabral Marques.

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Contents

ABSTRACT

Asthma is a very common chronic disease. This pathology is treated with inhaled corticosteroids which are very effective. Nowadays, inhaled corticosteroid (ICS) drug such as ciclesonide (CIC) are focus of many researchers, because they showed promising results in clinical trials. The solubility of CIC in water is very low. Cyclodextrins (CDs) are capable of forming inclusion complexes with many drugs by taking up a whole drug molecule, or some part of it, into their hydrophobic cavity. Complexation of CIC with different cyclodextrins (γ-CD, HP-β-CD) was attempted in order to improve its solubility.

Molecular modelling was performed to help in the selection of the best CD for CIC. Higher phase solubility equilibrium constant of CIC with HP-β-CD than with γ-CD was determined. Therefore, the present study was concentrated on exploring HP-β-CD as a complexing agent. Solid HP-β-CD/CIC complexes were prepared by an aqueous precipitation method. The solubility of CIC in HP-β-CD complexes were significantly improved. In this thesis work, HP-β-CD/CIC complexes were characterized by infrared spectroscopy, differential scanning calorimetry, scanning electron microscopy and twin impinger.

In the infrared spectra of HP-β-CD/CIC sample characteristic peaks of free CIC were no longer visible and in DSC as well. The results from IR and DSC tests showed that some complexes are formed. Information of the HP-β-CD/CIC particles morphology was obtained by scanning electron microscopy (SEM). The aerodynamic form and particle size of 1-5 μm are the most significant factors for delivery of drugs to the lungs. Aerodynamic performances were carried out on twin impinger comparing the deposition of HP-β-CD/CIC and CIC adsorbed on α-lactose monohydrate. The inhaling device Rotahaler® was used for discharging the powder from the gelatine capsules at a flow rate of 60 ± 5 l/min. Respirable fraction and emitted dose were determined.

Formulations with HP-β-CD/CIC inclusion complexes did not exhibit better aerosol performances compared to CIC on lactose monohidrate.

Keywords: ciclesonide, hydroxypropyl-beta-cyclodextrin, complexation, solubility, twin impinger, powder for inhalation.

POVZETEK

Astma je kronična bolezen. Zdravijo jo z inhalacijskimi kortikosteroidi, ki so se izkazali za zelo učinkovite. Inhalacijski kortikosteroidi, kot je ciklesonid (CIC), so priljubljeni, saj so pokazali zelo dobre klinčne rezultate. CIC je skoraj netopen v vodi. Ciklodekstrini (CD) so spojine, ki tvorijo komplekse s številnimi zdravilnimi učinkovinami, z vključevanjem cele molekule ali samo dela v svojo hidrofobno votlino. V diplomski nalogi smo CIC kompleksirali z γ-CD in HP-β-CD, da bi izboljšali njegovo topnost.

Molekularno modeliranje naše učinkovine z različnimi ciklodekstrini smo uporabili, da bi lažje izbrali najprimernejšega. Pri HP-β-CD smo določili višjo ravnotežno konstanto topnosti kot pri γ-CD. Zato smo v nadaljnih poskusih raziskave usmerili samo na HP-β-CD kot kompleksirajoč reagent. Trdne HP-β-CD/CIC komplekse smo pripravili z metodo precipitacije kompleksa v vodi. Topnost CIC se je v HP-β-CD/CIC kompleksih signifikantno povečala .V tej nalogi smo ovrednotili HP-β-CD/CIC komplekse še z infrardečo spektroskopijo, diferenčo dinamično kalorimetrijo, vrstična elektronska mikroskopija in Twin Impingerjem.

V IR spektrih HP-β-CD/CIC kompleksov niso več opazni karakteristični piki za sam CIC, prav tako ne v DSC termogramih. Rezultati testov topnosti, IR in DSC so pokazali, da so nastali komplesi med CIC in CD. Informacije o HP-β-CD/CIC morfologiji kompleksov smo dobili z vrstično elektronsko mikroskopijo (SEM). Aerodinamična oblika in velikost delcev 1-5 μm sta najpomembnejša dejavnika za dostavljanje zdravil v pljuča. S twin impingerjem smo določili aerodinamične lastnosti praškov, in sicer smo primerjali porazdelitev HP-β-CD/CIC kompleksov in CIC adsorbiranega na α-laktozo monohidrat. Inhalator Rotahaler® smo uporabili za izmet praškov iz želatinskih kapsul s pretokom 60 ± 5 l/min. Vsaki formulaciji smo določili procent delcev iz inhalatorja za suhe praške.

Izdelan HP-β-CD/CIC kompleks ni pokazal boljše porazdelitve v primerjavi z adsorbiranim CIC na α-laktozo monohidratu.

Ključne besede: ciklesonid, hidroksipropil-beta-ciklodestrin, kompleksiranje, topnost, twin impinge, praški za inhaliranje.

LIST OF ABBREVIATONS

CD	cyclodextrin
α-CD	alpha cyclodextrin
β-CD	beta cyclodextrin
BDP	beclomethasone dipropionate
BUD	budesonide
γ-CD	gamma cyclodextrin
CFC	chlorofluorocarbons
CGT-ase	cyclodextringlucosyltransferase
CIC	ciclesonide
COPD	chronic obstructive pulmonary disease
CYP3A4	Cytochrome P_{450} 3A4
DES-CIC	desisobutyryl-ciclesonide
DPIs	dry powder inhalers
DSC	differential scanning calorimetry
FT-IR	Fourier transformed infrared spectroscopy
FP	fluticasone propionate
G2-β-CD	maltosyl-β-CD
HP-β-CD	hydroxypropyl-β-cyclodextrin
HFA	hydrofluoroalkane
HP-γ-CD	hydroxypropyl-γ-cyclodextrin
ICS	inhaled corticosteroids
IgE	Immunoglobulin E
IL_2	interleukin 2
IL_3	interleukin 3
IL_4	interleukin 4
IL_5	interleukin 5
IL_{13}	interleukin 13
KBr	potassium bromide

K_S	stability constant
LABA	long-acting-beta-agonist
MCT-β-CD	monochlorotriaziny beta-cyclodextrin
MDIs or pMDIs	metered dose inhalers
MF	mometasone furoate
RFE	respirable fraction of the emitted dose
RFN	respirable fraction of the nominal dose
RM-β-CD	randomly methylated β-CD
RRA	relative glucocorticoid-receptor affinity
S_O	solubility
SBE	sulfobutylether β-CD
SEM	scanning electron microscopy
TA	triamcinolone acetonide
Th	T helper cell
TSLI or TI	twin impinger
UV	ultraviolet

1 INTRODUCTION

Asthma is the most common chronic respiratory disease in childhood. The prevalence of asthma has increased in developed countries. Approximately 300 million people suffer from this disease worldwide, although differences exist between countries and its prevalence is rising. This pathology decreases the quality of life of the patient. Inhaled corticosteroids (ICS) are the most effective anti-inflammatory drugs currently available for persistent asthma (1). The therapeutic effect of these agents depends on the degree of pulmonary deposition and affinity for glucocorticoid receptors. ICS can be deposited in the oropharyngeal cavity and potentially can lead to local complications. Furthermore, they may be swallowed and absorbed into the systemic circulation. Therefore, the aerodynamic properties of drug substances are the most important factor to deliver the drug into the lungs (2).

1.1 RESPIRATORY SYSTEM

The human respiratory system is a composed of several organs used to carry air into and out of the lungs. The system consists of two regions: the conducting airway and the respiratory region. The airway is further divided into many folds: nasal cavity and the associated sinuses, the nasopharynx, oropharynx, larynx, trachea, bronchi, bronchioles. There is no gas exchange, only conduct air to and from the lungs. In conducting airway region obstructive diseases such as asthma and chronic obstructive pulmonary disease (COPD) manifest. Gas exchange takes place in the respiratory region, which consists of respiratory bronchioles, alveolar ducts, and alveolar sacs (3).

1.1.1 LUNG

The principal physiological function of the lung is to distribute inspired air and pulmonary blood to ensure efficient gas exchange that is oxygenation of the blood and removal of carbon dioxide from the body.The lungs have several properties: large surface area for absorption (~100 m^2), very thin absorption membrane (0.1 - 0.2 μm), high blood flow (5 l/min) and possibility to avoid first-pass metabolism. Due to this, they rapidly distribute molecules through the body. Nearly 95 % of the alveolar cells are Type I cells (5 μm), the

rest are Type II cells (10-15 μm) and secrete surfactants which are important for the function of the lungs. The main phospholipids of lung surfactants are phosphatidylcholine and phosphatidylglycerol. They deposit a monomolecular film on the alveoli, prevent pulmonary edema and provide protection against infections (3).

	Generation		Diameter (cm)	Length (cm)	Number	Total cross sectional area (cm^2)
Conducting zone	trachea	0	1.08	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
	terminal bronchioles	5 ↓ 16	0.35 ↓ 0.06	1.07 ↓ 0.17	32 ↓ $6\text{-}10^4$	3.11 ↓ 180.0
Transitional and respiratory zones	respiratory bronchioles	17 ↓ 19	↓ 0.05	↓ 0.10	↓ $5\text{-}10^5$	↓ 10^3
	alveolar ducts	20 ↓ 22	↓	↓	↓	↓
	alveolar sacs	23	0.04	0.05	$8\text{-}10^6$	10^4

Figure 1. Structure of the human respiratory tract according to the model of Weibl (4).

1.1.2 PULMONARY DRUG DELIVERY

Local pulmonary delivery is an important research area which includes the treatment of illnesses including asthma, cystic fibrosis, COPD or lung cancer. This type of drug application in the therapy of these diseases is also called a targeted drug delivery. Drugs are absorbed from the lungs mainly by passive diffusion. The drug absorption in the lung is effective because of its large surface area, good blood supply and low enzymatic activity.

Pulmonary drug administration has several advantages over other routes of drug administration: provides local action within the respiratory tract; enables rapid drug action; allows reduced dose; allows for a reduction in systemic side-effects; reduces extracellular enzyme levels compared to gastrointestinal tract due to the large alveolar surface area; reduces evasion of first pass hepatic metabolism by absorbed drug. On the other hand, there are some limitations in the use of the pulmonary delivery, such as short duration of activity due to the rapid removal of the drug and frequent dosing is necessary (5). Drugs

which are delivered via pulmonary route are for example beta agonists, anticholinergic drugs, mucolytics and anti-inflammatory drugs (3).

1.1.3 AEROSOLS

Aerosol preparations are stable dispersions or suspensions of solid material or liquid droplets in a gaseous medium. The drug delivered by aerosols is deposited in the airways by: gravitational sedimentation, inertial impaction, and diffusion. Mostly larger drug particles are deposited by first two mechanisms in the airways, while the smaller particles get their way into the peripheral region of the lungs by following diffusion. A number of pharmaceutical products are found in the form of aerosols. There are also aerosols used for the application on the skin or into available body apertures. Antiseptics, antimycotics, local anaesthetics and corticosteroids are usually delivered in the form of aerosols (6).

1.1.4 FACTORS AFFECTING PULMONARY DELIVERY

Important parameters for lung deposition are: particle size distribution; the respiratory manoeuvre, such as inhalation and exhalation flow rates, tidal volume, pause between inhalation and exhalation; shape of the upper airways. The structure of the bronchial and alveolar region in normal lungs is considered to be of minor influence (7).

The main factor affecting pulmonary delivery is the size of inhaled particles. The deposition size and the efficiency of inhaled aerosols in the respiratory tract are influenced by the aerodynamic diameter. This is determined by density of particles, its shape and its size. Other factors affecting pulmonary delivery are the diseases of the respiratory tract and hygroscopicity of the powders (6).

The particles in the aerodynamic size range of about 3.5-6.0 μm can penetrate beyond the central airways into the peripheral region of the lungs. Particles less than 3.5 μm and greater than about 0.5 μm will mostly come to the respiratory bronchioles or deeper during inhalation and penetrate almost entirely to the deep lung. Smaller particles (with aerodynamic diameters less than 0.5 μm) are dominated by thermal interactions with the air molecules and will diffuse to the respiratory tract surfaces during inhalation. Larger particles are dominated by their inertial mass and will impact in upper airways due to their inertia (5).

Therefore, to optimize pulmonary drug deposition and to reach the lower respiratory tract, particles need to be smaller than 5 μm and larger than 0.5 μm. Particles larger than 5 μm usually deposit in oropharynx from which there are easy cleared (8).

1.2 PULMONARY DRUG DELIVERY SYSTEMS

Three types of conventional methods of inhalation delivery for the treatment of respiratory diseases are developed: Nebulizers, Pressurized Metered-dose Inhalers (MDIs or pMDIs) and Dry Powder Inhalers (DPIs); each class presents unique strengths and weaknesses (9).

1.2.1 NEBULIZERS

Nebulizers were the first devices developed for inhalation. They are electric- or battery-powered machines. Oxygen, compressed air or ultrasonic power are used to break up solutions/suspension into small aerosol droplets that can be directly inhaled from mouthpiece of the device. The user breathes in the mist through a mouthpiece or facemask. Devices are expensive, not transferable and are linked to hospital use. Nevertheless, nebulization has disadvantages such as low efficiency, poor reproducibility and great variability. Further improvement in aerosol delivery systems with greater efficiency and portability and shorter administration time could improve patient quality of life (10).

1.2.2 PRESSURIZED METERED-DOSE INHALERS (MDIS OR PMDIS)

The metered dose inhalers (MDIs) accounts for 70 % of all inhalation therapy.

MDIs are pressurised devices that use propellants to deliver doses of medication to the lungs of a patient. The drug represents about 1 % of the content, while the propellants are greater than 80 % of the content, by weight. A pMDI comprises several parts: the canister, the actuator, and the metering valve. The canister is made of aluminium, which is light and compact. It is attached to a plastic actuator, so when the canister is pushed down, a valve delivers a measured dose of medicine in a fine mist. The amount is controlled by a metering valve in the actuator into which the canister nozzle is located (11).

The pMDI contains 100-400 doses in a small and portable device that can be easily kept in a pocket. Using such an inhaler device can require considerable coordination, but it is important that the correct technique is used. If not, the patient won't fully inhale the correct dose of the medicine. This not only makes it less effective, but may also result in the medicine ending up in your mouth or throat, where it can cause side effects such as throat irritation. Losses are greater than 70 % and can exceed 90 % (12).

Recent changes in drug formulation related to the application of hydrofluoroalkane (HFA) propellant replacing the chlorofluorocarbon (CFC) have taken place due to concerns about the latter's damaging effect on the ozone layer. The Montreal Protocol, adopted in 1987, mandated a complete elimination of CFCs. Depletion of the ozone layer will permit the transmission of ultraviolet-B radiation through the stratosphere and increase the risk for disease, induce global warming, and destabilize ecological equilibrium (12).

1.2.3 DRY POWDER INHALERS (DPIS)

Despite the fact that the pMDIs are still the most commonly used worldwide, the ban on the use of chlorofluorocarbons (CFC) in MDIs has forced the pharmaceutical industry to introduce dry powder inhalers (DPIs) as an alternative system which utilizes drugs in dry powder form (13).

Various factors such as the poor coordination between inspiration and dose emission, which commonly happens by using MDIs, can be avoided by application of DPIs (8).

A Dry powder inhaler (DPI) is a device that delivers medication to the lungs in the form of a dry powder. DPIs can be further classified as either single dose (Spinhaler, Rothaler) or multi-dose devices (Turbuhaler, Servent Diskus Inhaler). There are two types of multi-dose devices, reservoir type devices and multi-unit dose devices. The multi-dose reservoir type device stores the formulation in bulk, and has a built in mechanism to meter individual doses from the bulk upon actuation. Single dose inhalers are characterized by that the substance and the carrier for improving the flow properties (usually lactose) are filled in the hard gelatin capsules. Micronized powder particles themselves are strongly autoadhering, which makes them difficult to use in dry powder inhalation formulations. Therefore they are mixed with an inert excipient such as lactose monohydrate or glucose to give an interactive powder mixture. Prior to use, the capsule is inserted into the inhaler,

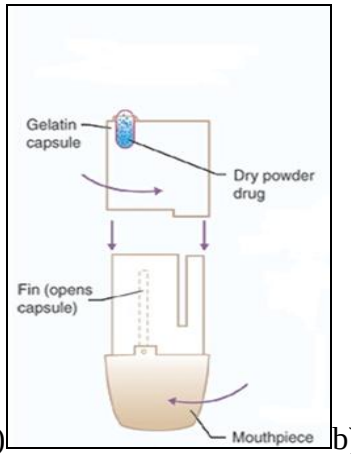
then the device vary depending on how inhaler activation mechanism releases drug from the capsule (9).

The drug is loaded into the inhaler within a capsule or blister, and aerosol is generated by air turbulence, as the patient inhales, drawing the powder through a plastic mesh or grid, thus breaking up larger particles and ensuring adequate dispersion of the aerosol. To be effectively delivered into the lung, drug particles are generally required to fall in the size range between 1 and 5 μm. Normally the powder is delivered during a single, large breath. Feature that it is activated by breath, is also one of the disadvantages. Required air flow is 60 l/min, which asthmatic people and especially children cannot always reach. Patients also have problems charging the inhaler, which is special undesirable during asthmatic attacks (11).

The advantages of dry powder inhalers are: they do not contain propellant, they enable the pulmonary delivery of higher dose and act locally.

The development of dry powders for inhalers should consider the following properties:

- easy for patients to use with minimal instruction (activated with only one operation)
- good aerosolization with minimal asthmatic effort
- minimum losses due to deposition of particles in mouth or throat physical and chemical stability of powders
- accuracy and reproducibility of the dosing
- protection against higher dose
- compact and economical production (14).

a) 

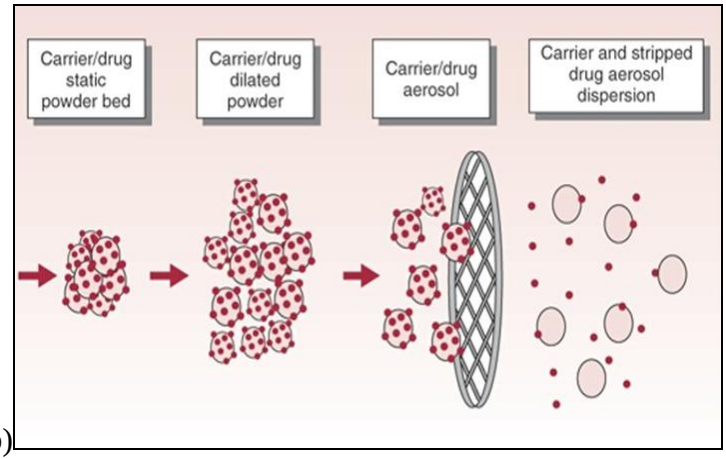
b) 

Figure 2. a) Rotahaler and b) aerosolization (15).

1.3 ASTHMA

1.3.1 EPIDEMIOLOGY

Asthma is one of the most common chronic inflammatory disorders of the airways and is characterized by airway obstruction, inflammation and hyperresponsiveness resulting from complex interactions among inflammatory cells, mediators, and the cells and tissues of the airways (16).

Bronchial asthma is defined by three main characteristics:

- *reversible airway obstruction*: widespread across the lung, with more or less frequent acute exacerbations, after which airway obstruction disappears either automatically or after treatment,
- increased nonspecific airway responsiveness to various factors, for example cold air or increased respiration,
- chronic inflammation of the airways.

Asthma is a heterogeneous disease that can be divided into two main categories.

Extrinsic asthma appears in 2/3 cases. It is atopic allergic disease based on IgE and the first type hypersensitivity caused by inhaled allergens, which are generally present in our environment (for example mites and pollen of grasses and trees). It is determined by the sensitivity tests and determination of IgE (17).

One third of the patients with asthma do not experience any signs of atopic reaction. The disease is called intrinsic asthma, whose cause is not well defined. It appears later in life. Skin tests are always negative and an increase in IgE is not present. In intrinsic asthma, there is probably a disorder in the development of immune response in the first years after birth. T lymphocytes ('helper'- Th) are responsible for the way of immune response. After the penetration of antigen, immature T cells are differentiated in the two subtypes of cells, which differ in the two types of cytokines that are released. Lymphocytes Th1 form interleukin-2 (IL-2), γ- interferon, meanwhile lymphocyte Th2 release IL-3, IL-4, IL-5 and IL-13 (17).

Fetus has more Th2 cells in the blood, but after birth there is as strong dominance of Th1 subtype. Patients with atopic asthma have still more Th2 cells. However, asthma is present only in 7-11 % of the population, which clearly shows that environmental factors are necessary for asthma development. Th2 precursors promote formation of IgE antibodies and lead to a chronic inflammation of the airways in patients with asthma.

Asthma symptoms are often "triggered" by environmental stimuli (smoke, perfumes, dust mites, animals, fungi/moulds, cold air) and aggravating conditions (viral upper respiratory infections or URIs, rhinitis, sinusitis, gastroesophageal reflux, stress, exercise). Such triggers may be more important for some asthma phenotypes than others (17).

Mechanisms of airway obstruction

There are three mechanisms involved in the airway obstruction during acute exacerbations:

- contraction (spasm) of muscles around the air passages
- swelling of the airway lining due to airway inflammation
- excessive mucus in the airways

Contraction of muscles is always the result of simultaneous action of two factors: direct effects of humoral factors on smooth muscle cells and neurogenic mechanisms (vagal reflex) (18).

Vagal reflex is important in neurogenic mechanisms. Because of bronchial deformation and bronchoconstriction triggered by inflammatory mediators, vagal receptors stimulate lung irritation. Through the vagal reflex (mediator acetylcholine), further bronchial smooth muscle contraction is caused (17).

1.3.2 PATHOGENESIS OF ACUTE EXACERBATIONS OF ALLERGIC ASTHMA

Acute exacerbation of asthma induced by inhalation of a single allergen manifests in three phases in most hypersensitive people.

Early phase: starts 5-15 minutes after contact with an allergen and takes about 2 hours. Mast cells start to release histamine and also at the same time metabolic processes are activated. Secondary mediators (prostaglandins and leukotrienes) are produced during

metabolism. Altogether, they cause bronchial smooth muscle contraction, which further strengthens vagal reflex. They also act as inflammatory mediators and promote the secretion of bronchial mucus.

Late phase: begins approximately 4 hours after exposure to an allergen and lasts 12-24 hours. Characteristics of this phase are the bronchial mucous edema, increased mucous secretion and exudation of plasma proteins. Inflammatory process is a pathogenetic process in asthma and it is characterized by the accumulation of eosinophils in inflammatory space. Th2 lymphocytes and cytokines are very important for inflammation:

- IL4: promote IgE synthesis in B lymphocytes and stimulates the production of Th2 cells themselves (chronic inflammation)
- IL3: increase the number of mast cells and activates
- IL5 and IL13 promote maturation, chemotaxis and activation of eosinophils in the airways (18).

Increased bronchial responsiveness phase: begins at the time of the late phase and lasts for a few days to several weeks. It is characterized by eosinophilic leucocytes infiltration and focal destruction of bronchial epithelial cells. Toxic alkaline proteins are secreted by activated eosinophils and cause destruction of epithelial cells. Airway responsiveness is also increased due to activation of the Vagus nerve. With the increase in bronchial responsiveness in asthma patients daily fluctuations in diameter of the airways is also strongly increased, which may result in nocturnal asthma (17).

Although asthma is characterized by reversibility of airway obstruction, in contrast with chronic bronchitis, is not progressive, but at least some asthmatics (usually severe), eventually develop irreversible component of the obstruction, which cannot be resolved with bronchodilators (17).

1.3.3 TREATMENT

Asthma cannot be cured. With regular use of drugs a significant reduction in symptoms, frequency of exacerbations and reduction of disease progression could be achieved. It is also necessary to avoid causative allergen as much as possible. Achieving and maintaining asthma control is the major goal of asthma care (18).

There are two basic asthma treatments. Long term control medications are used on a regular basis to prevent attack, as opposed to quick relief (rescue) medications which are used to relieve symptoms during an attack.

Long term control medications include:

- inhaled steroids (e.g., mometasone furoate (Asmanex), ciclesonide (Alvesco), budesonide (Pulmicort), fluticasone propionate (Flixotide), prevent inflammation
- leukotriene inhibitors (e.g., montelukast (Singulair), zafirlukast)
- long-acting bronchodilators (e.g., salmeterol (Serevent)) help open airways
- cromolyn sodium or nedocromil sodium
- aminophylline or theophylline (not used as frequently as in the past)
- combination of anti-inflammatory and bronchodilator, using either separate inhalers or a single inhaler

Quick relief (rescue) medication in use are:

- short-acting bronchodilator (e.g. salbutamol (Ventolin))
- oral or intravenous corticosteroids (prednisone, methylprednisolone) stabilize severe episodes
- anticholinergics (e.g. ipratropium bromide)

There is a recommendation to treat mild asthma with low-dose of ICS. Moderate asthma may be treated either medium-dose or low-dose of ICS with long-acting-beta-agonist (LABA). However, patients with persistent asthma are treated with medium or high-dose of ICS in combination with LABA. Omezalib is recommended, and possibly addition to LABA or oral corticosteroids, if control is not achieved (19).

1.3.4 INHALED CORTICOSTEROIDS

Inhaled corticosteroids have the central role in treating asthma. They are the most effective controller medication. Clinical studies showed high antiasthmatic efficacy and improved safety profile compared to oral corticosteroids. They are available in the form of sprays (aerosols) and as powders for inhalation.

Inhaled corticosteroids are important in the treatment of chronic inflammation, reducing inflammation in the airways and thus preventing asthma symptoms. Corticosteroids come

through cell membrane, where they bind to a glucocortisteroid receptor in cytoplasm. This corticosteroid-receptor complex come in the cell nucleus through plasma receptors, where they modulate gene transcription and thus promote the synthesis of active inflammatory proteins. The result is the increase or decrease of proteins, which in different ways inhibit inflammation.

A number of ICS have been developed and made available to treat asthma over the last 30 years. They include: beclomethasone dipropionate, triamcinolon, flunisolide, budenoside (Budiair, Pulmicort), fluticasone (Flixotide), mometasone (Asmanex Twisthaler) and ciclesonide (Alvesco).

ICS are deposited in the upper airways and lung. A large portion of the drug may be deposited in the oropharynx and swallowed. It can bring side effects, if drug is absorbed into the systemic system. It all depends on the formulation and the inhaler device.

Anti-inflammatory activity of inhaled glucocorticosteroids:

- reduce vascular vasodilation and liquid exudation with direct-vasoconstriction of smaller arteries
- inhibit mucus glycoproteins secretion
- reduce accumulation of leukocytes in inflammatory space, particularly difficult entry of eosinophils into the lung
- reduce the synthesis of IL-3, which regulates the production of mast cells
- inhibit the production of bronchoconstrictors and vasodilators
- inhibit T lymphocytes and their proliferation by inhibiting production of IL-2, therefore production of cytokines is reduced
- reduce secretion of histamine from basophils
- release toxic oxygen radicals from neutrophils and macrophages

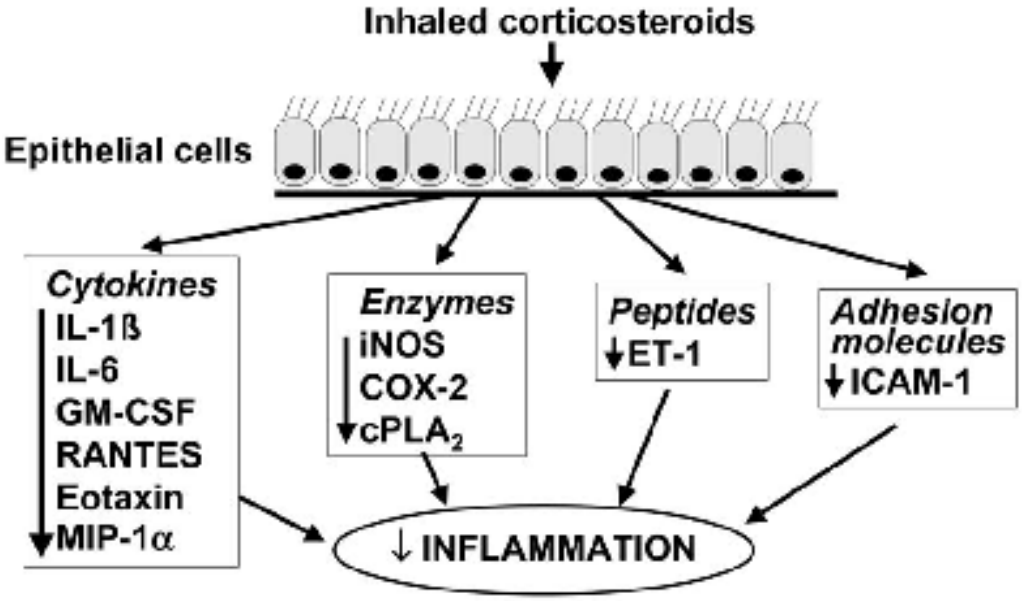


Figure 3. Inhibition of inflammation by inhaled corticosteroids (20).

Effectiveness of inhaled glucocorticoids is expressed in one week in the fast-acting, or after three months for the slow speed-ones, approximately. It is important to know that they should be used regularly every day, even when the patient has no symptoms.

The occurrences of adverse effects are considered to be related to both the dose administered and the duration of treatment. Long used of oral steroids or ICS leads to a higher risk of systemic side effects including adrenal suppression and insufficiency, growth suppression, bruising, osteoporosis, cataracts, glaucoma, metabolic abnormalities (glucose, insulin, triglycerides), psychiatric disturbances (euphoria, depression). The most common local side-effects of inhaled corticosteroids are: dysphonia, oropharyngeal candidiasis and cough.

Some of the goals in the development of new ICSs would be to improve therapeutic indexes, particularly at higher dosages; to have less frequent dosing intervals to encourage patient adherence; and maintain clinical effectiveness and potency.

1.4 CICLESONIDE

1.4.1 PHARMACOKINETICS AND PHARMACODYNAMICS

Ciclesonide ([R]-11β, 16α, 17, 21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with cyclohexanecarboxaldehyde 21-isobutyrate; CIC) is a new generation corticosteroid that has been developed for treatment of asthma and allergic rhinitis. It is inhaled into upper and lower airways, where it is converted to its pharmacologically active metabolite desisobutyryl-ciclesonide (des-CIC) by local esterases thus maximizing local effects (21). This onsite activation reduces oropharyngeal exposure and subsequent side effects.

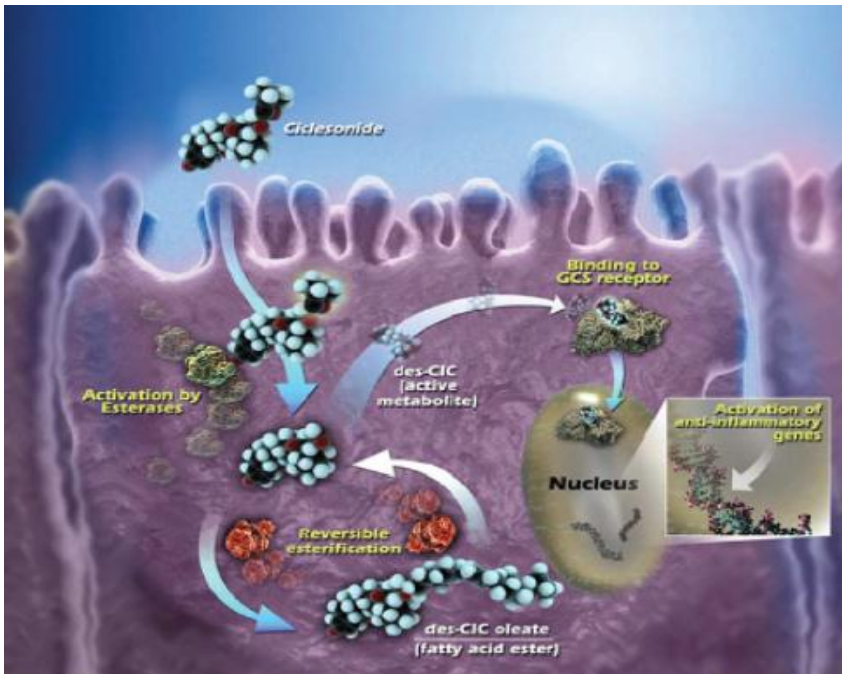


Figure 4. Intracellular activation of ciclesonide and reversible esterification of desisobutyryl-ciclesonide (des-CIC) (22).

Ciclesonide (CIC) is a prodrug with almost no receptor binding affinity, while other CS bind directly to corticosteroid receptor. The CIC molecule has a chiral centre in the acetal side chains. The two epimers of the compound are clearly different in their receptor affinities and metabolism rates. The R-epimer of CIC has a significantly higher binding affinity to the glucocorticoid receptor as compared to the S-epimer. Therefore only R- CIC is developed for clinical use (23). Des-CIC possesses 100-fold greater glucocorticoid-receptor-binding affinity than the parent compound (24). Its relative receptor affinity

(RRA) is similar to beclomethasone monopropionate (RRA=935) and its RRA is higher than budenoside, lower than mometasone furoate and fluticazone propionate. The potency of an ICS is evaluated in terms of its RRA versus dexamethasone, which is assigned a value of 100[4]. The higher the RRA, more systemic activity and more incidences of systemic side effects are observed, as glucocorticoid is expressed in almost all tissues and cells.

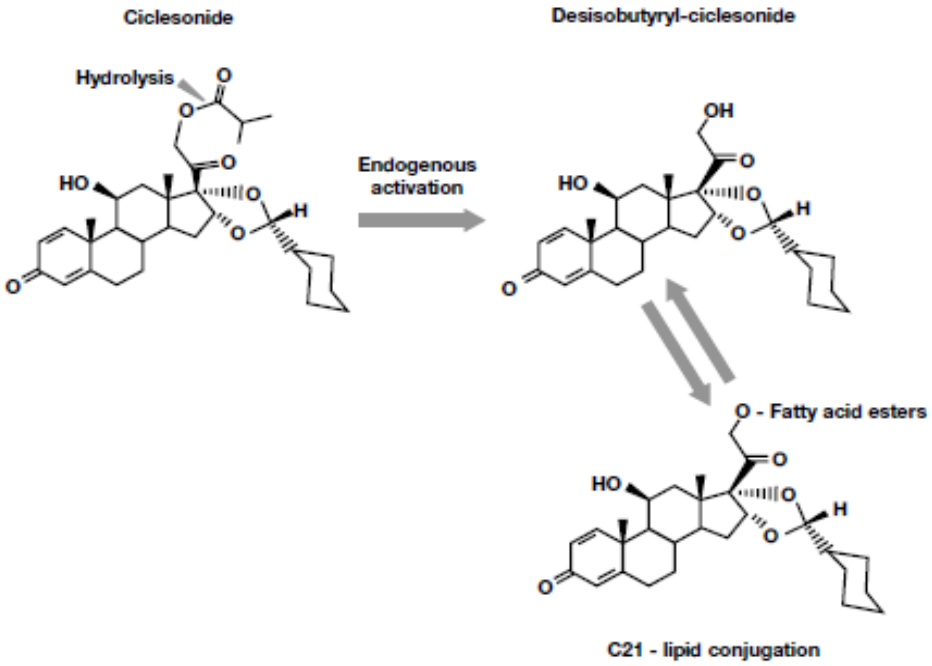


Figure 5. Bioactivation of ciclesonide to its active metabolyte desisobutyryl-ciclesonide (des- CIC) by endogenous esterases in nasal and bronchial epithelial cells and formation of fatty acid esters of des-CIC (25).

Des-CIC undergoes reversible esterification to fatty acid conjugates in the lung. A hydroxyl group at position C-21 is required for the formation of the ester bond between the corticosteroid and the fatty acid (26). The formed fatty acid conjugates decrease diffusion into systemic circulation and may prolong the local anti-inflammatory activity by slowly releasing active drug from the fatty ester depot (27). Furthermore, circulating Des-CIC is rapidly hydroxylated to inactive metabolites by the cytochrome P-450 enzymes, mainly CYP3A4 in the liver (28). After oral administration, CIC is excreted mainly in feces (67 %), which means that bile excretion is the main route of elimination.

Des-CIC stimulated release of inflammatory mediators such as granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein (MCP)-1, γ-interferon, interleukin (IL)-2, IL-4, IL-5, IL-8, and tumour necrosis factor (TNF)-a. CIC and/or des-CIC were effective in inhibiting proinflammatory functions, including the stimulated expression of intracellular adhesion molecule-1, they also inhibited the induced proliferation of immune cells such as peripheral blood mononuclear cells, CD4 lymphocytes, and human airway smooth muscle cells. Several studies have shown that treatment with CIC reduced the percentage of eosinophils in induced sputum of patients with asthma and also reduced levels of exhaled nitric oxide (NO) in patients with mild to moderate asthma (28).

The low oral bioavailability of CIC (approximately 1 %), high protein binding (99 %) and high hepatic clearance (396 l/h) contribute to the favourable safety profile of CIC (29).

CIC has several favourable properties, detailed below (30):

- a smaller particle size for greater lung deposition and less oral exposure
- greater affinity to the glucocorticoid receptors than current inhaled corticosteroids
- an ozone-safe propellant
- greater than 50 % of the inhaled dose delivered directly to the lung
- a conversion to its active form by esterases in the lung
- lipid conjugation for prolonged anti-inflammatory effects and once-daily dosing
- high protein binding for systemic safety
- only 1 % available for systemic exposure
- quick first-pass metabolism

1.4.2 CIC COMPARISON WITH OTHER ICS

CIC has been compared with other ICS in many studies. Relative topical potencies of ICS are as follows: FP – CIC > BUD > BDP > TA (31).

Pharmacokinetic profile (i.e. high clearance rate, high volume of distribution, and a high level of protein binding, together with a short elimination half-life and a long terminal half-life) have the potential to offer an improved therapeutic index as a result of increased receptor affinity, slower absorption from the lung following inhalation, and rapid systemic clearance (32).

Table 1. Comparison of the pharmacokinetic and pharmacodynamic features of a number of ICS. Modified from Cerasoli F. Developing the ideal inhaled corticosteroid. Chest, 2003, 130:54S–64S (32).

Parameters	Beclomethasone dipropionate	Budesonide	Fluticasone	Ciclesonide	Mometasone furoate
Oral bioavailability	<1 %	11%	<1 %	<1 %	<1 %
Pulmonary deposition	51 %	28 %	16 %	52 %	14 %
On-site activation	Somewhat	No	No	Yes	No
Receptor binding affinity	53	935	1,800	12	1,235
Esterification	No	Yes	No	Yes	No
Lipophilicity	Moderate	Low	High	High	High
Protein binding: free fraction (%)	87;13	88;12	90; 10	99; 1	99; 1
*$T^1/_2$ h	0.5	2.8	7.8	0.36	4.5
Vp, L	20	183	318	207	
Clearance, L/h	15	84	69	152	53.5

*T1/2 h = half-life / Vd = volume of distribution

1.4.3 PRECAUTIONS

CIC is not indicated for treating status asthmaticus or for primarily treatment of acute bronchospasm and there are no adequate and well-controlled studies in pregnant women (33).

1.4.4 DOSAGE AND ADMINISTRATION

CIC is available as a hydrofluroalkane pressurized metered-dose inhaler in two doses, 80 mcg/activation and 160 mcg/activation, administrated twice-daily. However, several studies showed that there is no improvement in lung function when a dose of 640 mcg/day was given (20 mcg twice daily), but it leads to decrease in the frequency of exacerbations. For some patients, decrease in dose to 80 mcg once daily, can be an effective maintenance dose.

The recommended starting dose for patients receiving inhaled bronchodilators alone is 80 mcg twice daily to a maximum dose of 160 mcg twice daily. Patients, who are receiving inhaled steroids, the starting dose is 80 mcg twice-daily with a maximum dose of 320 mcg twice-daily. For patients receiving oral corticosteroids, it is recommended that patients start at the maximal dose of 320 mcg twice daily with taper of oral prednisone not exceeding 2.5 mg/day on a weekly basis, starting at least 1 week after beginning of CIC therapy (34).

1.4.5 SAFETY AND ADVERSE EFFECTS

CIC has several pharmakokinetic properties, which are desirable for a drug safety profile.

The safety and tolerability of CIC have been examined in a variety of preclinical tests investigating acute and chronic toxicity in different species. CIC was well tolerated when it was administered to healthy subjects in clinical phase I studies. Suppression of the endogenous cortisol release was minimal.

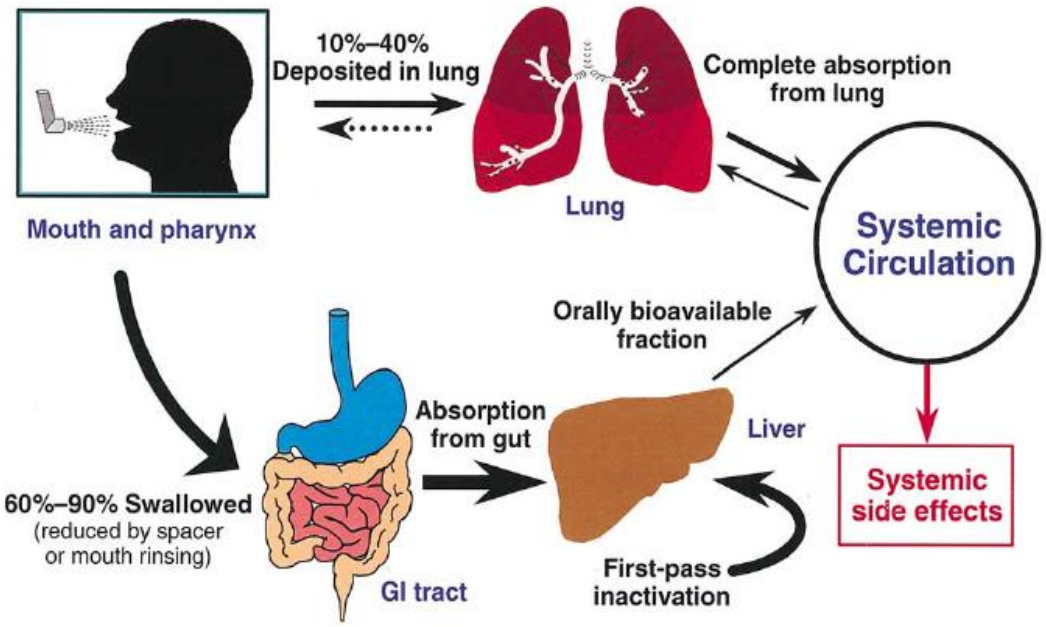


Figure 6. The fate of ciclesonide (35).

1.5 CYCLODEXTRINS

Cyclodextrins (CDs) are a group of structurally related cyclic oligosaccharides, very useful pharmaceutical excipients and nowadays very attractive.

Lately, they are popular because of their ability to change undesirable characteristics of the drugs, such as unpleasant taste and smell, low aqueous solubility, to improve physical, chemical and enzymatic stability of drugs, decrease local irritation of drugs and improving drug bioavailability by inclusion complex formation. Due to their biocompatibility, CDs may be used in different routes of drug application: oral, intravenous and topical, to improve the physiochemical and biopharmaceutical properties of drugs (36).

1.5.1 HISTORY OF CYCLODEXTRINS

Cyclodextrins (CDs) are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. In 1891, Villers discovered bacterial metabolite, which was isolated from starch. Experiments have shown that the substance was dextrin. Villiers named it cellulosin. Later, the Austrian microbiologist F. Schardinger described two crystalline substances α-dextrin and β-dextrin which he had isolated from bacterial digest of potato starch. These substances are now called CDs (37). In 1935, γ-CD was discovered by Freudenberg and Jacobi (38). The first CD-related patent was issued in 1953 to Freudenberg, Cramer and Plieninger (39,40). However, pure CDs that were suitable for pharmaceutical applications did not come available until about the middle of 80s and at the same time the first CD containing pharmaceutical product was marketed in Japan.

1.5.2 CHEMICAL STRUCTURE AND PROPERTIES OF CDS

Generally, CDs can be divided into naturally occurring and chemically modified CDs (41). Naturally occurring CDs consist of 6, 7 or 8 α- (1,4) linked D-glucopyranose units. They differ in their ring size and solubility.

Table 2. Some characteristics of α-, β-, γ-, and δ- CD (32,42).

Type of CD	Diameter	Weight	Water solubility (g/100 ml)
α-CD	4.7-5.3	972	14.5
β-CD	6.0-6.5	1135	1.85
γ-CD	7.5-8.3	1297	23.2
δ-CD	10.3-11.2	1459	8.19

The α-CD has six glucopyranose units and several names: Schardinger's α-dextrin, cyclomaltohexaose, cyclohexaglucan, cyclohexaamylose, αCD, ACD and C6A.

The β-CD has seven glucopyranose units and also several different names: Schardinger's β-dextrin, cyclomaltoheptaose, cycloheptaglucan, cycloheptaamylose, β-CD, BC, and C7A. The γ-CD has eight glucopyranose units and is also known as Schardinger's γ-dextrin, cyclomaltooctaose, cyclooctaglucan, cyclooctaamylose, γCD, GCD and C8A.

CDs with fewer than 6 units cannot be formed due to sterical hindrances, while the higher homologs with 9 or more glucose units are very difficult to purify (43).

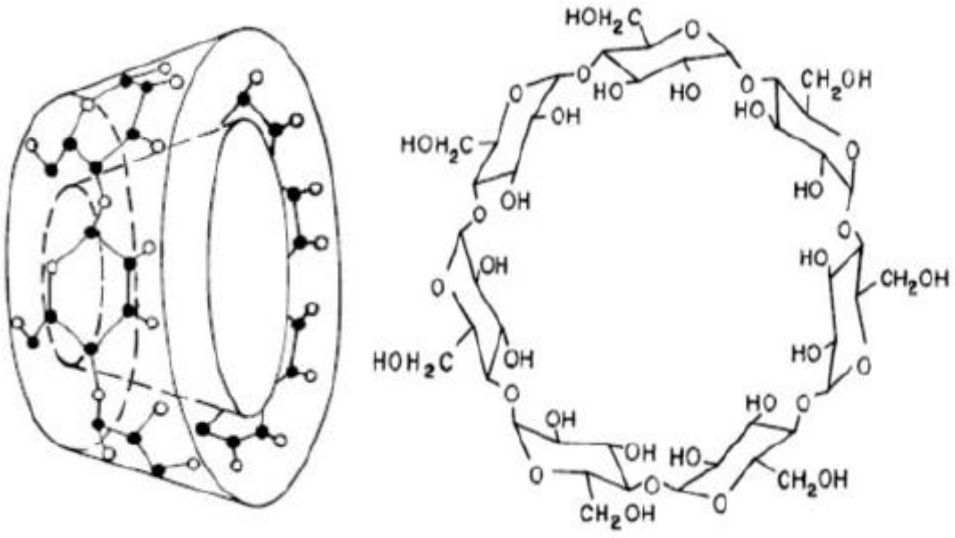


Figure 7. The chemical structure of β-cyclodextrin molecule (36).

CDs are crystalline, homogeneous and nonhygroscopic, but form various stable hydrates. They take the shape of a truncated cone or tours instead of a perfect cylinder because of the chair conformation of the glucopyranose units. Due to the chair formation of the glucopyranose units, CD molecules are shaped like cones with secondaryhydroxyl groups extending from the wider edge and the primary groups from the narrow edge. The central cavity of the molecule is lined with skeletal carbon and etheral oxygen atoms of the glucopiranose units, which gives it a lipophilic character (Figure 7) (44).

The cavity of the CDs is occupied by included water molecules both in crystalline state as well as in aqueous solution. These water molecules are in direct contact with the apolar wall of the CD cavity and this polar-apolar interaction results in an energetically unfavoured state.

'Guest' molecules which are less polar than water can easily substitute the included water molecules. One, two or three CD molecules contain one or more entrapped guest molecules. And the most frequently CD:drug ratio is 1:1. The aqueous solubility of naturally CD (α, β and γ-CD) is much lower than linear dextrins. This is thought to be due to relatively strong binding of the CD molecules in the crystal state (45).

CDs are formed by enzymatic degradation of starch by cyclodextrin glucosyltransferase (CGTase) (46). The natural α-CD and β-CD, unlike γ-CD cannot be hydrolyzed by human salivary and pancreatic amylases; though all three are subjected to fermentation by the intestinal microflora. CDs are large (MW ranging from almost 1000 to over 2000 Daltons) and hydrophilic ($\log P_{o/W}$ between -3 and 0) with a significant number of H-donors and acceptors. Due to this, CDs are not absorbed from the gastrointestinal tract in their intact form. Therefore, it is predicted that CDs do not penetrate biological membrane (47). CDs are only able to enhance permeation of the drugs that are in the form of complexes.

1.5.3 INCLUSION COMPLEX FORMATION

The most notable feature of CDs is their ability to form solid inclusion complexes (host-guest complexes) with a very wide range of solid, liquid and gaseous compounds by a molecular complexation (48). Complexation is formed by taking up a drug molecule or, more frequently, some lipophilic moiety of the molecule, into the central cavity. One, two or three CD molecules contain one or more entrapped "guest" molecules (drugs). Usually

the host:guest ratio is 1:1, however there are some cases when it is 1:2, 2:1, 2:2 or even more complicated complexes. It is an equilibrium process where free guest molecules are in equilibrium with the molecules in the complex. Most often the nature of CD to choose depends on the future role of the inclusion. Drug molecules, that have suitable geometry and are less polar than water, will form inclusion complex with CD (49). The main driving force leading to the complex formation is in the release of enthalpy rich water molecules from the central cavity of the molecule. During inclusion complexation no covalent bonds are formed or broken. Complexes are usually obtained either in solution or in the crystalline state. Water is typically the solvent of choice (48).

Many methods have been described for the preparation of inclusion complex such as: co-precipitation, slurry, paste, damp mixing and heating, extrusion, co-evaporation, spray-drying, freeze-drying, kneading, sealed-heating and supercritical carbon dioxide (50).

By forming inclusion complexes, the physicochemical (solubility, stability) and biological properties of the drug are improved. Another goal that might be achieved by inclusion complex formation is the reduction of unpleasant drug's taste or smell and it may protect chemically labile drug molecules from surrounding environment (44).

Factors that influence inclusion complex formation are: type of CD, performance of drug/CD complexes, temperature, method of preparation, etc. (36).

1.5.4 CYCLODEXTRINS DERIVATIVES

Chemically modified CD derivatives have been prepared to extend physicochemical properties and inclusion capacity of parent CDs. Various CD derivatives have been developed by the chemical substitution of the primary and secondary hydroxyl groups of natural CD molecules.

An industrially produced and marketed CD-derivatives need to have the following characteristics (51):

- has to be produced by a simple reaction
- retains complex-forming capacity
- must be non-toxic, when used as recommended and
- possesses particularly advantageous properties for some specific application.

More than 1500 different CDs derivatives have been synthesized and described in the literature (42). The known derivatives can be classified according to their substituents, their polarity, size, biological activity, etc. As for their practical uses, they are classified as follows (52):

- carriers (solubilizers, stabilizers) for biological active substances
- enzyme models
- separating agents (for chromatography or batch-processes)
- catalysts and additives (as detergents, viscosity modifiers, etc.)

Derivatives of CD which are of pharmaceutical interest are hydroxypropyl derivative of β-CD and γ-CD (i.e., HP-β-CD and HP-γ-CD), the randomly methylated β-CD (RM-β-CD), sulfobutylether β-CD (SBE β-CD), monochlorotriaziny beta cyclodextrin (MCT-β-CD), and the so called branched CDs such as maltosyl-β-CD (G2-β-CD).

1.5.5 HYDROXYPROPYL BETA CYCLODEXTRIN

The chemically modified hydroxypropyl-β-cyclodextrin (HP-β-CD) has higher water solubility, greater solubilizing and complexing properties than β-CD. It is obtained by partial etherification of the crystalline parent CDs with a hydroxyalkyl group usually using propylene oxide (53).

It is a cyclic oligosaccharide, amorphous white powder containing seven D-(+)-glucopyranose units, with an average of one hydroxypropyl group per unit. The non-polar aromatic portions of the compound tend to enter the nonpolar interior of the HP-β-CD molecule, when a compound with appropriate geometry and HP-β-CD are in the same solution. Drug aqueous solubility increases due to this complexation isolates the total drug or only the aromatic and heterocycle ring portion of the molecule from the water (54).

HP-β-CD preparations are very soluble in water as solutions can be prepared up to 75% w/w. They are also 50-60 % soluble in ethanol (95%), and samples with degrees of substitution less than 7 have limited solubility in acetone (55). The solubilizing property of HP-β-CD depends on the properties of the guests.

Based of toxicological studies, HP-β-CD is safe in most species, particularly if dosed orally. It shows limited toxicity, depending upon dose and route of administration. It is also

well tolerated in humans. The main adverse effect is diarrhoea with no effects on kidney function (56). However, since it is not nephrotoxic it has been suggested for use in parental formulations.

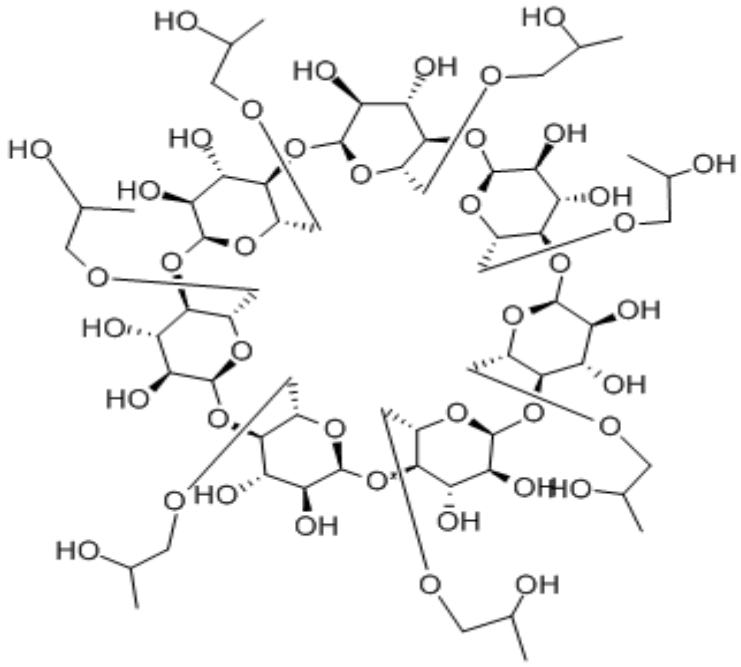


Figure 8. Structure of hydroxypropy-β-CD (57).

2 AIM

The objective of this graduation thesis was to develop and validate a new formulation with corticosteroid ciclesonide (CIC), for pulmonary administration. CIC is effective long–term control drug, used for the prophylactic treatment of mild to severe asthma in adults and children. But it is practically insoluble in water. Thus, the aim of this study was to evaluate if CIC solubility and its aerodynamic properties would be improved in the form of inclusion complexes with hydroxypropyl-β-cyclodextrin (HP-β-CD) as dry powder for pulmonary delivery.

The first part of the thesis was focused on the inclusion of the drug CIC to CDs (HP-β-CD and γ-CD), molecules with cavity size suitable for hosting large molecules or significant parts of these molecules. Phase solubility test was used to determine apropriate CD. Further work was focused only on HP-β-CD due to better stability constant than with γ-CD with CIC. HP-β-CD/CIC complexes were made. Infrared spectroscopy, differential scanning calorimetry, scanning electronic microscopy and molecular modelling were used to provide evidence of complex formation.

In the second part of the thesis *in vitro* aerosolisation behaviour of the formulations were tested. It was evaluated using the Rotahaler device. *In vitro* deposition properties of complexes and CIC were characterised by Twin Impinger based on the uniformity of emitted dose and aerodynamic particle-size distribution (respirable fraction (RF), as a percentage of nominal dose (RFN) and emitted dose (RFE)).

3 MATERIALS

DRUG

- **Ciclesonide (CIC)** (M=540.6876 g/mol) was a generous gift from Hovione Sociedade Química SA, Portugal, (water solubility is 1.57 e^{-03} g/l)
- **HP-β-CD** (KLEPTOSE® HP, MS= 0.85) (M=1476.6 g/mol) from Roquette, France
- **γ-CD** (CAVAMAX® W8 PHARMA GAMMADEX) (M= 1297 g/mol) Wacker-Chemie GmbH, Germany
- ethanol (96 %)
- demineralized water
- **α- lactose monohydrate** (Granulac 70) from Meggle, Germany

EQUIPMENT

- Infrared spectrophotometer: IMPACT 400, Nicolet
- Spectrophotometer: HITACHI U-2000
- Differential scanning calorimeter, DSC Analyzer from TA instruments (DSC Q200)
- Shaking water bath, The Mickle Laboratory Engineering CO.LTD, serial number 966 038 754, Gomshall, Surrey, England
- Sieve shaker AS 200 Digit, Retsch
- Scanning electron microscope (SEM), Supra 35 VP, Carl Zeiss, Germany
- Rotahaler®
- Twin Impinger**,** Copley, UK
- Ultrasonic water bath
- the electronic balance, AG204 delta range model

LABORATORIAL MATERIAL

Measuring cylinders (10, 50, 250 ml), volumetric flasks (10, 25, 50, 100), test tube support, test tube with caps, 3 ml transfer pipettes, 10 ml clear flat bottom headspace vials, automatic pipettes, tips for pipettes, microtubes, microplates, millipore filters 0,45, 5 ml syringes, beakers (50, 100, 250 ml), Erlenmeyer flasks, plastic wash bottles, ethanol wash

bottle, glass funnels, thermometer, mortar and pestle, watch glasses, spatulas, Al foil, Eppendorfs, baskets for laboratory dishes, racks for test tubes, bags for wrapping material.

4 METHODS

4.1 CICLESONIDE CALIBRATION CURVE

Calibration curve was constructed for the drug ciclesonide (CIC) to establish a linear working range. As CIC is poorly soluble in water, the drug was weighed in a volumetric flask and distilled water/ethanol (50:50) was added as the media for the drug. Stock solution (also called ″mother solution″) was submitted to ultrasound. From each ″mother solution″, six solutions with different concentrations ranging from 0.008 to 0.021 mM were prepared. All solutions were prepared in duplicates. The linearity was presented by measuring the absorbance of each solution that has been prepared from the main one. The absorbance was determined by an UV-detection spectrometer at 245.0 nm wavelength.

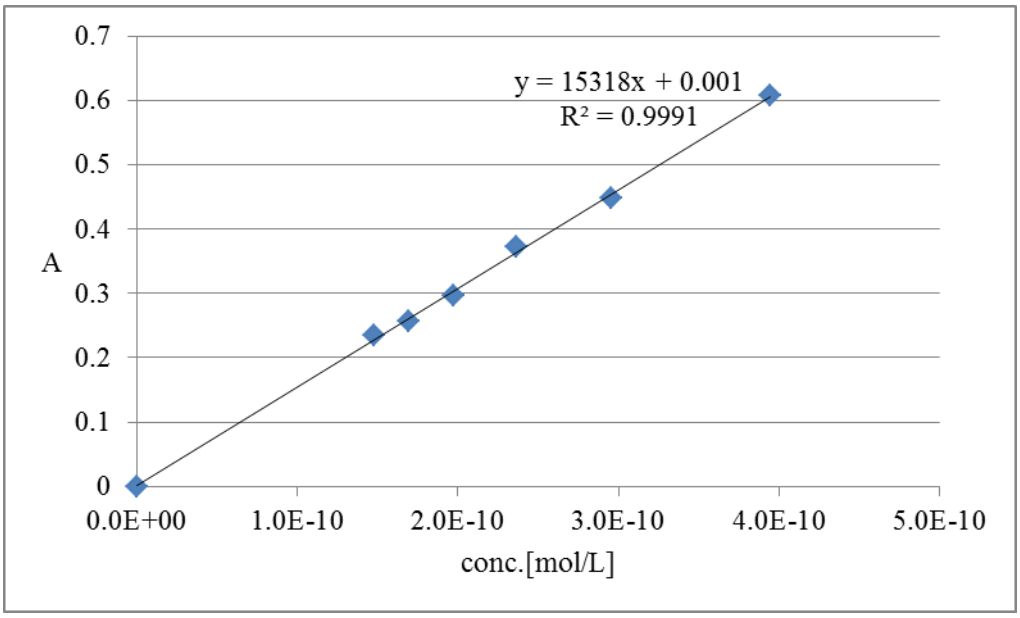


Figure 9. Calibration curve for ciclesonide.

4.2 PHASE SOLUBILITY TEST

The complex formation stability constant of ciclesonide (CIC) and the two CDs (*γ*-CD and HP-*β*-CD, n=2) were studied by phase-solubility techniques developed by Higuchi and Connors (59). This method is based on the effect of a complex forming ligand, *e.g.* CD, on the solubility of the drug. It can also be used for slightly soluble drugs with intrinsic solubility in the low-mM or nM range. The apparent stability constant (Ks) were calculated from the linear part of the phase-solubility using the equation where, S_0 is the solubility of the drug in the absence of CD (58).

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})},$$

Equation 1: Stability constant (Ks) for 1:1complexes.

An excess of drug (3 mg) was added to each one of 5 flasks. In the first flask 10 ml of distilled water was added and in the next four flasks different amounts of HP-β-CD (2.5 ml, 5 ml, 7.5 ml or 10 ml) were added. Distilled water was used as a medium to complete 10 ml and flasks were sealed and placed in waterbathshaker. All the above solutions were shaken for 24, 48, 72 h at 100 shaks/min. Samples were made in duplicate. After shaking, the sample of each flask (3 ml of solution) were taken and filtered through milipore filter (0.45 μm). Prior to use each sample of filtered solution was centrifugated. The filtered solution was diluted in 10 ml volumetric flask with water/ethanol (50:50). Concentration of CIC was analysed by UV Spectrophotometer (HITACHI U-2000) at 245.0 nm. The work was carried out at 25 ± 1 ºC. The same procedure was used for *γ*-CD. The solubility of CIC in every HP-β-CD and γ-CD solution was calculated, and phase solubility diagram was drawn.

4.3 PREPARATION OF HP-β-CD/CIC COMPLEXES

Complexes were prepared by adding HP-β-CD to a small amount of water till dissolution and then adding into the mixture the same molar proportion of CIC. The flask has been shaking for 48 hours in a water bath at 25 °C (agitation 150/min). The same procedure was used for γ-CD. After, the samples were placed in an oven for another 48 hours at 40 °C and then pulverized in a mortar. Small, clearly white particles were observed.

4.4 PREPARATION OF THE PHYSICAL MIXTURE

A physical mixture consisting of CIC and HP-β-CD in the same weight ratio was prepared by weighing on a precise scale, mixed together in an eppendorf and were shaken by hand for 10 minutes to obtain a homogenous blend. The resultant mixture was stored in a desiccator.

4.5 FILLING CAPSULES

Monohydrated lactose was sieved by a seive shaker AS 200 Digit, Retsch to obtain the same mean particle diameter. The fraction size 63-90 was collected.

CIC/HP-β-CD complexes and lactose were mixed in a mortar to a fill weight equivalent to a middle dose of 160 μm of CIC or to the highest dose 320 μm. The procedure was repeated several times and in the end we got around 100 gelatin capsules (number 2). Each gelatin capsule contains 20 mg ± 1 mg or 40 mg ± 1 mg. Filled capsules were stored in desiccator.

4.6 MOLECULAR MODELLING

All molecular modelling calculations were carried out using Macromodel 9.11 and MMFFs force field by prof. Mire Zloh from University of London. The implicit solvent

representation was achieved using generalised Born/surface area continuum (GB/SA) method with a constant dielectric function (ε=1). An extended nonbonded cut off (van der Waals: 8 A° ; electrostatics: 20 A°) was used. The modelling of complex formation was carried out using conformational search and torsional sampling (MCMM). The CIC was positioned in four different starting configurations in respect to the initial model of all cyclodextrin molecules and 5000 steps of Monte Carlo conformational search were carried out for each configuration. The ensembles of generated structures were clustered and analysed using the cluster analysis program Xcluster.

4.7 DETERMINATIONS OF SOLID HP-β-CD/CIC COMPLEXES

4.7.1 INFRARED SPECTRAL STUDIES

The FT-IR spectra of all samples (CIC, HP-β-CD, the respective complexes HP-β-CD/CIC complexes and physical mixture) were measured using FT-IR spectrophotometer (Impact 400). Some particles of dried sample were mixed with some amount of KBr by using a clean pestle and mortar. The procedure consisted of placing a sample of the neat powder dispersed in KBr into the sampling cup, smoothing the powder into a thin bed, and compressing the powder bed into the holder using a compression gauge. The sample was placed in the light path and the spectrum was obtained from 500 to 4000 cm^{-1}. FT-IR was performed in duplicate for each of the samples.

The spectral changes were evaluated by subtraction of the spectrum of HP-β-CD from the spectra of the samples.

4.7.2 DIFFERENTIAL SCANNING CALORIMETRY

To determine calorimetric measurements of HP-β-CD/CIC complexes, physical mixture of CIC with HP-β-CD, HP-β-CD and CIC were performed on a DSC Q200 Analyzer from TA instruments. For the calibration of DSC instrument indium was used as a standard. CIC (2.4 mg), HP-β-CD (6.5 mg), CIC/HP-β-CD complexes (8.9 mg) physical mixture (8.9 mg) were accurately weighed into a aluminum TG pan. Covers of the pans were closed by the press. Pans were transferred into DSC apparatus, which was heated in the temperature

range from 0 °C to 300 °C at a rate of 10 °C/min in a nitrogen atmosphere (flow rate 10 ml/min). An empty aluminum pan was used as a reference.

4.7.3 SCANNINIG ELECTRON MICROSCOPY

The particle morphology, volume size, surface features and blend uniformityof the CIC, HP-β-CD and HP-β-CD/CIC complexes was evaluated by scanning electron microscopy (SEM).

The powder samples were fixed on aluminum carriers and placed to metal poles with a bilateral conductive carbon tape (diameter 12 mm, Oxon, Oxford Instruments, Great Kingdom) and observed with a scanning electron microscope Supra 35 VP (Oberkochen, Zeiss, Germany) with an accelerating voltage of 1.00 kV and the use of secondary detector.

4.8 AERODYNAMIC CHARACTERISTICS AND AEROSOL PERFORMANCE

The aerodynamic characteristics and the aerosol performance of the formulations were tested by an impaction-based apparatus – Apparatus A (Glass Impinger), also known as a twin stage liquid impinger (TSLI) or simply twin impinger (TI)(59).

4.8.1 TWIN IMPINGER

The twin impinger is a two-stage cascade impinger which was produced specifically to determine the delivery efficiency of oral inhalation delivery devices (60). It operates by dividing the dose emitted from a dry powder inhaler into particles which fall within theoretical respirable range and those which do not. Further characterization of respirable particles is not possible.

Twin impinger is schematically shown in Figure 10. The instrument is designed from a series of glassware components and containing 7 and 30 ml of solvents for stages 1 and 2, respectively. Air is flown through the instrument at a flow rate 60 l/min by means of a vacuum pump at the outlet for 5 s.

Aerosol spray is produced at the inlet and passes through a glass bulb which is meant to simulate oropharynx. Then it passes into the upper impinger stage, which consist of nozlle and a bulb containing a measured amount of liquid.

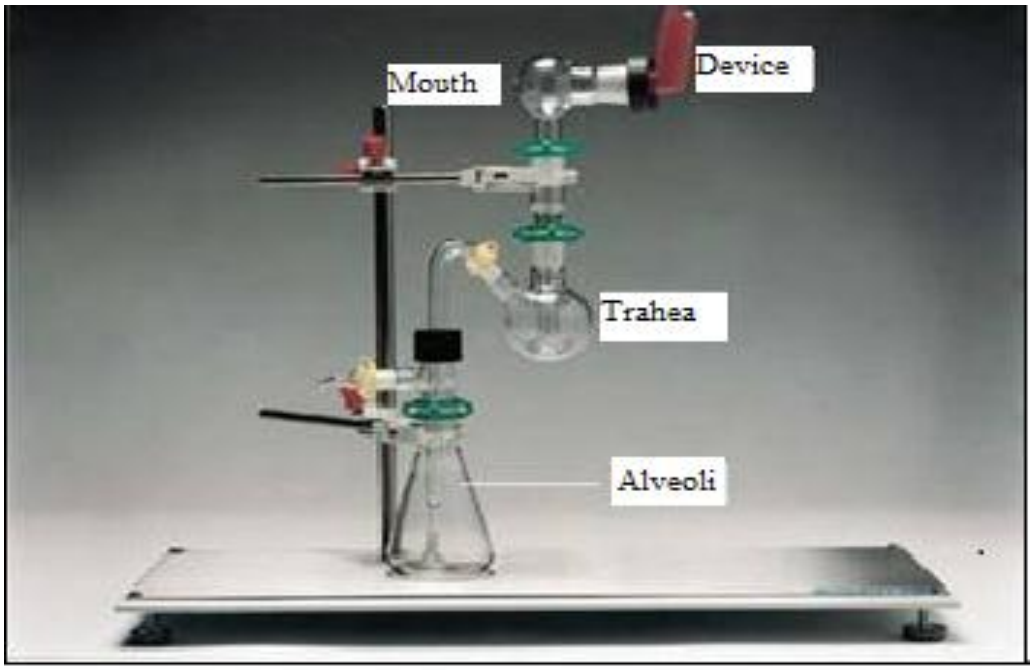


Figure 10. Twin Stage Impinger Apparatus B.P. (61).

The quantity of particles collected in various parts of the instrument is determined by washing the particles out of these parts with wash solution water:ethanol (50:50). The wash solutions were diluted to appropriate volumes and the drug contents were determined by spectrophotometry and the concentration was calculated.

4.8.2 INHALER:

A DPI Rotahaler was used to deliver powder in the simulating lung apparatus and thus, to evaluate the aerosol performance of the formulations. By rotating this device, the caspule is opened or smashed and the powder countinued through the sieve device. When a patient actuates a DPI, the flow of air both lifts the powder out of the inhaler (fluidisation) and causes the separation of drug from carrier (de-agglomeration).

4.9 DELIVERY OF THE INHALATION DOSES AND ASSESSMENT OF THE RESPIRABLE PARTICLES

To perform a test, the flow rate was set to the preestablished value 60 ± 5 l/min.

Capsula was precisely put in inhaler device Rotahaler by pincete. The device to be tested was placed into a mouthpiece attached to the throat of the TI. The pump was switched on for 5 seconds. Particles are introduced to the inlet of the impinger for a 5 seconds like our breath lasts. After the pump was switched off, the inhaler was removed and loaded with the next capsule. This procedure was repeated a further four times (0.160 mg or 0.320 mg of CIC in capsules) doses. Particles which do not exit, are collected from the device and from the capsula and quantified. After a run, the quantity of particles collected in the various parts of the instrument were determined by washing the particles out of these parts with wash solution water:ethanol (50:50).

After having discharged the final dose, the apparatus was dismantled. The following steps were:

1. The emptied capsules and the inhaler device were washed with mixture of ethanol/water (50:50). The washings were collected in a volumetric flask marked ‘D’(device) and diluted to 25 ml with ethanol/water (50:50).

2. The mouthpiece was washed with ethanol/water (50:50) and washings were collected in a volumetric flask signed ‘M’ (mouth) and diluted to 25 ml with ethanol/water (50:50).

3. The content of the upper impingement chamber (representing trachea) was transferred to a volumetric flask marked ‘T’ (trachea). The chamber and neck were washed with ethanol/water (50:50). The washings were collected in flask T and diluted to 25 ml with ethanol/water (50:50).

4. The content of the lower impingement was transferred to a volumetric flask marked ‘A’ (alveoli). The chamber and coupling tube were washed with ethanol/water (50:50). The washings were collected in flask A and diluted to 50 ml with ethanol/water (50:50). Each determination was repeated.

Before measurements, samples of the solutions were filtered through a 0.45 µm filter and UV spectrometer detection at 245 nm was performed. The concentration was calculated from the following formula, got from the calibration curve: $y=15318x+0.001$ ($R^2 = 0.9991$). These determinations were carried out at 25 ± 1 º C.

Emitted fraction (as percentage of CIC nominal dose that leaves device):
*Emitted dose of CIC (compart. M+T+A)*100/Nominal dose*

Respirable fraction (as percentage of the nominal dose (RFN) and as percentage of the emitted dose (RFE) of CIC that reaches the A compartment):
*Dose of CIC into compart. A *100/ Nominal Dose*

The same procedure was used for the Twin Impinger determinations of CIC.

5 RESULTS AND DISCUSSION

5.1 PHASE-SOLUBILITY TEST

A HP-β-CD/CIC and γ–CD/CIC complexes were studied by phase solubility test in order to choose the best carrier for lung delivery.

A phase solubility study was performed by gently shaking the vials containing an excess amount of CIC and solutions of HP-β-CD in ethanol/water (50:50), where concentrations of CIC ranged from 0.00 to 0.04 mM. The same procedure was used for CIC and mixture of γ–CD in ethanol/water (50:50). It was observed at low concentrations of HP-β-CD, that CIC was settling on the top and suspension was nearly clear. At high concentrations of HP-β-CD, supernatant was more turbid and less particles of CIC were settling to the bottom of vial. This was due to the increase on the solubility of CIC by forming inclusion complexes with HP-β-CD. The same observation was noted also in vials with CIC and mixture of γ–CD and ethanol/water (50:50).

Higuchi and Connors (58) characterized two main phase solubility profiles in Figure 11 obtained from the interaction between the guest and the host-CD when they are in solution. Type A is typically defined for the water soluble CD derivatives and type B is noticed for the less-soluble natural CDs. In type A, the solubility of the drug increases with increasing CD concentration, while B-type phase-solubility profiles reflect the formation of complexes with limited solubility in aqueous medium. Type A has three subtypes (A_n, A_l and A_p). When the complex is first order with respect to ligand and first or higher order with respect to substrate then A_l-type phase-solubility profile is obtained. If the complex is first order with respect to the substrate, but second or higher order with respect to the ligand then A_p-type phase solubility profile is obtained. It is difficult to interpret the A_n-type phase-solubility profile. The negative deviation from linearity may be associated with CD induced changes in the dielectric constant of the aqueous complexation media, changes in complex solubility or self-association of CD molecules.

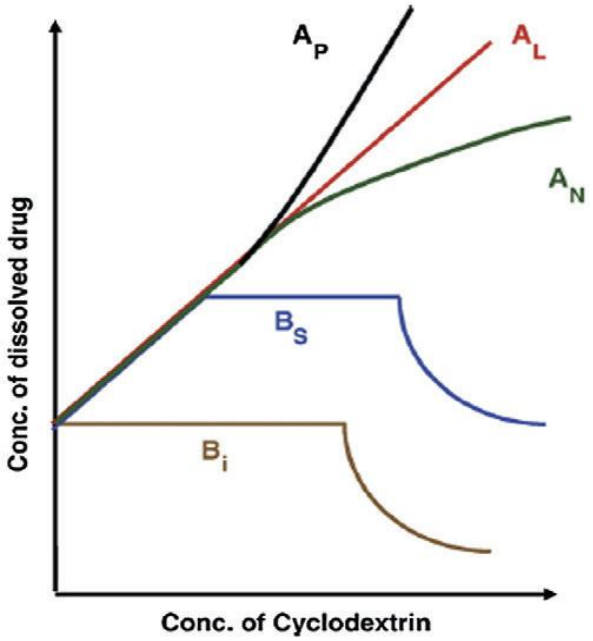


Figure 11. Theoretical phase solubility diagram (62).

CIC solubility increased linearly as a function of γ-CD concentrations and thus the solubility curves were classified as A_l type (Figure 12) (r^2 from linear regression of 0.99987), indicating the formation of 1:1 drug:CD complexes, when one drug molecule forms a complex with one CD molecule. A slope of <1 for a type A_l diagram does not necessarily indicate that only a 1:1 complex is formed, although this is the most usually assumed.

The phase solubility diagram of CIC with HP-β-CD showed a positive deviation from the straight line, i.e., an A_p-type Higuchi phase solubility diagram (r^2 from linear regression of 0.9791) (Figure 13). Complexation between more than one HP-β-CD molecule and one guest molecule (i.e. CIC) was likely to have occurred at the higher concentrations of HP-β-CD. As a result, the complexes formed, in the A_p-type phase solubility diagram were presented to a higher order than one in the host molecule (i.e. HP-β-CD). Aditionally, this may indicate the formation of 1:1 and 1:2 stochiometric ratios of CIC/HP-β-CD complexes (58). These results are similar to those from an early study (63), which showed that steroid cholesterol with HP-β-CD form A_p-type and may occur by the formation of 1:1 and 1:2.

Figure 12 and 13 present the phase solubility diagram of CIC and γ–CD and of CIC and HP-β-CD. The solubility of CIC increases with increasing CD concentration. HP-β-CD typically give A type of a curve, when they show up with inhaled steroid (63).

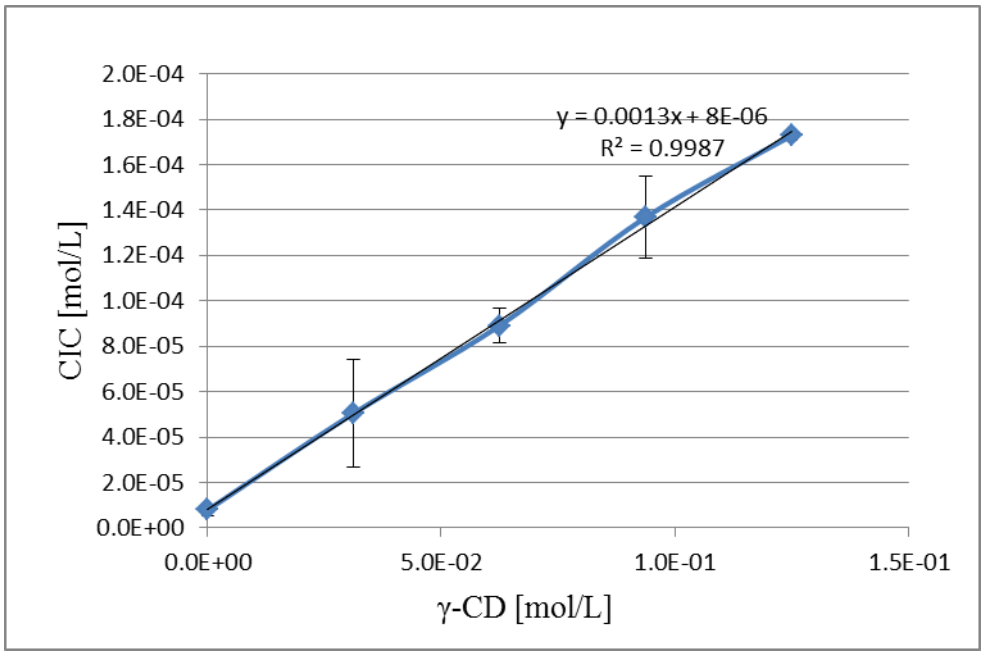


Figure 12. Solubility test of ciclesonide with γ-CD after 48 hours.

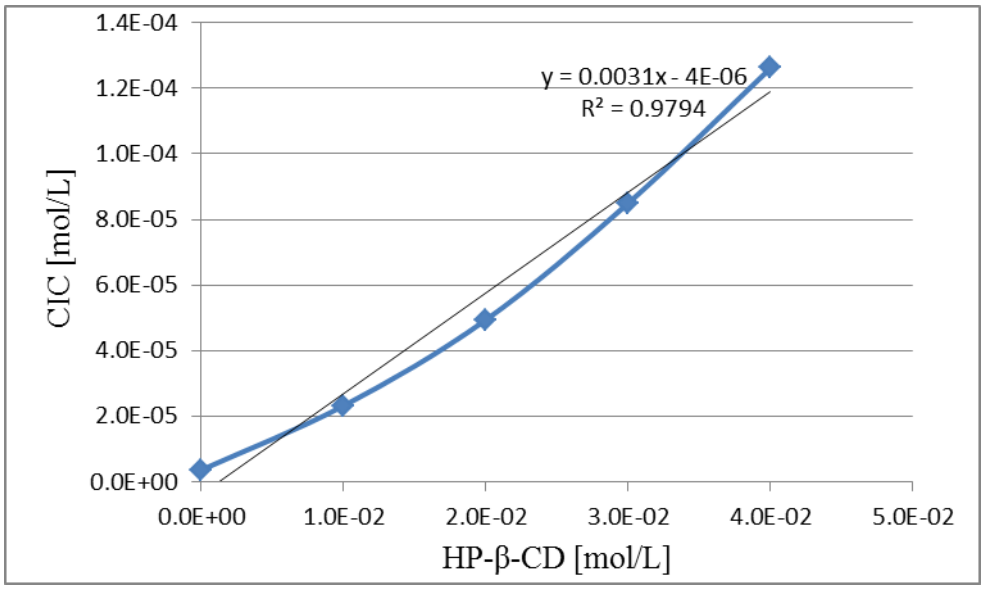


Figure 13. Solubility test of Ciclesonide with HP-β-CD after 48 hours.

The value of the complex stability constant K_s was calculated from the slope and the intrinsic solubility of the drug in the aqueous medium.

In the current studies, HP-β-CD was selected for preparation of the complex with CIC as it revealed higher stability constant (K_s =743.77 M^{-1}) than that of γ–CD/CIC (K_s=149.77 M^{-1}) (Figure 14). HP-β-CD is more suitable for forming complexes with CIC. Thus, in further studies only complexes HP-β-CD/CIC were used.

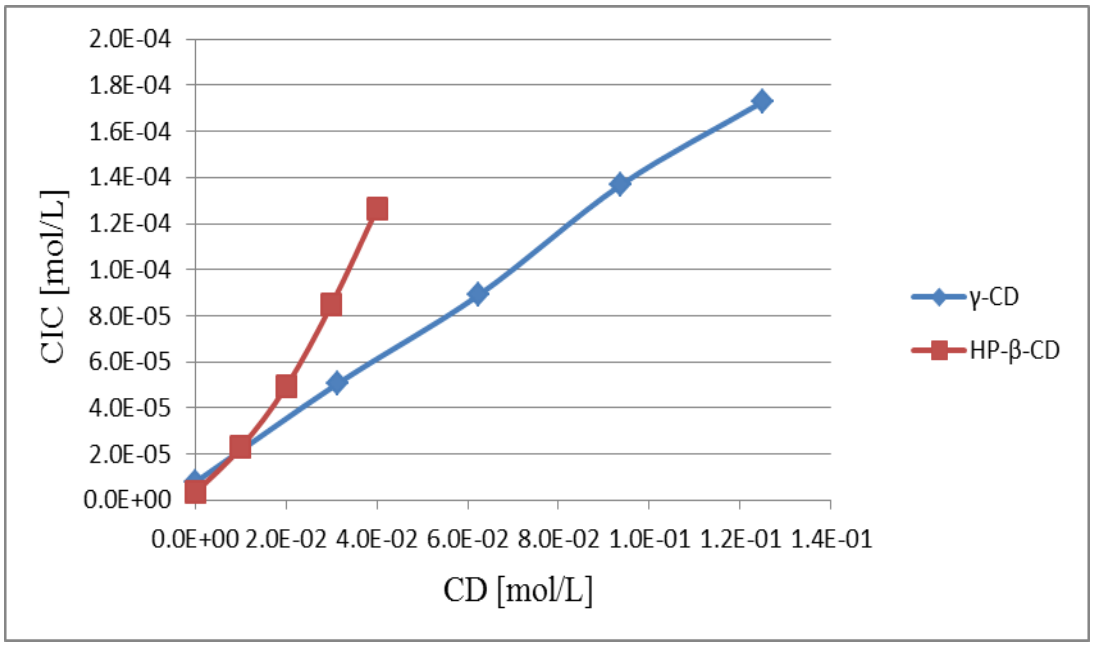


Figure 14. Solubility test of ciclesonide with HP-β-CD and γ-CD after 48 hours.

5.2 CHARACTERISATION OF HP-β-CD/CIC COMPLEXES

5.2.1 INFRARED SPECTRAL STUDIES

FT-IR is a useful technique to confirm the formation of an inclusion complex. FT-IR spectra of CIC, HP-β-CD, physical mixture and prepared inclusion complexes of CIC with HP-β-CD are shown in Figure 16.

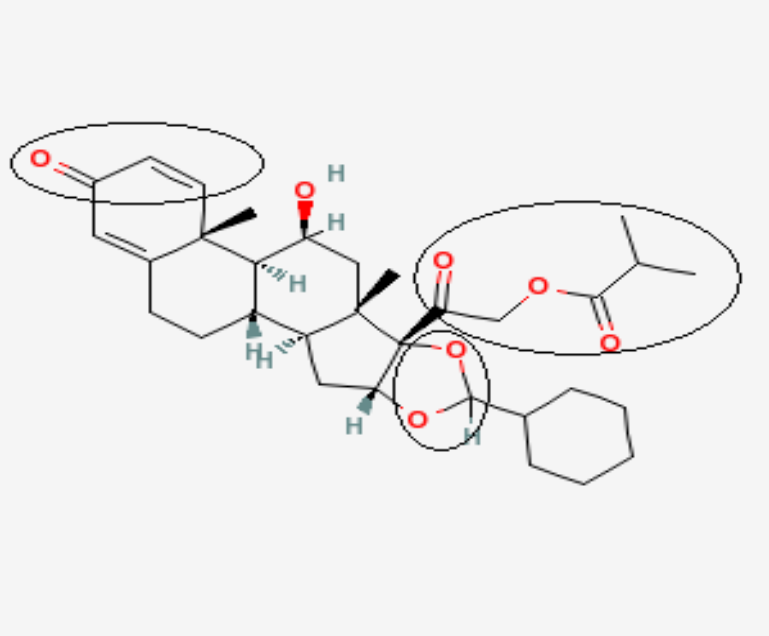


Figure 15. Chemical structure of ciclesonide.

CIC FT-IR (Figure 16 (a)) presents bands at about 1121 cm^{-1}, 1063 $cm^{-1}$and 1178 cm^{-1} due to C-O stretching vibrations in secondary alcohols and asymmetric C-O-C-O-C stretching vibrations in acetals. The spectrum within the range around 1242.55 cm^{-1} can be attributed to C-O stretching vibrations in esters in CIC. Absorption peak in the region at 1402.13-1465.96 cm^{-1} can be assigned to CH_2 and CH_3 asymmetric and symmetric deformation vibrations of methyl, isopropyl, and cyclohexyl groups and the peak at 1663.83 cm^{-1} presents C=O stretching vibration of α, β-unsaturated carbonyl functions. Absorption peak around 1753.19 cm^{-1} is due to C=O stretching vibration of keton and esters. CH_2 and CH_3 asymmetric and symmetric stretching vibration were observed at 2920 cm^{-1}. This is also presented in the literature (64).

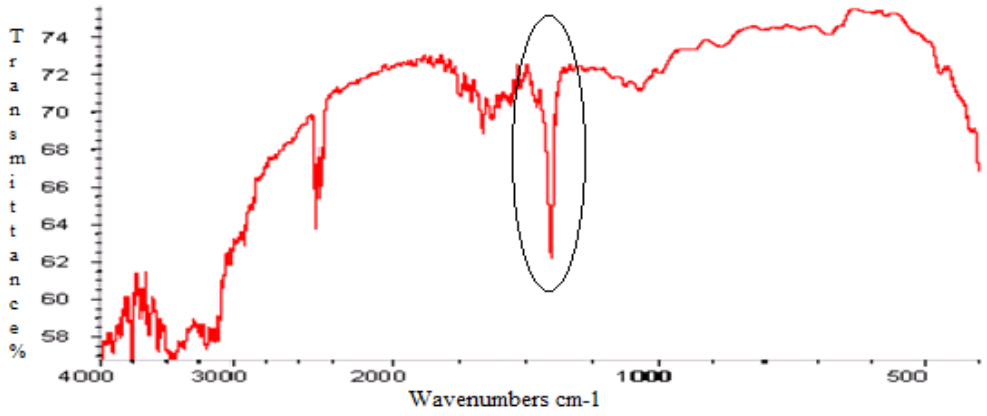
The IR spectrum of HP-β-CD (Figure 16 (b)) indicated OH group of the HP-β-CD in the range of 3200–3600 cm^{-1} wave number regions (65). The HP-β-CD spectrum showed prominent peaks at 2945.71 cm^{-1} (C-H), at 1651.02 cm^{-1} (H-O-H bending), and vibration bands of C-O and O-H groups at 1165.96 cm^{-1} and 1044.68 cm^{-1} respectively. Absorption peak at 1020 cm^{-1} was due to the C-O stretching band present in HP-β-CD.

As displayed in Figure 16 (c), in FT-IR spectra of physical mixture, were some significant difference from the respective spectra of each of the pure components as seen in Figure 16 (a), (b). It was observed that some of the peaks (at 2945.71 cm^{-1};1408.51 cm^{-1} and 1038 cm^{-1}) appeared with decreased intensity. The peak at 1165.96 cm^{-1} and 1044.68 cm^{-1} was shifted to 1159.57 cm^{-1} and 1038.30 cm^{-1} respectively. Some of the absorption peaks of CIC (at 1121.28 cm^{-1} and 1063.83 cm^{-1}) were not significant in physical mixture. This result suggested that strong physical interaction of CIC with HP-β-CD occurred.

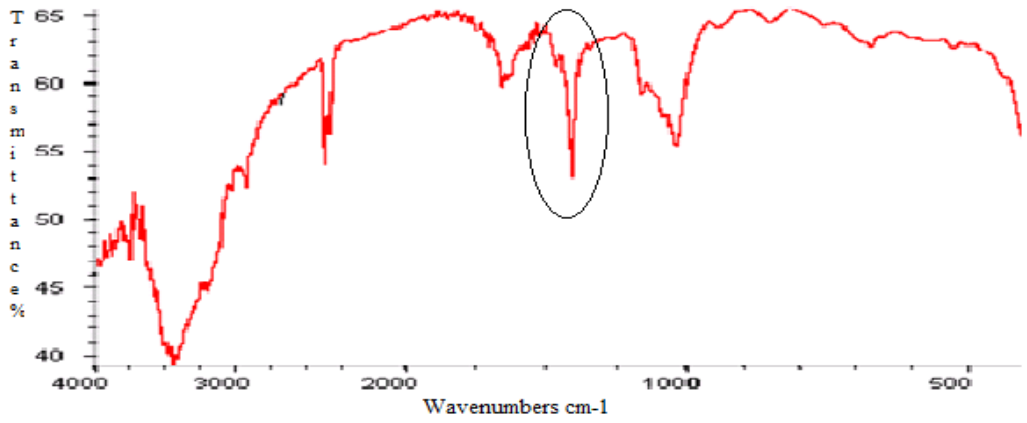
However, more significant differences could be seen in the FT-IR spectrum of the inclusion complex as shown in Figure 16 (d). Minor peaks and peak shifts were characterised in the complex spectra when comparisons were made to the corresponding pure drug. No peaks of CIC were observed at about 1121 cm^{-1}, 1063 cm^{-1}, 1178 cm^{-1} indicating the possibility of bonding C-O in secondary alcohols and asymmetric C-O-C-O-C in acetals of CIC with C-O and O-H bond of HP-β-CD. Disappearance of the active ingredient at 1663.83 cm^{-1} wave number region could be due to the bond of α,β–unsatured carbonyl group in CIC with hydrated bonds of HP-β-CD. The absence of the absorption peak at 1753.19 cm^{-1} on FT-IR complex signify the inclusion of the two carbonyl groups of CIC in the CD-ring cavity.

Intensity of the HP-β-CD peak at 1651.06 cm^{-1} did not decrease after the formation of complexes what means that drug can not produce complexes in solid state.

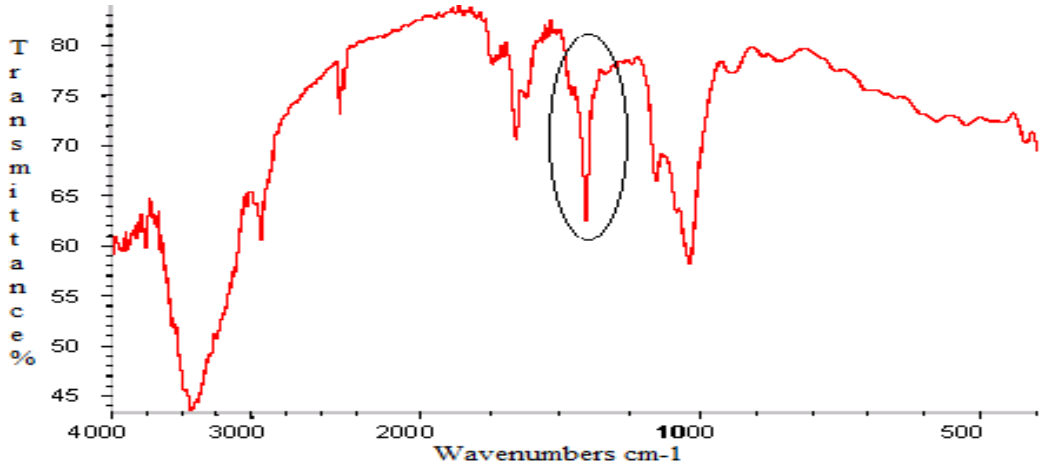
Additionally, the broader peak of OH stretching in the range of 3000 to 3600 cm^{-1} was found from the inclusion complex and physical mixture spectra, and this corresponded to the multiple OH functional groups of HP-β-CD molecules.


Transmittance %
74
72
70
68
66
64
62
60
58
4000
3000
2000
1000
500
Wavenumbers cm-1

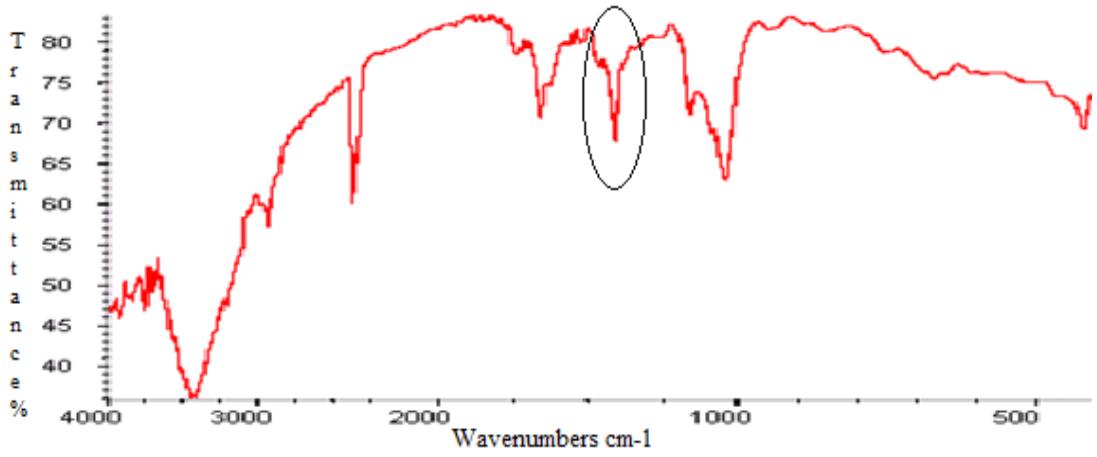
(a)


Transmittance %
65
60
55
50
45
40
4000
3000
2000
1000
500
Wavenumbers cm-1

(b)


Transmittance %
80
75
70
65
60
55
50
45
4000
3000
2000
1000
500
Wavenumbers cm-1

(c)



(d)

Figure 16. FT-IR spectrophotometer of (a) ciclesonide, (b) hydroxypropylbeta cyclodextrin, (c) physical mixture (d) CIC/HP-β-CD complexes.

5.2.2 THERMAL ANAYSIS OF INDIVIDUAL COMPONENTS AND COMPLEXES

Differential scanning calorimetry (DSC) is the most useful method of thermal analysis. By using DSC analysis, the formation of inclusion complexes may be observed. Structural changes are accompanied by heat flow to/from the sample. Samples absorb heat during the melting (endothermic reaction), while during the crystallization heat is released (exothermic reaction).

DSC method was used to analyze each component of the complexes. Curve shapes and peaks were observed to study melting temperature and enthalpy.

The method is applicable for determining the interactions between components as well as crystallness and amorphousness and other structures in which they are located. The DSC curves can explain or confirm a number of results that were obtained by other methods.

The thermal behaviour of all components were detected. Figure 17 shows the DSC results for CIC, HP-β-CD, physical mixtures and complexes HP-β-CD/CIC. A small peak around 65 °C is observed in every component, perhaps due to impurities. An endothermic peak for CIC was observed at 203.60 °C, which corresponded to the melting point. The melting point for pure CIC was reported to be between 203 °C and 213 °C (66). In the thermogram of HP-β-CD, three endothermic peaks are observed. Endothermic peak near 164°C probably corresponds to HP-β-CD fusion. DSC method shows that there were some

complexes in mixture which were prepared by complexation. Melting point for complexes (190.615 °C) was lower than physical mixture and CIC, what means that there were some interactions (possibly complexes formation). It is known, that the higher is reduction curve, the greater are interactions.

DSC alone cannot characterise the complex formation since the absence of the drug melting peak in the thermogram can result either from drug complexation or from its transformation to the amorphous state in a physical mixture.

The observed thermoanaytical properties depend on water content, crystal structure, heating rate, and atmospheric conditions.

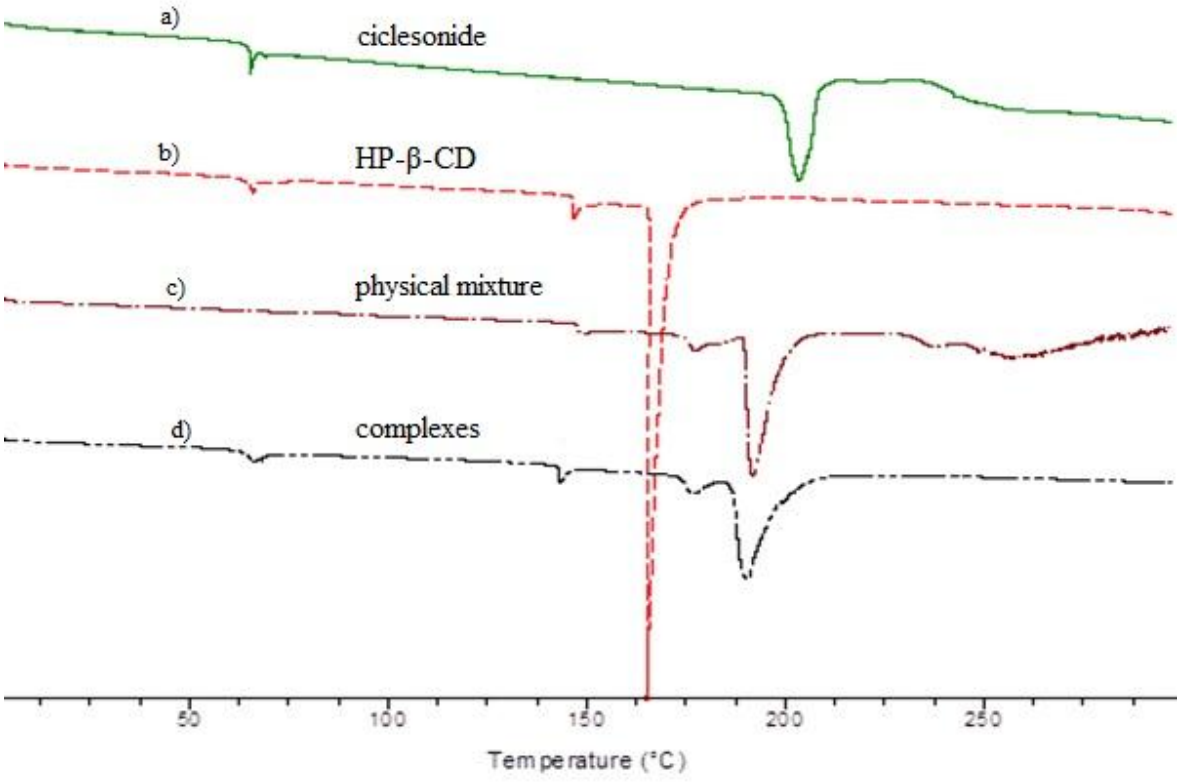


Figure 17. DSC thermogram of a) CIC b) HP-β-CD c) physical mixture and d) HP-β-CD/CIC complexes.

5.2.3 SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (SEM) gives qualitative information of the particles morphology but cannot be used as an indicative method for complex formation. SEM was used to visualize the particle diameter, structural and surface morphology of the powders.

The scanning electron microphotographs of CIC and HP-β-CD in Figure 18 and Figure 19 were taken before any procesing are performed. CIC were in the form of irregular particles. HP-β-CD appeared as amorphous spheres with concaves shapes. In contrast, the shape and morpholgy of the HP-β-CD/CIC complexes (1:1) changed distinctly in Figure 20.

Since aerosol particles are small, the total surface area of a powder is very large. A large surface area renders the particles subject to greater potential for charging and moisture uptake. In addition, the size of the particles renders them more susceptible to the influence of van der Waals forces. That is the reason why aggregats are formed.

In order to reach the alveoli, aeorsol particle size should be 5 μm (or lower) (35). Resulting powder was not so spherical particles, which are needed for inhalation delivery (67). Particles are bigger than expected and we could say that aggregats are observed in the Figure 20. CIC, HP-β-CD and HP-β-CD/CIC complexes were evaluated by SEM some months later by prof. Odon Planinšek at Faculty of pharmacy, University of Ljubljana, Slovenia. The changes in particles could appear because of transportation and other many factors, such as temperature and humidity leading to aglomerats.

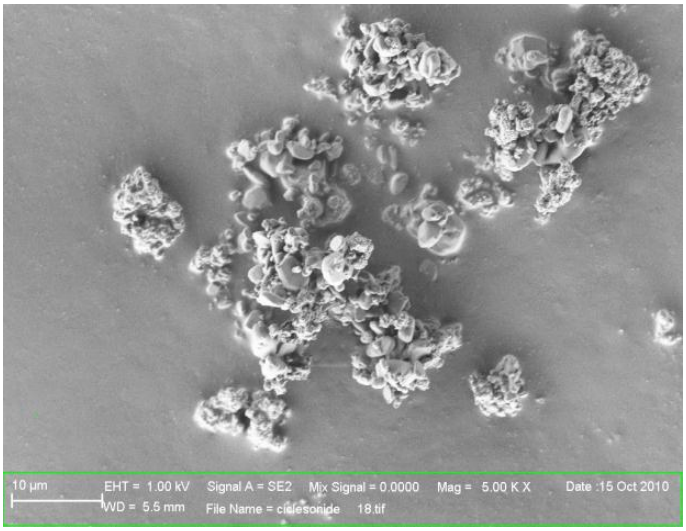


Figure 18. Representative scanning electron microscopy image of the ciclesonide.

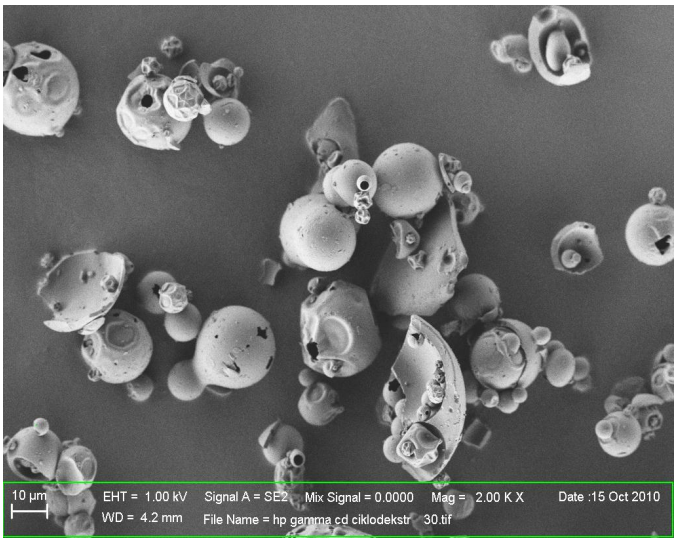


Figure 19. Representative scanning electron microscopy image of the HP-β-CD.

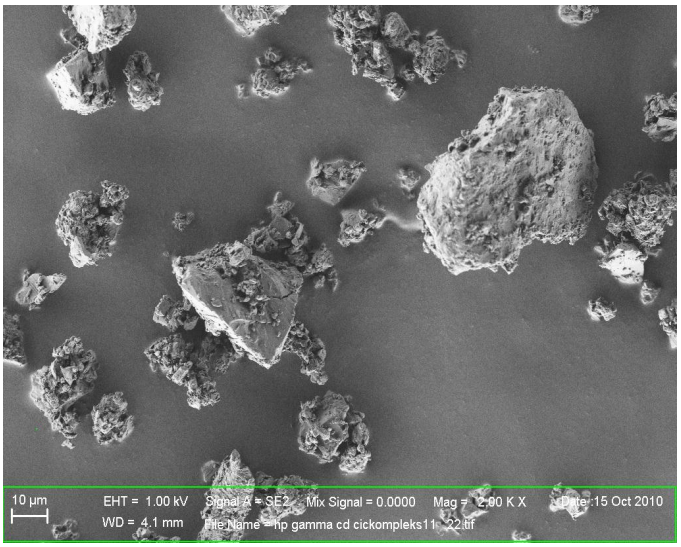


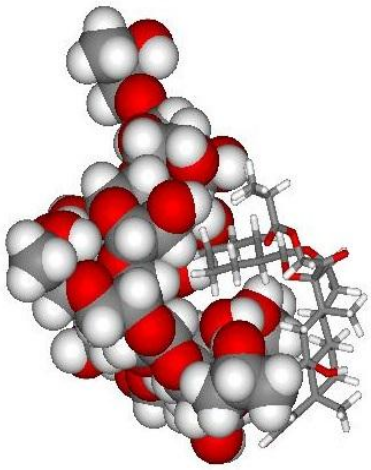
Figure 20. Representative scanning electron microscopy image of the HP-β-CD/CIC complex.

5.2.4 MOLECULAR MODELLING

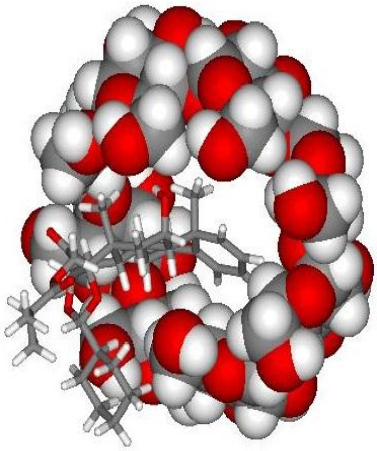
The conformational ensembles of CIC and HP-β-CD inclusion complexes that have been generated in this study were calculated using the MacroModel V9.11 suite software. Nonbonded interactions within 8 A ° for van der Waals' and 20 A ° for electrostatic interactions were included in all calculations. All the molecules were built in the builder subprogram of the Macromodel computational package. Monte Carlo Multiple Minimum (MCMM) conformational searching with 2000 steps was employed to find the lowest energy structures for the complex starting from four different configurations. The combination of MMFFs force field and GB/SA implicit representation of water as the

solvent were used. The same method was also used for γ-CD/CIC and β-CD/CIC inclusion complex.

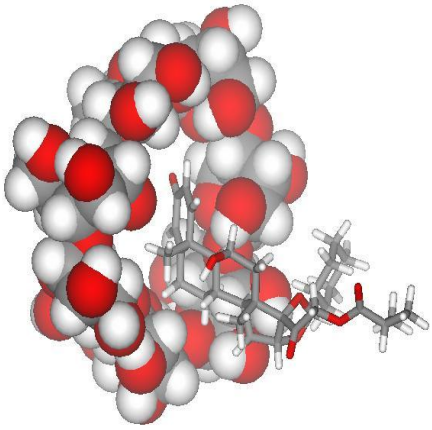
The ligand has shown favourable interactions with all cyclodextrin molecules and the propensity for complex formation that could improve solubility. The lowest energy (most stable) conformers of the HP-β-CD/CIC, γ-CD/CIC and β-CD/CIC inclusion complex are shown in Figure 21.



(a)



(b)



(c)

Figure 21. Molecular structure of (a) HP-β-CD/CIC complexes, (b) γ-CD/CIC complexes (c) β-CD/CIC complexes.

The intermolecular interaction between different cyclodextrins and ligand molecules are driven by the size of the cleft. The γ-CD cleft is the largest with the diameter of 1 nm and it is possible to accommodate the bulky part of the molecule including the hydrophobic, while the cyclohexane ring is interacting with the surface of the γ-CD. The size of the cleft of the β-CD is smaller with the diameter of 0.78 nm, which allows only a ring to protrude into the cleft. The rest of the molecule is exposed to the solvent. The HP-β-CD presents the smallest cleft, which is not deep enough to accommodate the ring. The rest of the CIC ligand interacts with the surface of HP-β-CD.

The qualitative assessment of the interactions observed between CIC and different cyclodextrins could be used to provide some information about rational for differences in observed solubility of CIC in presence of different CDs. Closer inspection of the complexes, it can be observed that CIC exposes hydrophobic surfaces to the aqueous environment, which is not an optimal conformation of the drug. The hydrophobic interactions in HP-β-CD/CD complexes are more prominent and more of the hydrophilic surfaces of CIC are exposed to the water. This may result in better solubility of CIC in presence of HP-β-CD.

This is in a general agreement with our experimentally observed data, and will need further computational experiments, such further conformational searches using different software and molecular dynamics simulation in presence of explicit solvent.

5.3 AERODYNAMIC CHARACTERISTICS AND AEROSOL PERFORMANCE

Dry powder for inhalationis is generally characterized as a powder mixture of coarse carrier and micronized particles (respirable particles) of drug (68). Micronised powder particles are strongly autoadhering, which makes them difficult to use in dry powder inhalation formulation. Due to their poor flowability properties, they are mixed with coarse carrier such as lactose monohydrate or glucose to give and interactive powder mixture. Furthermore, coarse particles (median size 20-100 μm) or inert carrier particles (usually lactose monohydrate, in typical weight ratio 67.5:1; carrier:drug) used to obtain uniform filling of fine drug particles that adhere to inhalation. In principle, the coarse carrier can also be respirable, but more commonly its particle size is much larger so that it is not inhaled but goes to the GI tract. In the design of dry powder inhalations with carrier particles, it is important to consider how to obtain good flow, the packing properties of the carrier/drug powder mixture and to obtain a good outflow from capsules and devices.

Drug amounts delivered to the lung are generally lower than then the amounts given orally. Inhaled drug particles are deposited in various regions of the respiratory tract, depending on the aerodynamic diameter of the particles. Coarse particles larger than respirable sizes are generally deposited in the upper respiratory tracts such as throats by the inertial impaction. During inhalation, coarse particles may help the emission of the drug from the device or capsules and improves its inhalation properties such as the delivery of the drug particles to the bronchi or alveoli (69).

Micronized particles must have an aerodynamic diameter of 1-5 μm to reach lower part of the lungs-alveoli, where deposited drugs can act directly on the lung tissue, be absorbed into the blood tissue or both. If particles are bigger than 5 μm, they can be deposited in oropharynx and caused side effects. The lung composition of HP-β-CD/CIC complexes was *in-vitro* evaluated by using Twin Impinger (TI).

Recommended starting dose of commercial drug CIC is 80 mcg twice daily and the highest recommended dose is 0.320 mcg also twice daily (70).

The amount of particles collected in the device, mouth, upper part–trachea and lower part-alveoli are shown in Figure 22 (a), (b), (c) and (d).

The data of the present work showed differences between aerodynamic particle size distribution when 0.160 mg or 0.320 mg CIC was submitted to the TI. When only CIC (40 mg) was in the capsule around 23.5 ± 2 % of the drug come to the lower part of the lung (alveoli). That is a little less than it is prescribed but anyway acceptable. In the Figure 22 (a), (b), (c) and (d) is shown that 50 % of CIC or more stay in a device and around 47 % of all emitted CIC come to inspiratory tract.

There is a difference between 0.160 mg and 0.320 mg HP-β-CD/CIC complexes filled with lactose till 40 mg in 1:1 proportion. Formulation with 0.320 mg HP-β-CD/CIC complexes in 40 mg capsules showed poorer inhalation performance than formulation with 0.160 mg of HP-β-CD/CIC complexes in 40 mg capsules. Three times higher RFE was evidenced in gelatin capsules with 0.160 mg of HP-β-CD/CIC complexes than in gelatin capsules with 0.320 mg of HP-β-CD/CIC complexes. More than a 50 % of complexes stay in trachea, in the case when 0.320 mg of complexes were filled with lactose till 40 mg.

When 0.160 mg of HP-β-CD/CIC complexes were filled with lactose monohydrate to fill a weight 20 mg capsules, the most particles stay in trachea and around 17 % of particles come to lowest part of the lung-alveoli. In the case when 20 mg gelatin capsules contain 0.360 mg of HP-β-CD/CIC complexes, the RFE was nearly 8 %.

RFE values were not so high as expected. Complexation of CIC with HP-β-CD does not improve the aerolisation behaviour of the active ingredient in the lungs. The poor physical fit between Rotahaler and twin impinger may have contributed to the low respirable fraction. High moisture content flow, wet capsules or powders, inaccurate washing and many more reasons have influenced results.

Because of limited research concering this topic, comparisement to other studies is not possible.

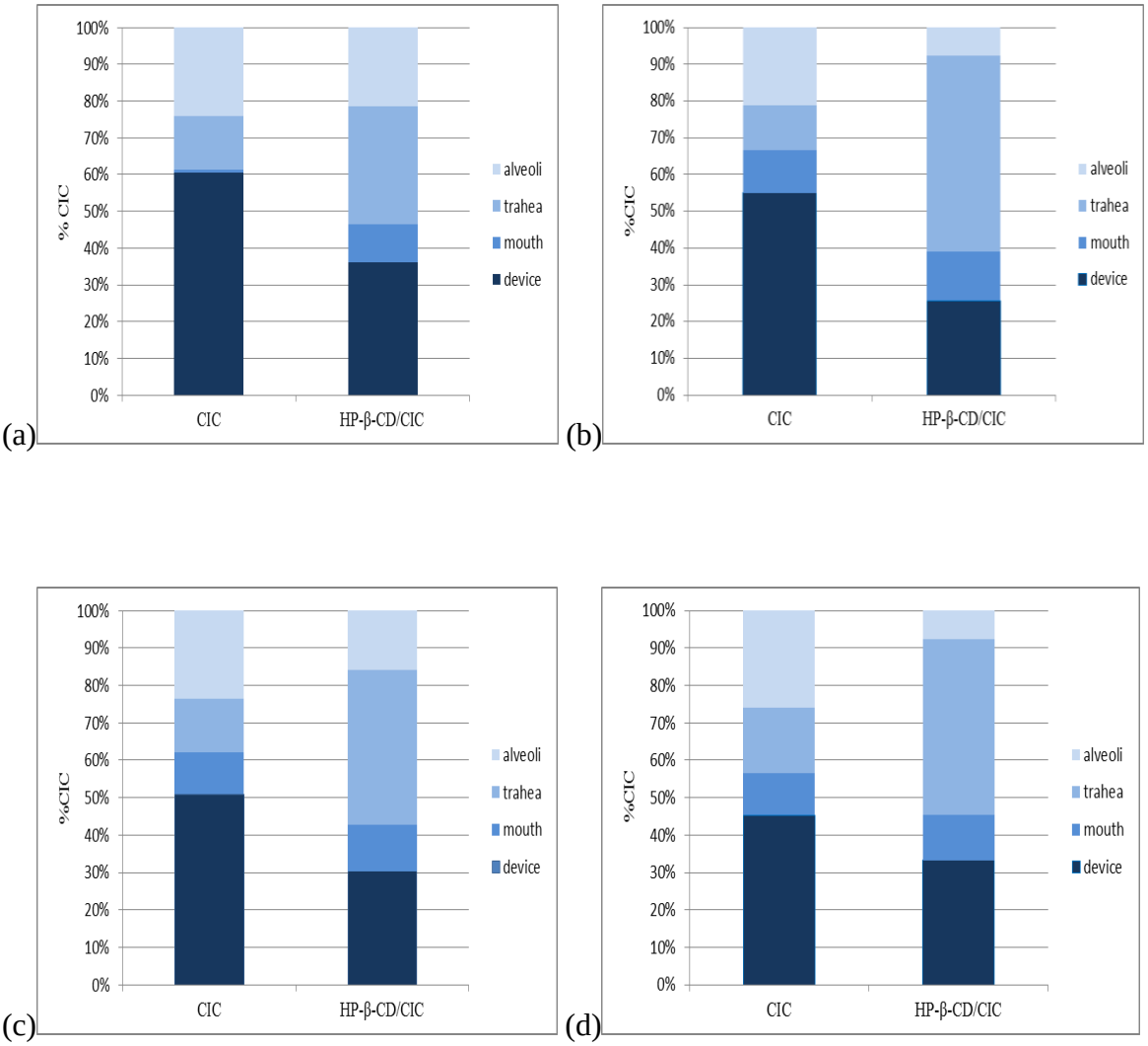


Figure 22. (a) 0.160 mg of HP-β-CD/CIC 40 mg capsules, (b) 0.320 mg of HP-β -CD/CIC 40 mg capsules, (c) 0.160 mg of HP-β -CD/CIC 20 mg capsules, (d) 0.320 mg of HP-β-CD/CIC 20 mg capsules.

6 CONCLUSIONS

The pulmonary administration of corticosteroids, such as CIC, has shown to be efficient in asthma therapy. CIC has low water solubility ($1{,}53\times e^{-3}$) g/l and favourable pharmacodynamic and pharmacokinetic profile, with potential for reduced local and systemic adverse effects. It was presumed that the formation of CD/drug complexes will improve the delivery of the active ingredient into the lungs. In this thesis work, HP-β-CD/CIC and γ-CD/CIC complexes were prepared and characterized by IR, DSC, SEM and twin impinger.

Molecular modelling calculations were used to foresee the possible orientations of and inside the HP-β-CD, β-CD and γ-CD cavity by prof. Mire Zloh, Faculty of Pharmacy, University of London. From the 3D picture of HP-β-CD/CIC complexes, it is signified that some part of the drug come into the CDs cavity. The hydrophobic interactions in HP-β-CD/CD complexes are more prominent and more of the hydrophilic surfaces of CIC are exposed to the water. This may result in better solubility of CIC in presence of HP-β-CD.

Results show that aqueous solubility of CIC was successfully increased by complexation CIC with HP-β-CD after 48 hours. The solubility constant of HP-β-CD was higher than in the case with γ-CD/CIC complexes. Due to this, it was decided to use HP-β-CD in the further research. The changes appearing in the IR revealed the complex formation. Some of the CIC peaks were shifted and some of them dissapeared. In the differential scanning calorimetry, the disappearance of the melting point of the pure CIC was not observed clearly. Images of scanning electron microscopy showed that complexes exist in aggregates of particular units. We expected smaller particles than we got.

Aerodynamics characterstics and aerosol performance, *in vitro,* tested by Twin Impinger showed that the respirable fractions of the emitted CIC dose comparable for both, CIC adhered to lactose monohydrate and HP-β-CD/CIC complexes adhered to α-lactose monohydrate. Better RFE (23 %) was shown in the capsules with 0.160 mg HP-β-CD/CIC complexes filled with lactose till 40 mg. Only CIC adhered to α-lactose monohydratepresents better delivery to alveoli. Nevertheless, it was proved, that CIC with HP-β-CD complexes are delivered in low quantity in the alveoli.

The main limitation of the developed formulations was CIC retained into the capsule and in the inhalation device, which could be due to the moisture content. In future studies, we should try to use any other complexation methods with drug and CD, such as supercritical fluids, slurry method, precipitation, co-evaporation, spray-drying, freeze-drying and sealed-heating. Studies should also consider methods to visualizing particle deposition, structure and change with respect to time.

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