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MASTER THESIS FOR THE DEGREE MASTER OF PHARMACY

Development of orodispersible films: A new age-appropriate dosage form for children

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Abstract (English)

In the recent years, governmental institutions have given incentives to the pharmaceutical industry in order to develop age-appropriate dosage forms for pediatric use. This has resulted in an interest in design of new and improved dosage form for pediatric patients. One of the many recent approaches is to take advantages of ODFs. Taste masking is a crucial step in the design of not only ODFs but all drug formulations intended for pediatric use. Many taste masking approaches has been reported, among them is the complexion of cyclodextrines with API.

The overall aim of this study was to investigate ODF as a new age-appropriate dosage form for children. The working hypothesis was that use of Hydroxypropyl cyclodextrines (HP-CD) as means to mask the bitter taste of drugs would not have a negative impact on the quality of the films. Inclusion complexes of the model drug Furosemide with HP-β-CD and HP-γ-CD were prepared by the shake flask method, and the equilibrium kinetics of complexation and the phase solubility was studied, and stability constants were estimated.

A design based on several 2^2-full factorial designs with center point was applied to investigate factors influencing the properties of ODFs. Solvent casting was used as manufacturing method for ODFs. Three different water-soluble film-forming polymers (Lycoat RS 720, Hydroxypropyl methyl cellulose and Hydroxypropyl cellulose) were evaluated. Glycerol was used as plasticizer and studied on different levels (0.2-1 % w/w), together with the two types of drug-inclusion complexes at different levels (0.1-1 % w/w). The prepared films were characterized with respect to physical and mechanical properties, and the results were analyzed with the help of multivariate data analysis (MVA) to investigate the effects of the different variables on films' quality.

The results showed that inclusion complexes were successfully incorporated in ODFs. Statistical analysis revealed that the incorporation of FR: HP-CD inclusion complexes in ODFs did not have a significant effect on the quality of ODFs, and Lycoat RS 720 appeared more suitable for ODFs than HPMC and HPC at investigated conditions.

Based on this study Lycoat RS 720 films seems interesting to be taken in an optimization design to investigate the optimum settings in which they provide desired ODFs for pediatric use.

Abstract (Norwegian)

I løpet av de siste årene har myndighetene innvilget økonomisk støtte til legemiddelindustrien til å formulere alderstilpasset doseringsform for pediatrisk bruk. Dette har skapt stor interesse for å utvikle eller forbedre doseringsformer for pediatriske pasienter. En av flere tilnærminger som brukes for å formulere legemidler til bruk hos barn er å utnytte fordelene "orodispersible" filmer" (ODF) tilbyr som legemiddel formulering. Smaksmaskering er et avgjørende trinn i formuleringen ikke bare av ODFer, men alle legemidler formuleringer som er beregnet for pediatrisk bruk. Mange smakmaskering tilnærminger har blitt rapportert blant dem er dannelse av inklusjon komplekser mellom cyklodekstriner og API.

Det overordnede målet med denne studien var å utforske ODF som en ny alderstilpasset legemiddel formulering for barn. Arbeidshypotesen var at bruk av hydroksypropyl cyklodekstriner (HP-CD) for å maskere den bitre smaken av legemiddel ikke ville ha en negativ innvirkning på kvaliteten på filmene. Inklusjonskomplekser av modell-legemidlet furosemide med HP-β-CD og HP-γ-CD ble fremstilt ved ristemetoden, og likevektskinetikk for kompleksdannelse ble studert i tillegg til faseløselighet. Stabilitetskonstanter for de to inklusjonskompleksene ble estimert.

Et design basert på flere 2^2-full faktorielt design ble brukt til å studere hvordan ulike variable påvirker egenskapene til ODFer. Støping ble brukt som fremstilling metode for ODFer. Tre forskjellige vannløselige filmdannende polymerer (Lycoat RS 720, hydroksypropylmetylcellulose og hydroksypropylcellulose) ble evaluert. Glycerol ble benyttet som mykgjører, og undersøkt på forskjellige nivåer (0,2 til 1% vekt / vekt), sammen med de to typer inklusjonskomplekser på forskjellige nivåer (0,1-1% w / w). .De fremstilte filmene ble karakterisert med hensyn til fysikalske og mekaniske egenskaper, og resultatene ble dataene analysert ved hjelp av multivariat dataanalyse (MVA) for å undersøke effektene av de ulike variablene på filmenes egenskaper.

Resultatene viste at inklusjonskomplekser ble inkorporert i ODFer. Statistiske analyser viste at inkorporering av FR: HP-CD inklusjonskomplekser i ODFer ikke hadde en signifikant effekt på kvaliteten av ODFer, og Lycoat RS 720 var tilsynelatende mer egnet for ODFer enn HPMC og HPC under studerte forhold.

Baserte på denne studien er Lycoat RS 720 en interessant polymer å studere videre i et optimalisering design for å finne den optimale sammensetningen som gir ønskede ODFer for pediatrisk bruk.

1   Introduction

From 1968 when Shirkey published the paper addressing children as "therapeutic orphans" due to the lack in clinically tested drugs for children(2), until 2007 when the European Union (EU) came with a new legislations on medicinal products for paediatric patients, it took about 40 years to acknowledge that most of the drugs used in paediatric are «off label». This means that drugs are used outside their marketing authorization, and are therefore not tested for safety, dosing, and efficacy in children, or are available in an appropriate dosage form for use in this population (4, 5). Formulating appropriate dosage form for paediatric use has been proven to be challenging (4). Some of these challenges will be presented below.

1.1   The pediatric population

The paediatric population is not a monolithic population. This population can be divided into different subpopulation (6):

- Preterm new-born infants
- Term new-born infants, neonate (0-27 days)
- Infants and toddlers (28 days-23 months)
- Children (2-11 years)
  - Preschool children (2-5 years)
  - School children (6-11 years)
- Adolescent (12-16/18 years)

This means that a formulation which is suitable for one subgroup might not be the best choice for another subgroup. Therefore each subgroup might require different appropriate drug formulation. For example formulation that is suitable to adolescent might not be suitable for infants and toddlers and vice versa.

1.2   Biological and pharmacological development

It is well established that children are not small adults, but rather distinct entities with regards to pharmacotherapy. They differ from adults with regards to their physiological and psychological development, their toxicity related to medicines and their taste preferences. The ability to absorb,

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distribute, metabolise and eliminate drugs by the very young in the paediatric population is affected by a number of factors, among others the development and maturation of organs, the body fat distribution, the pH of the different segments of gastro intestinal tract etc. (5).

1.3   Oral administration

The major factors influencing patient compliance are the selection of the route of administration and the dosage form (7). In the general population, oral administration is the most common and preferable route of administration. This is due to the convenience and flexibility that this route of administration offers to patients. But that comes also with his own challenges (8). Tablets and capsules are the most frequently used oral dosage forms, partly because of properties such as stability, dosing accuracy, packing volume and opportunities for taste masking. Many children, however, will find that tablets and capsules are difficult to swallow, and alternative oral formulations will therefore be necessary. For some commonly used drugs in children, alternatives are liquid formulations, for example antibiotics mixtures (9).

Liquid formulations have major disadvantages such as chemical, physical or microbial instability, taste issues and lack of controlled release properties (10). Those disadvantages never been an obstacle for liquid formulation to be considered as the most suitable oral dosage form to children less than six years.

In 2008 a paradigm shift from liquid oral dosage form to solid dosage form for paediatric medicines was suggested by the World Health Organization (11). Orodispersible dosage forms was among the various recommended solid oral dosage form (12). Among the orodispersible dosage forms we find orodispersible tablets, lyophilised wafers and thin films. Orodispersible films, will when placed in the mouth disperse or melt rapidly on the tongue. Therefore they show great promises for children as they are easy to administer, do not require additional water and, as long as dispersion is rapid, are difficult to spit out and could provide a range of dosages appropriate for use in younger children (13).

1.4   Pediatric Dosage forms

In pharmacy practice, patient compliance is an important corner stone if not the most important. Patient compliance is not an easy task in the pediatric population therefore coming with ways and tools that will ease drug administration is very important for this group of patient. The World Health Organization (WHO) describes an ideal drug delivery system for children as follows (12):

- Acceptable palatability
- Possibility of weight-based-dosing and dose-titration
- Use of safe, well established and stable excipients

Other parameters that have to be taken into consideration when formulating dosage form for pediatric patients are (4, 11)

- Sufficient bioavailability
- The uniformity of the dose have to be within acceptable range
- Safe administration
- Socio-cultural acceptability
- Precise and clear information about the product and use
- Friendly to parent and caregiver.

Finally, an ideal pediatric dosage form should aim at reducing dosage frequency and provide reliable administration (10).

Oral dosage formulations available on the market for pediatric use are listed in table 1 (6). In the recent years, governmental institutions has given numerous incentives to the pharmaceutical industry to formulate age-appropriate dosage form for pediatric use. This resulted in an interest for the pharmaceutical industry to use new technology to design or improve dosage form for pediatric patients such as multi particles system (MUPS), mini tablets, orally disintegrating tablets, orodispersible films etc. These dosage form can be used to ease pediatric drug administration and increase patient compliance in the pediatric population (14).

Table 1: Oral pediatric dosage forms available on the market (8)

Dosage form	Formulations
Liquid	Solutions, syrups, suspensions
Solid	powders, granules, effervescent tablets, orodispersible tablets, orodispersible films, chewable tablets, mini-tablets, immediate and modified release tablets and capsules

1.4.1 Liquid dosage form

The major reasons behind the perception that liquids are appropriate dosage form for pediatric patients are dose adjustment flexibility and ease to swallow. Until the age of around five months toddlers can only swallow liquids due to the extrusion reflex (15).

Many drugs have a bitter taste, and are rejected by children because of the bad taste(16). Liquids are more challenging when it comes to taste masking compare to solid dosage form, because solutions come in close contact with the taste buds. The choice of excipients is restricted for use in pediatric formulation. Therefore taste masking can be more challenging with liquid dosage form. Other disadvantages of liquid dosage forms as compared to solid forms are stability, cost and dosing error (4, 12).

The solution to the disadvantages encounter with liquid dosage forms may be solid dosage forms.

1.4.2 Solid dosage form

Solid dosage forms offer several advantages over liquid dosage forms, such as(17):

- Possibility of using excipients that are not recommended for pediatric patient is low
- Low manufacturing costs
- Numerous ability to mask the taste

- Modification of drug release

- Stability

- Possibility of achieving higher content uniformity

- Easy to administer.

The main disadvantages include(17):

- Swallowing issues

- The need of water for swallowing

- Little dosage flexibility

- Variation in bioavailability

- The risk of choking.

Orally dissolving or dispersible drug formulations, such as orodispersible tablets also known as melting tablets and orodispersible films (ODF), can be used to overcome problems related to liquid and solid dosage form. Because they have many of the benefits of both liquid and solid dosage forms. Like a solid dosage form ODFs are stable, easy to administrate and as liquid dosage form ODFs are easy to swallow and flexible to dose.

1.5   Taste perception and palatability

«If it tastes bad if must be good for you», this quote is not valid for the paediatric population. To ensure patient compliance, paediatric dosage forms have to be formulated either by having a minimal impact on lifestyle or by having an appropriate appearance (colour, smell, texture and palatability), especially for oral liquids but also for orodispersible products and powders. It is often difficult to assess the taste attributes of the drug formulation, particularly in younger children who are not capable of expressing their taste sensations and mouth feelings adequately (18). The American Academy of Paediatrics did a survey to find out why children do not comply with their treatment regimens (19). They found that bad taste is the main reason in the same line with dosing frequency and side effects. Palatability is a major characteristic for all pediatric drug delivery system. It rank highest after efficacy and safety among parent when it comes to children medication (20). New research shows that the perception of bitter taste are age-dependent, and bitter blockers

have been found to be less effective as taste masking strategy in children than in adults(17). The bitter taste is thought to have evolved through evolution as a deterrent against ingestion of potentially harmful substances (17, 21). Taste masking is a major issue while formulating dosage form for the paediatric world.

1.6 Orodispersible film

Ph.Eur. defines ODFs as*: "single or multilayer sheets of suitable materials, to be paced in the mouth where they disperse rapidly"(22)*. The literature defines ODFs as strips or thin films which are intended to disintegrate in the mouth within seconds of being in contact with saliva on the tongue (13, 23). In the literature, various terms are used to refer to orodispersible film. . Some of those terms are; wafer, oral film, thin strip, orally dissolving film, flash release wafer, quick dissolve film and melt-away film (13, 24, 25). In this study it is referred to as orodispersible film. This novel drug delivery system provides some opportunities that can be taken advantages in pediatric drug formulation. However it comes with some drawbacks too. These drawbacks have to be taken into account while formulating ODFs. The advantages and disadvantages of ODFs are listed in Table 2 below

Table 2: Advantages and disadvantages of ODFs(13)

Advantages	Disadvantages
Rapid onset of action	Drug loading is limited max 62 mg
Patient complaint	Added cost for taste masking of bitter drugs
No need of water to swallow	Dose uniformity is technical challenging
Accurate dosing	Hygroscopic in nature
	Require special packaging
	Thermostable API

1.6.1 Manufacturing methods

There are two majors methods for manufacturing ODFs described in literature (17): solvent casting and hot met extrusion. However other techniques can be used as well like semisolid casting, solid dispersion and extrusion rolling. Solvent casting is the preferred method in the pharmaceutical industry because the content of uniformity is between 1 and 2 % (13, 26). In this study the method used was solvent casting. Briefly, solvent casting method is done as follows: dissolved or suspended API is added to a viscous solution made of film forming polymers. The solution is then poured into glass or teflon coated trays where the solvent is evaporated and the film is formed. The casting can also be done using a film casting apparatus equipped with a coating knife to homogenously distribute the solution on a release liner (Figure 1). After drying the Films are cuts into single dose units and packed separately (13, 27).
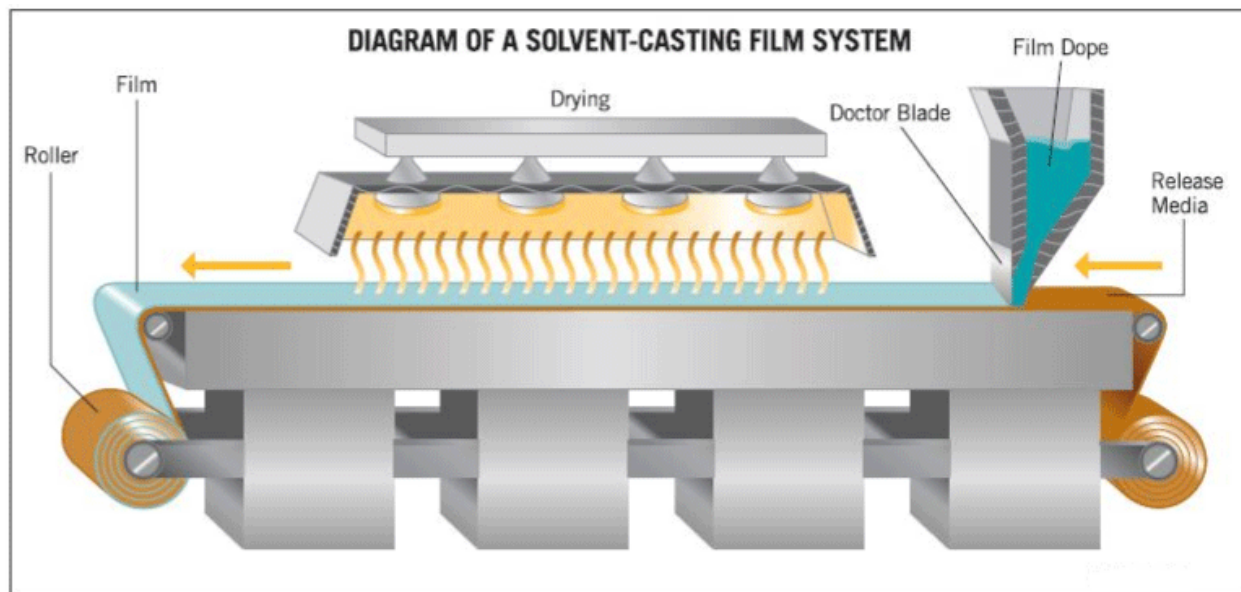
Figure 1: Picture of solvent casting apparatus(28)

1.6.2 Taste masking

Different type of taste masking agent and technique for masking taste of bitter API in ODFs are reported in the literature. Taste can be mask by the addition of sweeteners and flavors, or by

complexation of the bitter-tasting API with ion exchange resin or cyclodextrines (29-31). Another approach is to apply a physical barrier, for instance by film coating the API crystals (23).

In this study formation of inclusion complexes with cyclodextrines was used as taste masking strategy (see *Frame 1 Cyclodextrines*). Hydroxypropyl-beta-cyclodextrin (HP-β-CD) and Hydroxypropyl-gamma-cyclodextrines (HP-γ-CD) were used as taste masking agents (32). Inclusion complex formation between cyclodextrines and API has been proven effectively to mask the taste of bitter API in previous studies (33, 34). The effectiveness of a taste masking strategy can be assess by electronic taste sensing system (electronic tongue), human taste panel, disintegration time or spectroscopic drug dissolution (35, 36). It has been suggested that a drug release below 10% during the first 5 minutes of dissolution can be used as an indicator of successful taste masking (37). However this cannot be applied to disintegrating systems because they are meant to disintegrate faster. And a fast disintegration imply a fast dissolution.

Frame 1

1.6.3 Cyclodextrines

Cyclodextrines (CD) are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose (*Figure IIa*). Due to the restricted rotation about the bonds of the glucopyranose units, the cyclodextrines are not perfectly cylindrical in shape, but cone shaped or toroidal (*Figure Ib*).
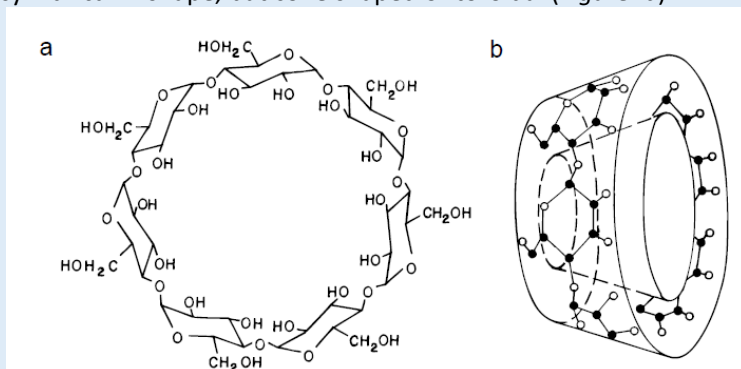
Figure 2: a) Chemical structure of β-cyclodextrine, b) toroidal shape (adapted

The central cavity is relatively hydrophobic, whereas the outer surface is hydrophilic. This gives cyclodextrines solubilizing properties. The hydrophobic parts of a poorly water-soluble guest molecule (e.g. drugs) can associate with the hydrophobic parts of the cyclodextrines host (*Figure III*). The stoichiometry of the formed inclusion complex may be 1:1 drug-CD or 1:2 drug-CD (*Figure IIIa-b*) or even higher order depending on the structure of the guest molecule (drug).

The most commonly used cyclodextrines are α-CD, β-CD, and γ-CD, which consist of six, seven, and eight glucopyranose units, respectively (3). Substitution of the glucopyranose units may be used to modify the aqueous solubility of the CD; the hydroxypropyl substitution of the cyclodextrines will increase the solubility as compared to the unsubstituted cyclodextrines.
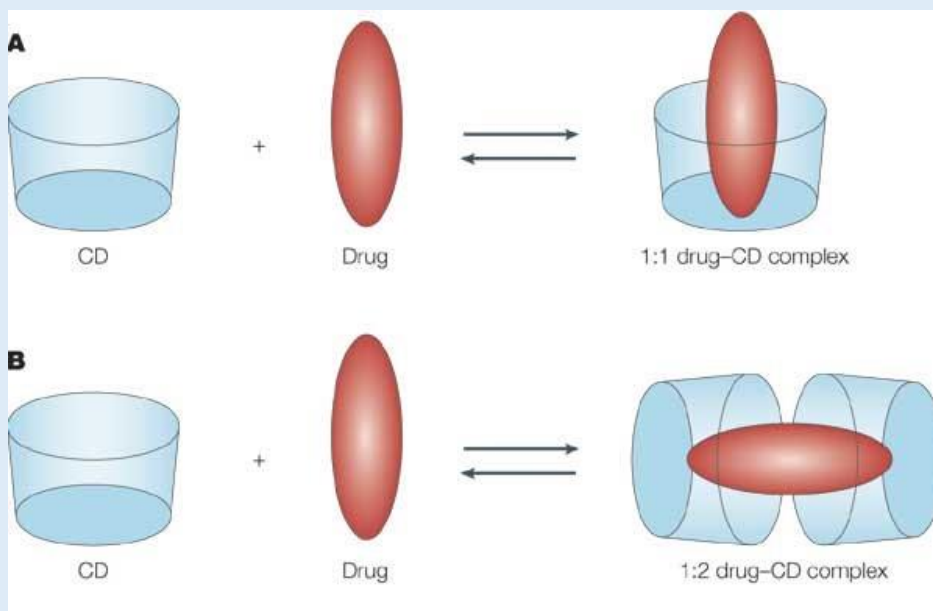
Figure 3: Schematic illustration of the association of drug and cyclodextrine (CD) to form inclusion complexes. a) 1:1 drug-CD complex, b) 1:2 drug-CD complex (adapted from(38))

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1.6.4   Film composition

The typical composition of an ODT consists of a water-soluble polymer as the film former, plasticizer, and the drug in the ratios shown in Table 3 (17). Sweeteners, flavors, colors and saliva stimulating agents are frequently added, also fillers and surfactants are used to manipulate with the disintegration time.

Table 3: Typical composition of ingredients in ODFs. The amounts are given as percentage of the dry film(13).

Ingredients	Amount(w/w)
Drug(API)	1-30%
Water Soluble Polymer	40-50%
Plasticizer	0-20%
Sweetener, Flavor, Color, etc	0-40%

1.6.4.1   Active pharmaceutical ingredient

In general, all API that can be administered orally are potentials candidates for ODFs, but the ideal API to incorporate in ODFs should have following characteristics (36, 39, 40):

- Pleasant taste
- Low dose, generally less than 30 mg per dose
- Low molecular weight
- Soluble and stable in water and saliva
- Partially unionized at pH of oral cavity
- Able to permeate oral mucosal tissue.

In this study Furosemide (FR) was used as API (see *Frame 2 Furosemide*).   FR fulfills most of the criteria listed above but it has a bitter therefore that have to mask.

Frame 2

1.6.4.1.1   Furosemide
IUPAC name

4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic (see Figure IV for chemical structure)
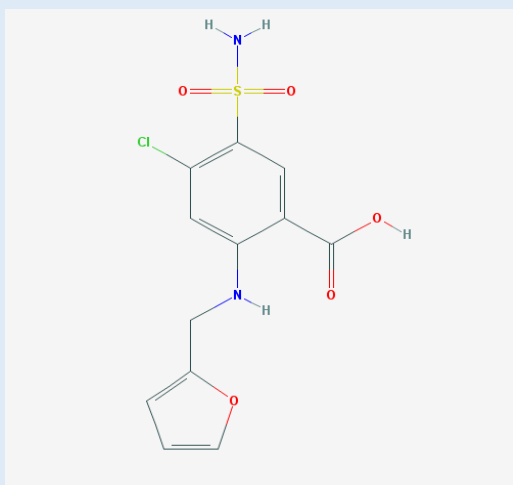
Figure 4: Chemical structure of furosemide (1)

Indications and pharmacological effects

Furosemide is a loop diuretic with fast onset and short duration that is used for edema in heart failure and chronic renal insufficiency. The physiologic effect of Furosemide is by means of increased diuresis at Loop of Henle(41).

Pediatric relevant dose

Oral administration according to BNF for children (41):

Children   1   month   –   12   years: 0.5-2mg/kg 2-3 times daily; higher doses may be required in resistant edema, not exceed 80 mg daily

Children   12-18   years: 20-40 mg daily, increased in resistant edema to 80-120 mg daily

1.6.5   Film forming Polymer

The film forming polymer is the main excipient in ODF. To obtain fast disintegration and pleasant mouth feeling the film former should be water-soluble. The film should also possess sufficient mechanical properties for handling packaging and storage. The properties of polymers depend on their molecular weight. Polymers with low molecular weight generally increase disintegration rate

(shorter disintegration times) as compared to polymers with high molecular weight, whereas the mechanical properties are generally is better for those with high molecular weight (17). Also viscosity of the film formulation increases with increasing molecular weight. This is an important parameter for the manufacture, as the viscosity should be high enough to prevent sedimentation during drying, but not too high to allow mixing and pouring and proper spreading during casting (17). Also the evaporation of solvent will be slower and the drying time longer if the viscosity is too high.

ODFs are typically prepared from water-soluble polymers, such as Hydroxypropylmethylcellulose (HPMC), Hydroxypropylcellulose (HPC), Carboxymethylcellulose (CMC), modified starches, Pullulan, Polyvinylpyrollidone (PVP), polyvinyl alcohol (PVA), pectin, gelatin, sodium alginate, and Maltodextrins (13, 39, 40). Also commercially available fast dissolving film forming polymers are used, such as Lycoat® a modified Hydroxypropyl starch from corn.

1.6.6   Plasticizer

Plasticizers are used to modify the mechanical properties of the films and ensure preparation of flexible and non-brittle films. Plasticizers are small molecules that intervene with the polymer chains of the film network and lower the glass transition temperature. Typical plasticizers used in ODFs are glycerol, propylene glycol, sorbitol, and low molecular macrogols (13). However, water molecules can also act as plasticizer in polymer films. Identification of the appropriate amount of plasticizer for the specific formulation is essential, because too high concentrations may result in stability problems, tacky and too flexible films (17).

1.7   Characterization of films

1.7.1   Thickness

ODFs should provide accurate dose. The accuracy of the dose in ODFs is correlated with the thickness of the film. Therefore ODFs thickness should be measured. This can be performed by using micrometer screw gauge. It should be done at least at three different places and the average of three values can be calculated (26, 36). The thickness of film should be in range 5-200 μm(23).

1.7.2   Disintegration

The Pharmacopoeias do not have a predefined test when it comes to disintegration of ODFs. Search in the literature point to numerous disintegration test who have been used for ODFs. The most prominent are the petri dish and the drop method.

1.7.2.1   *Petri dish method*
The petri dish method is characterized by fact that the disintegration time is the time that it takes for a piece of film to be completely disintegrate. Test is carry out by placing one piece of film in a petri dish then adding 2-3 ml of water or phosphate buffer (25, 42, 43).

1.7.2.2   *Drop method*
The drop method is characterized by the fact that the endpoint is the duration it takes for one drop of water or phosphate buffer makes a hole in the film or tears it apart. The test is carry out by placing a piece of film in a slide frame and applying a drop of water of phosphate buffer on it. (25, 27, 42).

These methods provide good ability to characterize the disintegration pattern of film with different thicknesses. They are no endpoint requirement when it comes to disintegration of ODFs. But some studies apply the endpoint requirement from to ODTs. The Ph.Eur require that ODTs should disintegrate within 3 minutes(44). In the other hand the Food and Drug Administration (FDA) in the United States of America require that the film should disintegrate within 30 seconds(45).

1.7.3   Moisture content

The residual solvent (moisture content) is wanted in the final film because it avoid film brittleness. But at the same time moisture content have a profound influence in the mechanical properties of the film and the stability. Therefore moisture content have to be measured. The literature describe different methods to measure moisture content. One of those method is by using infrared moisture analyzer(46).

1.7.4   Drug content
Single-dose preparations require an even distribution of the API in the polymer matrix. This can be achieve by dissolving the API in the matrix. But the solubility of the API in the polymer matrix

is not a requirement. In the literature they are numerous successful encounters of API suspended in polymer matrix in film formulations(47, 48).

An even distribution of the API is been proven defiant, therefore a dose uniformity testing is required. The most adequate test for this task is the test of uniformity of dosage units describe in the European Pharmacopeia.

The test is describe as follows in the monograph(49):

"10 individual film pieces with a single dose are required and completely dissolved. The drug content is determined according to the validated assay. The uniformity of dosage units is assessed calculating the acceptance value (AV)".

1.7.5   Mass

There is no standardized test nor requirement for ODFs to be weigh. But weighing ODFs is a useful quality control tool to ensure that excipients and API are evenly distributed in the film. An analytical balance can be used to weigh ODFs and average weight can be determined for each film. Ideals film should have nearly constant weight(50).

1.7.6   Mechanical properties

Characterization of films mechanical properties is performed not only to guaranty good manufacturing, packing but also to make sure that the product is not damage when it is handle by the patient. Another reason for characterizing films mechanical properties is that factors like moisture content, plasticizer, polymer type, thickness have on puncture strength and elongation(46).

 A puncture test is one of the test who can be performed on polymeric films to characterize mechanical properties. Puncture test is preferable to pharmaceutical films polymer compare to tensile test (51, 52). The puncture test is performed as follows:

A piece of film cut at a selected size is clamped in between two test plates with a cylindrical hole in the middle. The velocity in which the puncturing probe is moving toward the film surface is

predefine. Displacement and force applied on the piece of film are measured. Characteristic of the film like puncture strength and elongation are assessed based on these measurements (42, 46).

2 AIM OF THE STUDY

The overall aim of this study was to investigate ODFs as a new age-appropriate dosage form for children. The working hypothesis was that use of Hydroxypropyl cyclodextrines (HP-CD) as means to mask the bitter taste of drugs would not have a negative impact on the quality of the films. The study was divided in the following sub goals:

- Identification of suitable concentration ranges of water-soluble film forming polymers and plasticiser (glycerol) and drying conditions for the preparation of films with reasonable film thickness and disintegration time in phosphate saline buffer (PBS) pH 7.4 simulating saliva to serve as platform for ODFs. .

- Preparation of HP-CD inclusion complexes of model drug (Furosemide) using the shake-flask method, including the determination of time required to reach complex formation equilibrium and the phase solubility constant.

- Systematically study the influence of type of HP-CD inclusion complex, type of film forming polymer, concentration of inclusion complex and concentration of plasticizer (glycerol) on physical and mechanical properties of ODFs using design of experiment (DoE), and evaluate the effects by multivariate analysis (MVA).

- Characterization of prepared ODFs with respect to film thickness, disintegration time, dosage uniformity (uniformity of mass and content), rest moisture content, puncture strength and elongation to break.

3   Materials and Methods

3.1   Materials

3.1.1   Active pharmaceutical ingredient (API)

5-Aminosulfonyl, 4-chloro, 2-(2-furanylmethyl)-amino benzoic acid (99% purity), also known as furosemide (FR), Lot no MKBR8358V Sigma-Aldrich, Norway

3.1.2   Film forming polymers

Hypromellose 4000; Hydroxypropylmethylcellulose (HPMC), Lot no 08B052/3, Fagron, Norway

Hydroxypropylcellulose (HPC), Lot no 200063 Norsk Medisinaldepot, Norway

Lycoat® RS 720; Modified starch for immediate release film coatings, high viscosity type, Lot no E002R, Roquette Pharma, France

3.1.3   Plasticizer

Glycerol 85 %, Lot no 08B052/3 Norsk medisinaldepot (NMD), Norway

3.1.4   Taste masking agent

Hydroxypropyl beta cyclodextrin (HP-β-CD), Lot no73B025, Wacker chemie, Germany

Hydroxypropyl gamma cyclodextrin (HP-ɣ -CD), Lot nr 83P005, Wacker chemie, Germany

3.1.5   Dye

Brilliant blue R-250, Lot no BCK8393V, Sigma-Aldrich, Norway

3.1.6   List of solvents

Purified water

Phosphate Buffered Saline – tablet (PBS), pH 7.2-7.6, Lot no SLBJ117V, Sigma-Aldrich, Norway. One tablet dissolved in 200 mL of deionized water yields 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, at 25 °C.

Ethanol 96 % (v/v), Eterfabrikken, Norway

3.1.7 List of instruments and equipment

3.1.7.1 *Preparation of inclusion complexes*

Multi flask shaker VKS 75 A, Edmund Bühler GmbH, Germany

Freeze dryer, Christ Alpha 2-4 LD, United Kingdom

3.1.7.2 *Quantification of furosemide*

45µm light brown rim filter, SPARTAN 13/ 0.45 RC, GE Healthcare Life Sciences, Sigma-Aldrich, Norway

UV-spectrophotometer, SHIMADZU 1800, Japan

3.1.7.3 *Preparation of films*

Plastic petri dish, D x H 90 mm x 16,5 mm, Sigma-Aldrich, Norway

Analytical balance R200D, SARTORIUS, Germany

Precision balance, METTLER PC 4400, METTLER TOLEDO, Norway

Heating cabinets, Medcenter MMM GmbH, Germany

Magnetic stirrer, Multi-position magnetic stirrers, Ikamag® RO 5/10/15 Power series, Czech Republic

3.1.7.4 *Characterization of films*

3.1.7.4.1 Film thickness
Micrometer screw, Cocraft, Clas Ohlson, Norway

3.1.7.4.2 Moisture content
Moisture meter MA 30, SATORIUS, Germany

3.1.7.4.3 Dose uniformity
UV-spectrophotometer, SHIMADZU 1800, Japan.

Analytical balance, SARTORIUS, Germany

3.1.7.4.4 Disintegration

Electronic stop-watch and timer, Fine Sciences Tools GmbH, Germany.

Micropipette, BIO-RAD, Norway

3.1.7.4.5 Mechanical properties

Texture Analyser, TA-XTplus Stable Microsystems, United Kingdom

3.1.8 Multivariate analysis (MVA) and design of experiments (DoE) software

The Unscrambler 9.8, Camo ASA, Norway

3.2 Methods

3.2.1 Preparation of inclusion complexes by the shake-flask method

3.2.1.1 Quantitative analysis of furosemide

A stock solution of 100 µg/ml furosemide in PBS pH 7.4 was prepared. This stock solution was used to prepare standard solutions with the following concentrations: 10 µg/ml, 20 µg/ml, 30 µg/ml and 40 µg/ml. Equation (1) below was used to calculate the appropriate volume of stock solution needed to prepare standard solution of a given concentrations. Each standard solution was prepared in triplicate.

$$C_1 \times V_1 = C_2 \times V_2 \tag{1}$$

C_1 = the concentration in the stock solution.

V_1 = the volume of stock solution

C_2 = the new concentration.

V_2 = total volume needed at the new concentration.

The standard solutions were measured using a UV-VIS spectrophotometer at the absorption maximum found at 276 nm (53, 54). The absorption maximum was verified by scanning one of the standard solutions from 200 nm to 400 nm. Calibration curves were plotted ($R^2 \geq 0.9872$) and used for the quantification of samples.

3.2.1.2 *Kinetics*

To determine the time it takes to saturate the cyclodextrines with the drug, i.e. obtaining the equilibrium between the inclusion complexes formed and dissociated, kinetic studies were performed. Brown glass flasks (20 ml) were loaded with 50 mg furosemide. 20 ml of 10 % (w/w) HP-β-CD dissolved in PBS pH 7.4 was added. It is important to ensure that the drug is in excess throughout the whole experiment. The mixtures were shaken at room temperature until complex formation reached equilibrium. At predetermined time points (24, 48, 72, 96, 120, 144 and 168 hours) samples of 1 ml were withdrawn, and filtered with a 0.45 µm filter. 10 µl of the filtered mixture was diluted with PBS pH 7.4 to 10 ml in a volumetric flask. The diluted samples were quantified as described above (a) Quantitative analysis of furosemide).

The study was performed in 3 replications. Mean and standard deviation were calculated for each time point, and the time-concentration curve was plotted. The time point when drug concentration reached the plateau was identified as the time required to reach complex formation equilibrium.

The same study was also performed for furosemide with HP-γ-CD.

3.2.1.3 *Phase solubility*

The phase solubility study was carried out according to Higuchi & Connors method (55). This method is based on the changes in solubility of the drug as a response to an increased concentration of cyclodextrins. Addition of higher concentrations of cyclodextrin shifts the inclusion complexation equilibrium towards complex formation since the complex is more soluble than the drug itself. The overall result is an increased solubility of the drug (56, 57).

For this purpose, 20 ml brown glass flasks were loaded with 50 mg furosemide and 5 ml solutions of HP-β-CD in PBS (pH 7.4), in the concentrations 0, 2, 5, 7 and 10 % (w/w), were added. For each concentration 3 samples were prepared (n=3). The mixtures were shaken at room temperature for 7 days to ensure that the inclusion complex formation reached equilibrium.

The mixtures were filtered through a 0.45 µm membrane filter and the filtrates were collected. 10 µl of the filtrates were diluted with PBS (pH 7.4) in 10 ml volumetric flasks. The concentrations were determined using a UV-VIS spectrophotometer at 276 nm as described above. The same study was performed for furosemide with HP-γ-CD.

All the experiments were conducted under light protection (using brown glass or covered with aluminum foil) to prevent photo degradation of furosemide (58, 59). The products obtained from the phase solubility study were freeze-dried to preserve the inclusion complexes until further use in the film formulations.

The molar concentration of each series of cyclodextrin samples were plotted against the measured molar concentration of furosemide. The stability constant of the inclusion complex (Kst) was determined based on the following equation

$$K_{st} = slope/S_0(1 - S_0) \qquad\qquad (2)$$

where S_0 is the solubility of the pure drug (equal to the intercept of the diagram). The stability constant of the complex Kst is the ratio of $K_{formation}/K_{dissociation}$.

3.2.2 Preparation of films by the solvent casting method

The preparation processes for orodispersible film containing API-cyclodextrin inclusion complexes are schematically illustrated in Figure 5.
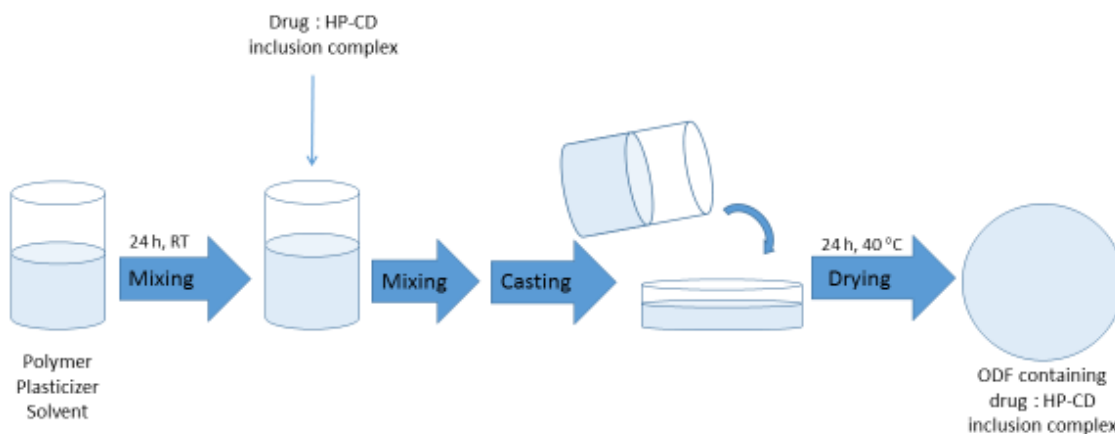
Figure 5: Schematic illustration of the preparation of orodispersible films containing drug-cyclodextrin inclusion complexes.

Dry polymer was weighed in 100 ml beakers, and wetted with glycerol. Appropriate amounts of the respective ingredients are specified in the experimental set-up (1.5.2). Polymer and glycerol concentrations were calculated based on the dry substances. The solvent (distilled water) was added gradually up to 100 g. The mix was stirred to a homogeneous viscous gel was obtained using a magnetic stirrer. Formulations based on HPMC required stirring overnight, whereas formulations based on Lycoat RS 720 and HPC were homogeneous after 30 minutes to 1 hour of stirring. When the gels appeared homogeneous inclusion complexes were added (where appropriate) as specified in the experimental set-up (1.5.2). The mixture was stirred again for some minutes to obtain a homogeneous distribution of the complexes in the formulation. The mixture was left on the bench for the air bubbles to disappear before casting of films. The viscous mixture was then poured into petri dishes (90 mm diameter) and left to dry either at room temperature or in heating cabinet at 40 °C overnight, only Lycoat RS 720 was dried in less than 24 hours (around 16 h). One batch (100 ml) was divided in five petri dishes and resulted in 5 pieces of film.

After drying, the films were covered with aluminum foil and stored at room temperature and ambient relative humidity (around 20 % RH) until further characterization was performed.

3.2.3 Characterization of the films

3.2.3.1 Film Thickness

The thickness of film was evaluated using a micrometer screw with the measuring range of 0-25 mm and the resolution of 0.01 mm. The film thickness was measured at predetermined positions. The average of 3 independent readings was taken. The thickness of samples from each of the three films from each formulation (or composition) were measured, and the mean and standard deviation for the compositions were calculated (n=9).

3.2.3.2 Moisture content

The amount of rest moisture present in the films was determined by using an infrared balance (Moisture meter). Samples of 5 cm x 5 cm from each of the films of the formulation were placed in the apparatus. The mass was recorded, and the sample was heated for thirty minutes at 130 °C. The loss on drying (LOD) was taken as evaporated water, and the water content in the film was

calculated in percentage (w/w). The mean and standard deviation for each formulation was calculated (n=9)

3.2.3.3 *Uniformity of mass of single-dose preparations test (Ph.Eur. 2.9.5)(60)*

Orodispersible films are single dose preparations. Film pieces of 1 x 1 cm were regarded as a single dose unit. Since there are no official monographs for the test of uniformity of mass for films, the test was performed according to the monograph for tablets (uncoated or film coated) of 80 mg or less.

Twenty individual films from each batch were weighed separately on an analytical balance and the average mass was calculated. The percentage deviation of each individual mass from the average mass was calculated. According to the monograph, not more than two of the individual masses should deviate from the average mass by more than ten percent and none should deviate by more than twice that percentage, i.e. twenty percent.

3.2.3.4 *Drug content and uniformity of dosage units test (Ph.Eur. 2.9.40)(49)*

Homogeneous distribution of the drug substance in the film should be achieved during manufacturing of the films. Film pieces of 1 x 1 cm were regarded as a single dose unit, and the drug content was determined in films as follows:

Ten individual films were completely dissolved in 3 ml of PBS-ethanol mixture in the ratio 2:1 (v /v). The drug content was determined spectrophotometrically at 276 nm as described in section xx. Calibration curve in PBS-etanol 2:1 (v /v) was used in the quantification. The content was calculated as mg per dose and % per dose, additionally, mean and standard deviation was calculated (n=10).

The test for uniformity of dosage units (Ph.Eur. 2.9.40) is considered to be most appropriate for validating dose uniformity of orodispersible films (Ph.Eur. 7.4). According to the monograph the uniformity of dosage units was assessed by calculating the acceptance value (AV) as follows:

$$AV = [M - X] + ks, \tag{3}$$

where M is the reference value given in the monograph, X is the mean of individual determined contents in percent of the label claim (here the theoretical content according to the experimental

set-up was used), k is the acceptability constant that varies depending on dosage units (2.4 for n = 10; 2.0 for n = 30) and s is the sample standard deviation. Reference value M differs depending on target drug content.

The formulation passed the test if L1 is less or equal 15. L1 is the maximum allowed acceptance value (AV).

3.2.3.5 *Disintegration Test*

3.2.3.5.1 Petri dish method

3 ml of PBS pH 7.4 was placed in a petri dish, a piece of film of 2 x 2 cm was added on the surface of the buffer, and the time required until the film dissolved completely was measured (27, 42). The test was carried out in triplicate for each film of the formulation (n=9 per composition). The mean and standard deviation was calculated.

3.2.3.5.2 Drop method

In this test a modified version of the method described by Preis et al. (42, 61) was used. One drop (200 µl) of PBS containing 0.1 % Brilliant blue R-250 was dropped onto the film. For this purpose, the films were placed on a petri dish. The time until the film breaks or a hole is formed in the film was measured. The test was carried out in triplicate for each film of each composition/formulation (n=9). The mean and standard deviation was calculated. The variation of the drop-method carried out in this study is illustrated in Figure 6 below.
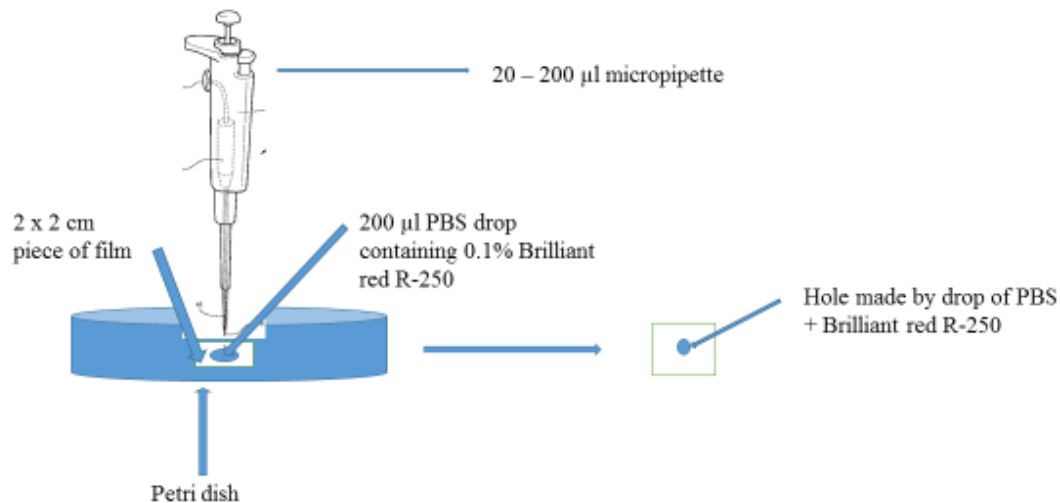
Figure 6: Schematic illustration of the variation of the drop-method carried out in the study

3.2.3.6 *Mechanical properties*

Puncture strength and percent elongation, area under the curve (AUC) and energy to puncture are various parameters used to assess the mechanical properties of orodispersible films (8). For this purpose, a puncture test was performed as described by Preis et al. (8). The puncture test was performed using a Texture Analyser TA-XTplus, with a 5 kg load cell and sensitivity of 0.001 N.

The films were cut into pieces of 2 x 2 cm. The film was fixed between two plates with a cylindrical hole of 13.97 mm diameter (Figure 7A and 7B). The area of the sample holder hole was 153.20 mm^2. Four pins stabilized the plates that were placed centrically under the punch of the Texture Analyser. The selected probe was a cylindrical flat-faced probe with a diameter of 7.03 mm.
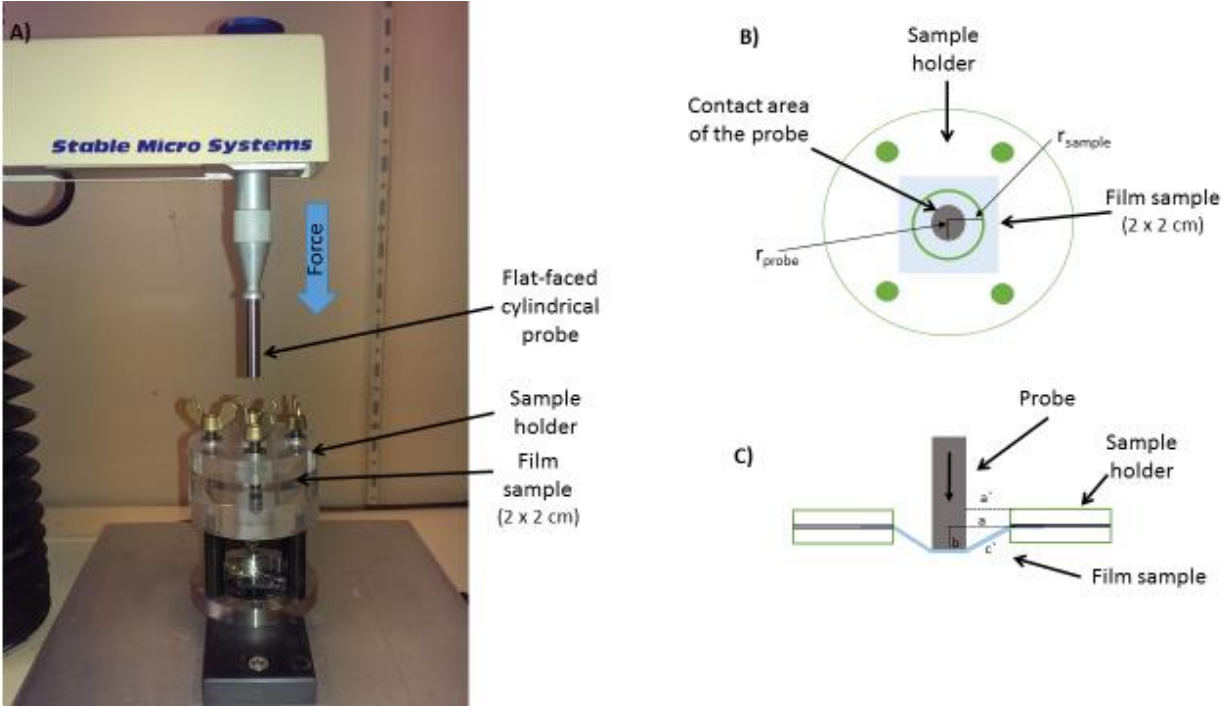
Figure 7: A) Picture of the experimental setup using Texture Analyser TA-XTplus, B) Sample holder for the puncture test) (r_s = radius of samples, r_p = radius of probe) and C) Determination of elongation to break: sample deformation before break (a = radius of the film in the sample holder opening, initial length; a' = initial length − radius of probe; b = displacement of the probe; c'+ r = length after strain; c' = length of a' after strain; r = radius of probe).

The pre-test velocity of the probe was set to 1.0 mm/s. Measurement started when the force was triggered (i.e. the probe is contact with the sample surface). The trigger force was set to 0.05 N. The test speed was 0.1 mm/s and constant until the film ruptured. The applied force and displacement (penetration depth) were registered. All experiments were conducted at ambient room conditions (21.8-22.8 °C, 23 − 25 % relative humidity).

The mechanical properties were calculated using the following equations:

$$\text{Puncture strength} = \frac{Force}{Area} \qquad (4)$$

where the force is the maximum force applied and recorded during strain. The area is the probe contact area with the film, which was 38.82 mm^2 for the 7.03 mm probe.

$$\text{Elongation to break} = \left(\frac{\sqrt{a'^2 + b^2} + r}{a} - 1 \right) * 100 \qquad (5)$$

where a represent the radius of the film in the sample holder opening $(a = 6.985$ mm$)$, a' represent the initial length of the film sample that is not punctured by the probe $(a' = 3.47$ mm$)$, and b represent displacement or penetration depth by the probe, and r represent the radius of the probe $(r = 3.515)$ (Figure 7C) (46).

The experiments were performed in triplicate for all films of the various combinations (n=9).

3.2.4 Experimental set-up

3.2.4.1 Preliminary tests

In order to gain some experience with plain films without inclusion complexes, a simple univariate screening of the film forming properties of three polymers (HPMC, HPC and Lycoat RS 720) was performed with glycerol as plasticizer. Different polymer concentrations (2- 5 % w/w) and glycerol concentration (5- 20% w/w) were tested to identify the outer boundaries of a suitable design space. Different drying conditions (room temperature, 40 °C) with subsequently varying drying times were tried out. In addition to the visual examination, the films were evaluated with respect to film thickness and disintegration time.

The purpose of the preliminary tests was to facilitate the design of the full study by identifying suitable polymer and plasticizer concentrations and drying conditions that gave films with reasonable disintegration times for an orally disintegrating film formulation.

3.2.5 Design of Experiment (DOE)

To study the influence of furosemide-cyclodextrin inclusion complexes on ODFs film quality, an experimental design was set up. The basis was a 2^2-factorial design with center point investigating the independent factors *concentration of inclusion complex* and *glycerol concentration* in the film formulation (Table 4).

Table 4: Investigated factors and their levels in the basic 22-factorial design with center point *

Factors	Levels	
	-1	1
Inclusion complex concentration (% w/w)	0.1	1
Glycerol concentration (% w/w)	0.24	1

* Center point: 0.55 % (w/w) inclusion complex and 0.59 % (w/w) glycerol

To investigate the additional factors *type of polymer* (HPMC, HPC and Lycoat RS 720) and *type of inclusion complex* (FR:HP-β-CD and FR:HP-γ-CD), the basic design was repeated with all combinations for the polymers with the respective cyclodextrins. This resulted in a total of 10 unique film compositions for each type of polymer, and a total number of 30 film compositions. However, several films (petri dishes) were prepared for each composition, so the total number of films prepared for each of the polymers was 34.

3.2.6   Multivariate analysis (MVA)

The influence of the design variables on the responses was evaluated by multivariate analysis using the Unscrambler 9.8 (Camo AS, Norway). Principal component analysis (PCA) was used to identify the most important factors, and groups or trends in the data matrix. Partial least square regression (PLS) was employed to quantify the effects. Prior to modeling, the variables were scaled by auto-scaling to unit variance (1/S.D). The models were calculated using systematic cross-validation and jack-knifing to estimate the approximate uncertainty variance of the PLS regression coefficients (62).

4 Results

4.1 Preliminary Test

Films were prepared using three film forming polymers in different concentrations and varying concentrations of glycerol as plasticiser. It was easier to prepare homogenous aqueous gels with HPC and Lycoat RS 720 than HPMC. Both HPC and Lycoat RS 720 gave moderately viscous solutions from 2 to 5 % (w/w), whereas HPMC resulted in highly viscous gels even at low concentrations, and it was impossible to obtain a homogenous gel at 5% w/w; therefore only 2% w/w was tested for this polymer. HPMC was the most challenging to work with, and it was important to completely moisten the dry powder with glycerol before water was added to avoid formation of gel lumps with dry powder inside.

It was observed that when drying at room temperature it required seven days for the solvent to evaporate and the film to be formed, whereas it took twenty four hours for the solvent to evaporate and the film to be formed at 40°C in the heating cabinets. Films based on Lycoat RS720 cracked when dried at 40°C for 24 h, and between 16 and 20 hours seemed more appropriate for this polymer. Figure 8 shows examples of films prepared by solvent casting.
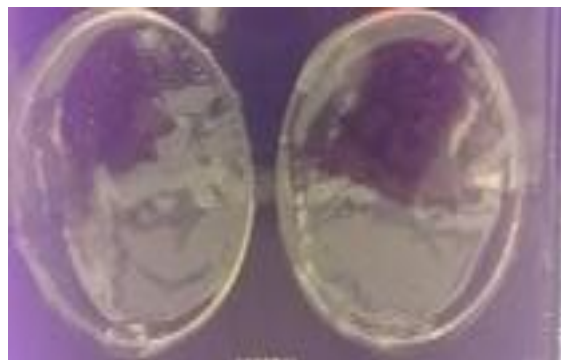
Figure 8: Examples of films prepared by solvent casting. The film to the right is from HPC and left is from Lycoat RS 720.

All films were characterized with respect to films thickness (Table 5) and disintegration time in PBS using the petri dish method (Table 6). The film thickness increased with increasing polymer concentration in the film formulation. Glycerol did not appear to have a particular influence on the

film thickness. The thinnest films were made from 2 % (w/w) Lycoat RS720, and the thickest from 5 % (w/w) HPC.

Table 5: Thickness of films of various compositions dried at room temperature and heating cabinet; mean ± SD (n=3)

Polymer type	Polymer concentration (% w/w)	Film Thickness (μm) Plasticizer (glycerol %, w/w)			
		0.25%	0.50%	0.75%	1%
HPC	4	147.5 ± 12.6	140.0 ± 8.2	152.5 ± 9.6	160.0 ± 8.2
HPC	5	187.5 ± 9.6	187.5 ± 15.0	190.0 ± 8.2	185.0 ± 8.2
HPMC	2	122.5 ± 9.6	127.5 ± 5.0	120.0 ± 8.2	135.0 ± 5.8
Lycoat RS 720	2	53.0 ± 9.6	48.0 ± 9.6	38.0 ± 2.9	53.0 ± 9.6
Lycoat RS 720	2	48.0 ± 9.6 *	58.0 ± 5.0*	50.0± 14.1*	50.0 ± 8.2*
Lycoat RS 720	5	135.0 ± 17.3	135.0 ± 30	153.0 ± 12.6	160.0 ± 8.2
Lycoat RS 720	5	135.0 ± 12.9*	138.0 ± 9.6 *	143.0 ± 12.6*	153.0 ± 9.6 *

* dried in heating cabinet 40°C for 24h

Disintegration time was different for the different polymers (Table 6). The longest disintegration times were found for HPMC films and the shortest for films prepared from Lycoat RS720. Again the polymer concentration was found to be important; therefore film thickness had an effect on the disintegration time. Glycerol did not appear to have an effect on disintegration.

Table 6: Disintegration time of films of various compositions in PBS pH 7.4 (Petri dish method); mean ± SD (n= 3)

Polymer type	Polymer concentration (% w/w)	Film disintegration time in (second)			
		Plasticizer (glycerol %, w/w)			
		0.25%	0.50%	0.75%	1%
HPC	4	65.0 ± 8.6	65.0 ± 12.2	65.0 ± 5	65.0 ± 8.7
HPC	5	128.0 ± 9.2	144.0 ± 4.4	134.0 ± 10.6	140.0 ± 5.0
HPMC	2	149.0 ± 8.0	150.0 ± 8.3	143.0 ± 1.5	147.0 ± 4.2
Lycoat RS 720	2	10.0 ± 2.0	12.0 ± 2.5	8.0 ± 1.5	7.0 ± 3.2
Lycoat RS 720	2	11.0 ± 1.2 *	9.0 ± 1.2*	10.0 ± 2.0*	11.0 ± 3.0*
Lycoat RS 720	5	27.0 ± 2.9	28.0 ± 2.9	37.0 ± 2.9	37.0 ± 2.9
Lycoat RS 720	5	33.0 ± 7.6 *	32 ± 7.6 *	37.0 ± 2.9 *	35.0 ± 5.0 *

* dried in heating cabinet 40°C for 24 h

The results indicate that there were no difference in thickness and disintegration between films dried at room temperature and those dried in the heating cabinet.

The knowledge acquired from the preliminary test, helped choosing the parameter settings when it comes to glycerol concentration, polymer concentration, and drying method in the main experimental design.

4.2 Furosemide Hydroxypropyl cyclodextrine inclusion complexes

4.2.1 Kinetics

Figure 9 shows the times required to reach the equilibrium between Furosemide and the two Hydroxypropyl cyclodextrines. A plateau can be observed from 120 hours. It means that it take 120 hours (5 days) to reach equilibrium between formed and dissociated inclusion complexes. Both

types of cyclodextrines required the same amount of time to reach equilibrium. The HP-β-CD was able to solubilize higher amounts of Furosemide in the inclusion complexes as compared to the inclusion complexes with HP-γ-CD.
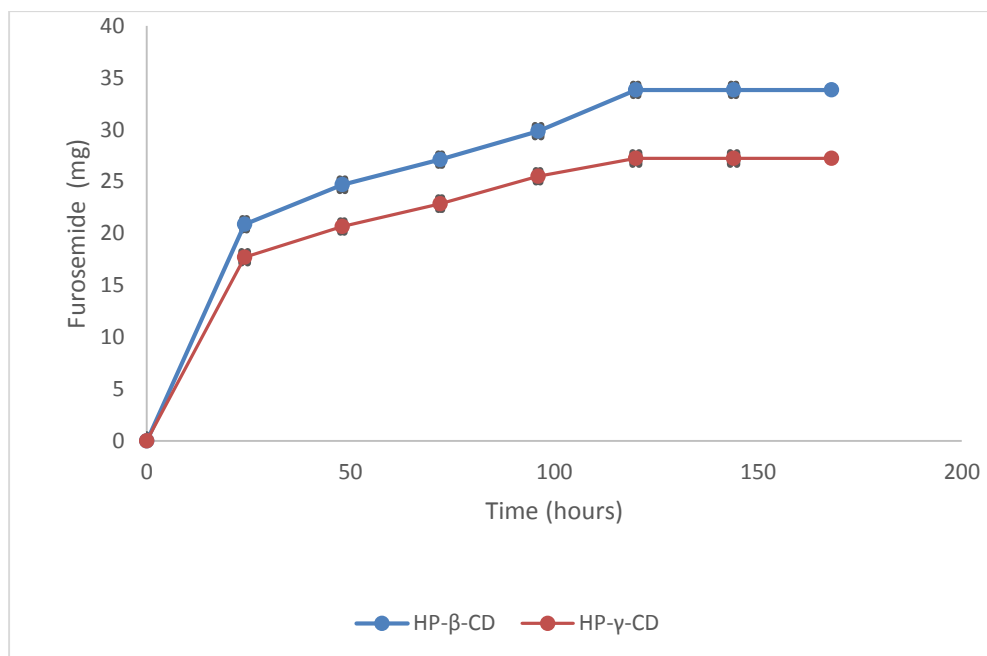
Figure 9: Kinetic study diagram of Furosemide with 10% HP-β-CD and 10% HP-□-CD; mean values ± standard deviation (SD) (n = 3).

4.2.2 Phase solubility

An increasing equilibrium solubility of furosemide was found with increasing concentrations of both HP-β-CD (Figure 10) and of HP-γ-CD (Figure 10). Furosemid was efficiently solubilized by both types of hydroxyprolyl cyclodextrines. Linear relationships were observed in both phase solubility diagrams, indicating that furosemide form 1:1 complexes with both HP-β-CD and HP-γ-CD.
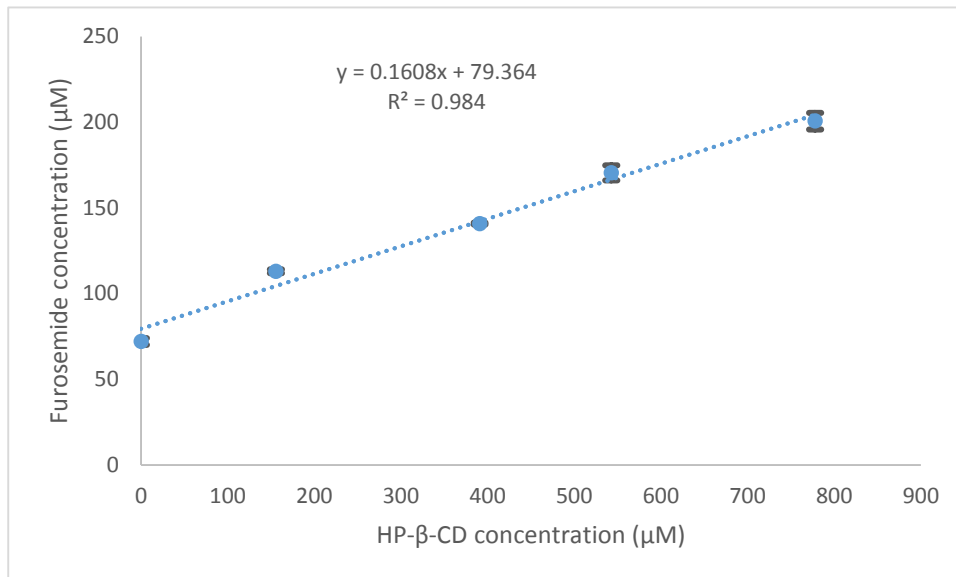
Figure 10: Phase solubility diagram of Furosemide with HP-β-CD; mean values ± standard deviation (SD) (n = 3)
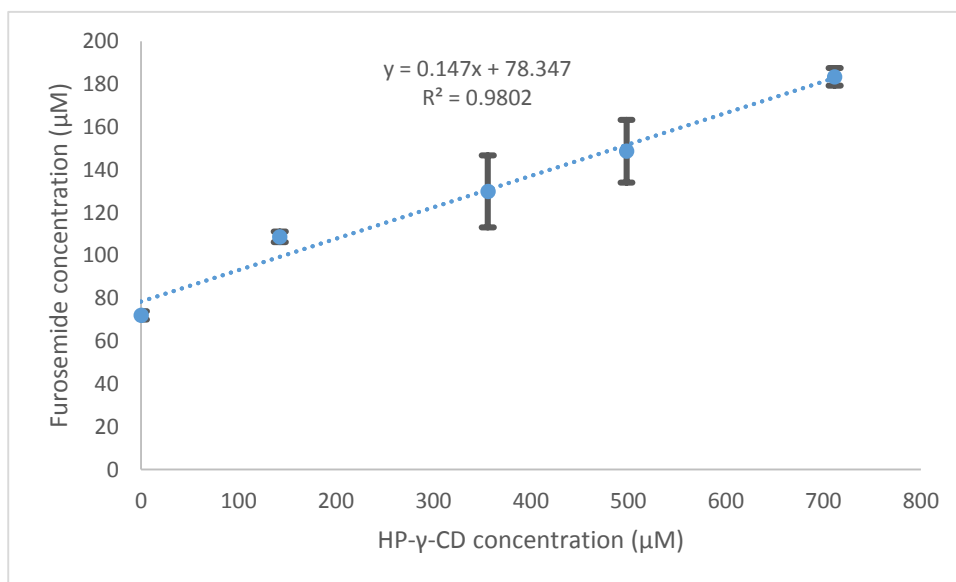
Figure 11: Phase solubility study diagram of Furosemide with HP-γ-CD mean values ± standard deviation (SD) (n = 3)

Based on the phase solubility relationship, the stability constant of the inclusion complexes were calculated (Table 7). The FR:HP-β-CD stability constant was higher than FR:HP-γ-CD stability

constant, meaning that the FR: HP-β-CD inclusion complex is more stable than the corresponding FR: HP- γ-CD inclusion complexes. The solubility of Furosemid (without cyclodextrin) can be extrapolated from the phase solubility diagrams as the intercept (S_0). As can be seen from Table 8 sligtly different S_0 values were found from the two studies, eventhough the value should in theory be identical.

Table 7: Calculated stability constants of inclusion complexes between FR: HP-β-CD and

FR: HP-y-CD

Inclusion complexes	S_0 (µM)	K_{st} (mol^{-1})
FR:HP-β-CD	79	3262
FR:HP-γ-CD	78.	2198

4.3 Statistical analysis of the data matrix from films characterization from experimental design

4.3.1 Exploring the data matrix using Principal Component Analysis (PCA)

A PCA was performed to explore the data matrix, extract information, remove noise and reduce dimensionality. A PCA bi-plot is shown in Figure 12.
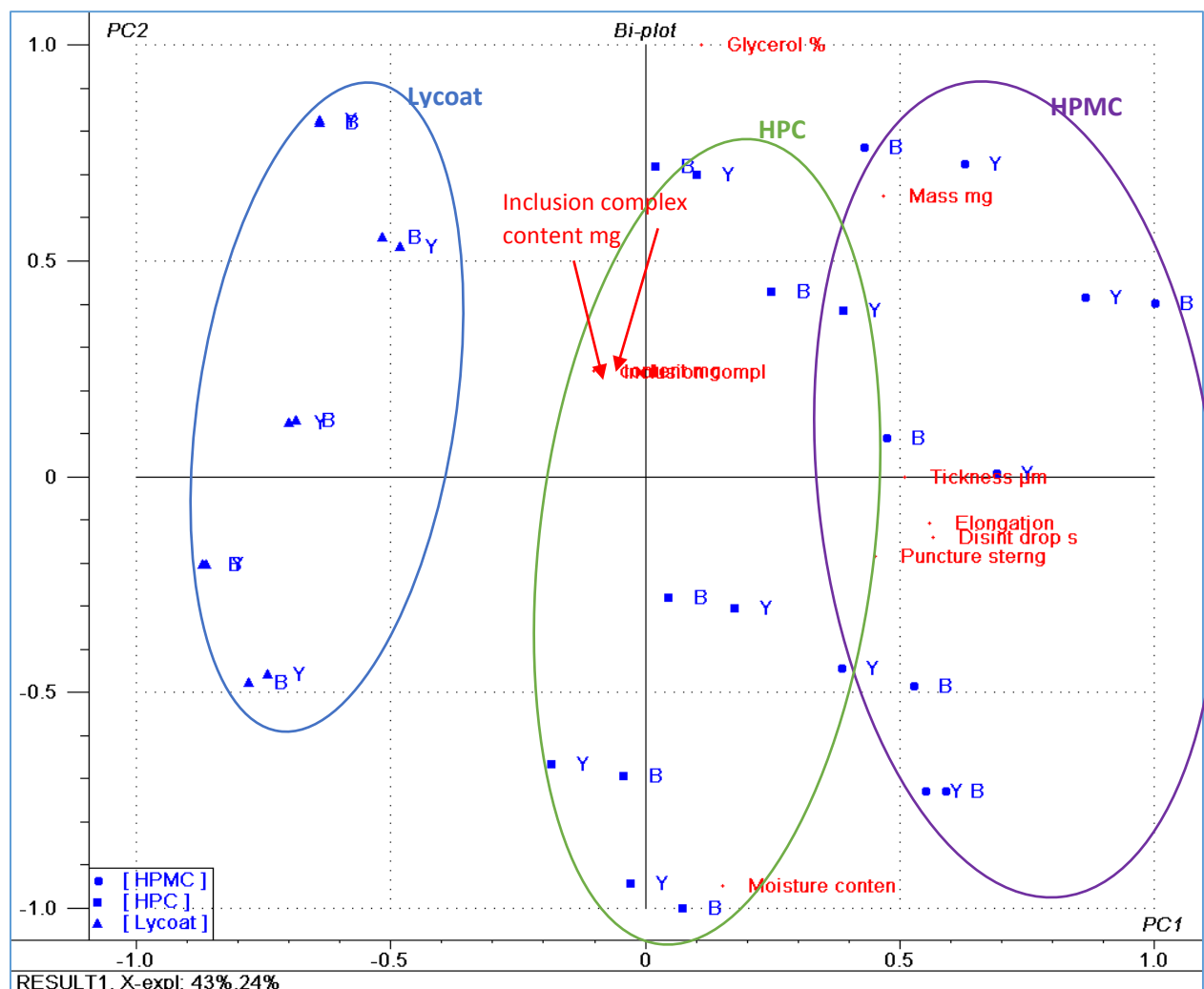
Figure 12: Bi-plot from a PCA of the full data matrix. Scores (sample) in blue symbols and loadings (variables) in red. (67% explained variance on PC1 and PC2). Clusters of films from the same film former marked with circles.

To explain the distribution of the samples in the score plot, information from the loading plot is needed. Therefore bi-plot is a good plot to aid the interpretation of the data. These are the main information, which can be extracted from this plot:

The first thing that can be derive from this plot is that 43% of variance is explained on PC1 whereas 24% of the variance is explained in PC2. More principal components are needed to describe all the variance in the data matrix and this case 6 PCs was suggested. However, the first principal component(s) describe the most of the variation in the data matrix and may therefore be regarded as the most important.

The type of Hydroxypropyl cyclodextrin do not have an effect on the data when it comes to Lycoat RS 720 because HP-CD are almost on top each other for the different films compositions from Lycoat RS 720 (Figure 12). However that is not the case for HPC and HMPC because HP-CD is scattered around (Figure 12).

The different polymer types can be found in clusters along PC1, spreading out on different levels along PC2. Lycoat films form a more distinct group whereas the clusters of HPC and HPMC films are overlapping (see Figure 12). This means that Lycoat films are different from HPC and HPMC films in the parameters investigated, and that the differences between HPC and HPMC films are less profound.

Moisture content and glycerol content both show high values on PC2, but in opposite direction, meaning that they are inversely correlated. Films with high glycerol content are associated with low rest moisture content.

Concentration of Inclusion complex and drug content are found on top of each other in the bi-plot, and therefore directly correlated. Moreover, they are located at relatively low values of both PC1 and PC2 (close to the center of the plot), meaning that they do not have influence on the variation of the data.

Film Thickness, disintegration time, puncture strength and percent elongation all show high values on PC1 and small or no values on PC2, and they are correlated.

The mass has the same high value on PC1 as thickness implying some correlation, but these factors are well separated along PC2.

The trends and relationships will be examined in more details with the help of correlation plots (scatter plot) and quantified using regression analysis (PLS).

4.4   Moisture content and film thickness of the films

All films were prepared from 4% (w/w) polymer solutions. The thickness of the films was mainly related to the type of film former used. HPMC gave the thickest films of around 190 μm, HPC films were roughly in the range 60-80 μm thick and films from Lycoat RS 720 were around 40-50 μm (Table 8).

Table 8: Mean and standard deviation values of moisture content and thickness from the different films compositions characterized in the main design (n = 9)

Comp.no.	Design variables				Moisture %		Thickness µm	
	Type of HP-CD	Type of Polymer	Inclusion complex % w/w	Glycerol % w/w	Mean	SD	Mean	SD
1	β	HPMC	0.1	0.24	11.47	0.05	186.11	3.85
2	β	HPMC	1	0.24	11.64	0.22	190.00	4.19
3	β	HPMC	0.1	1	6.41	0.20	185.00	1.92
4	β	HPMC	1	1	6.88	0.09	193.89	3.78
5	β	HPMC	0.55	0.59	8.23	0.09	192.00	7.49
6	γ	HPMC	0.1	0.24	11.60	0.27	184.44	3.47
7	γ	HPMC	1	0.24	11.53	0.30	188.89	4.41
8	γ	HPMC	0.1	1	6.27	0.10	187.78	3.47
9	γ	HPMC	1	1	6.48	0.19	191.67	11.34
10	γ	HPMC	0.55	0.59	8.50	0.26	191.67	7.88
11	β	HPC	0.1	0.24	13.74	0.31	66.11	6.01
12	β	HPC	1	0.24	13.34	0.17	62.78	4.81
13	β	HPC	0.1	1	5.59	0.25	72.22	7.58
14	β	HPC	1	1	5.73	0.08	69.63	3.47
15	β	HPC	0.55	0.59	11.74	0.36	73.00	3.47
16	γ	HPC	0.1	0.24	13.64	0.23	67.22	1.92
17	γ	HPC	1	0.24	13.64	0.22	66.67	1.67
18	γ	HPC	0.1	1	5.49	0.14	70.56	3.54
19	γ	HPC	1	1	5.40	0.16	79.44	4.86
20	γ	HPC	0.55	0.59	11.45	0.30	73.33	2.55
21	β	Lycoat	0.1	0.24	7.53	0.42	39.44	1.92
22	β	Lycoat	1	0.24	7.44	0.16	41.11	2.55
23	β	Lycoat	0.1	1	4.32	0.17	39.44	3.33
24	β	Lycoat	1	1	4.45	0.20	50.00	7.60
25	β	Lycoat	0.55	0.59	6.45	0.39	49.33	12.02
26	γ	Lycoat	0.1	0.24	7.18	0.21	50.00	3.33
27	γ	Lycoat	1	0.24	7.42	0.40	50.00	10.18
28	γ	Lycoat	0.1	1	4.48	0.32	51.11	9.62
29	γ	Lycoat	1	1	4.46	0.38	52.22	3.65
30	γ	Lycoat	0.55	0.59	6.53	0.34	41.00	3.57

Figure 13 illustrates that there were no strong correlation between thickness and moisture content of the films ($R^2 = 0.189366$). However, films from the different polymer types formed clusters indicating that the polymer type have an effect on moisture content in addition to the film thickness. In the same figure it can be observe that HPC have the films with the highest moisture content whereas Lycoat have the films with lowest moisture content and HPMC values are somewhere in between.
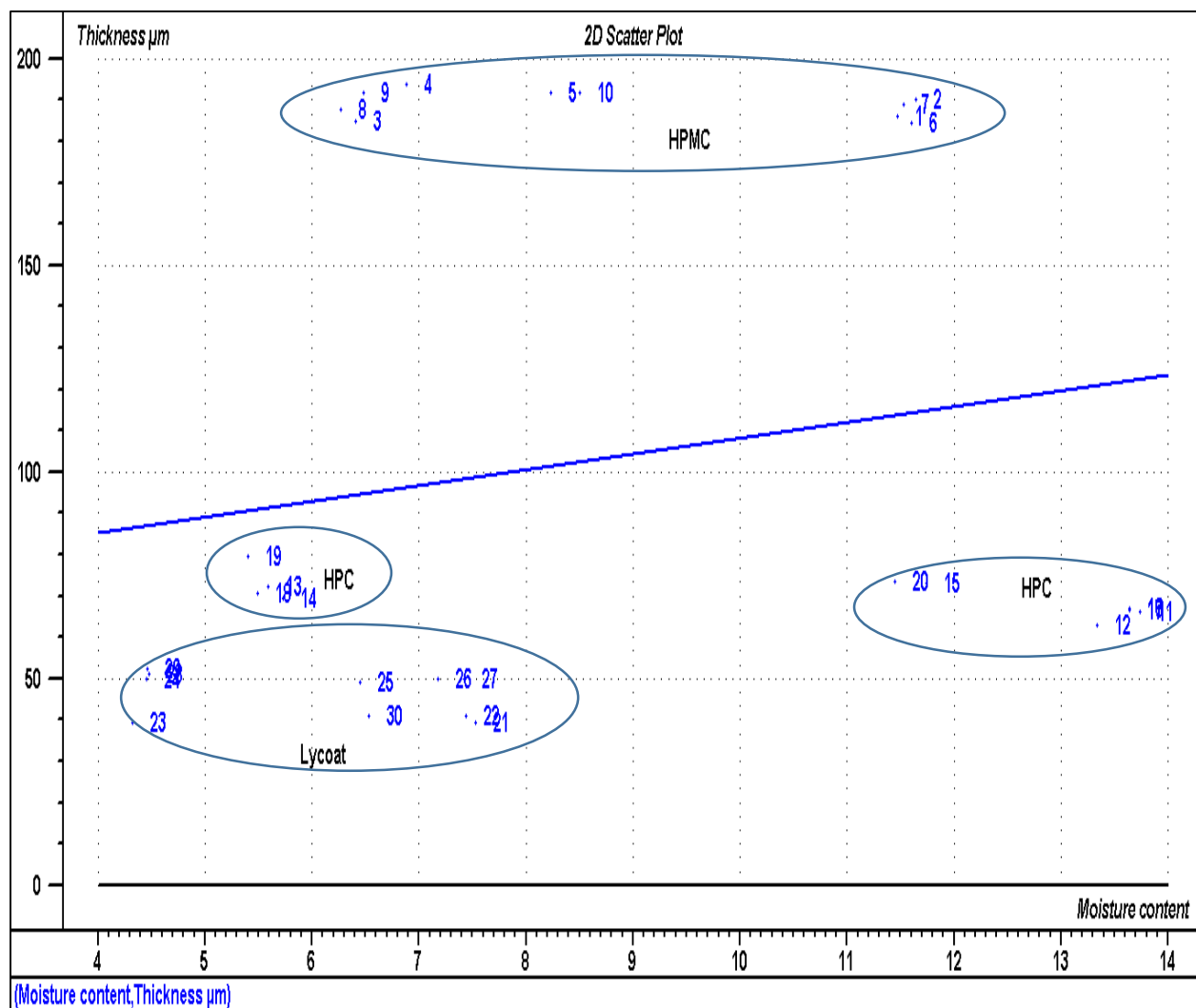
Figure 13: Film thickness versus moisture content (R2 = 0.189366). Clusters of films from the same polymer are marked with circles

A negative correlation was identified between moisture content and glycerol content (Figure 14). The higher the level of glycerol the lower is the rest moisture content after drying. Again the polymer type was found to have an effect on the moisture content.
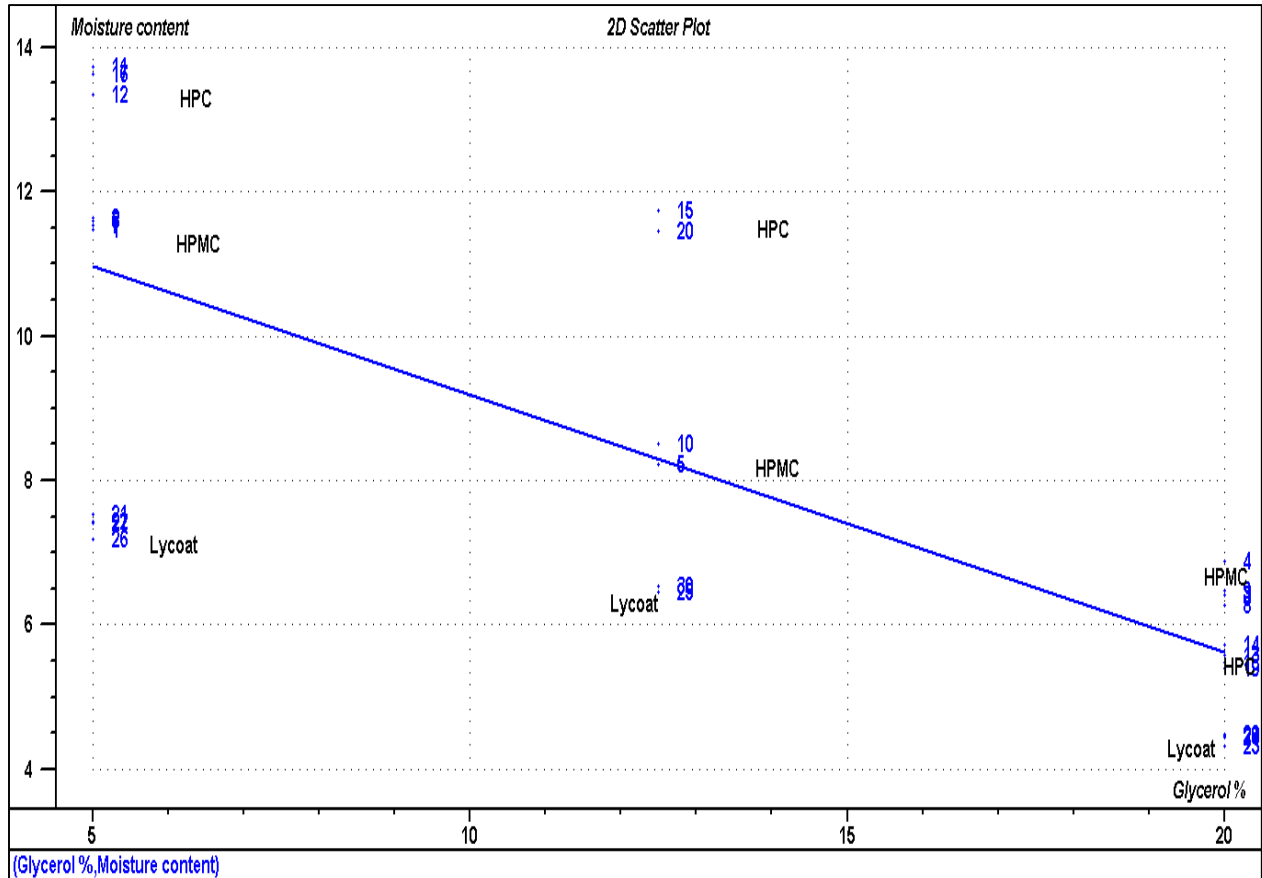
Figure 14: Moisture content of the film versus glycerol content (R2 = - 0.770292).

To further analyze and quantify the factors that are important for the rest moisture content of the films a PLS regression of all design variables (type of polymer, type of HP-CD, concentration of inclusion complex and concentration of glycerol) as x-variables, was performed. The regression coefficients are shown in Figure 15a. The significant variables (identified as error bars not crossing zero) were the three film forming polymers and the glycerol content, Use of HPC and HPMC contributed to high moisture content of the dried films, whereas Lycoat as film former and a low glycerol content contributed to low moisture contents. The variable with the strongest influence on the moisture content, i.e. the one with the highest regression coefficient, was glycerol content. The model explained 88% of the variation in Y using 40 % of the variation in X on two components.

The type of HP-CD and concentration of inclusion complex were found to be insignificant for the moisture content.

Since both film thickness and the moisture content were related to the type of film former, a PLS model was made including film thickness as one of the x-variables (Figure 15b). The two models were similar with respect to the influence of the variables, except that HPMC did not come out as significant in the latter, and film thickness did show a significant influence on the rest moisture content. The latter model explained 88% of the variation in Y using 50 % of the variation in X on two components, which was an increase in the X-variance as compared to the model without film thickness.

a)

RESULT6, (Y-var, PC): (Moisture content,2) B0W = 4.036394
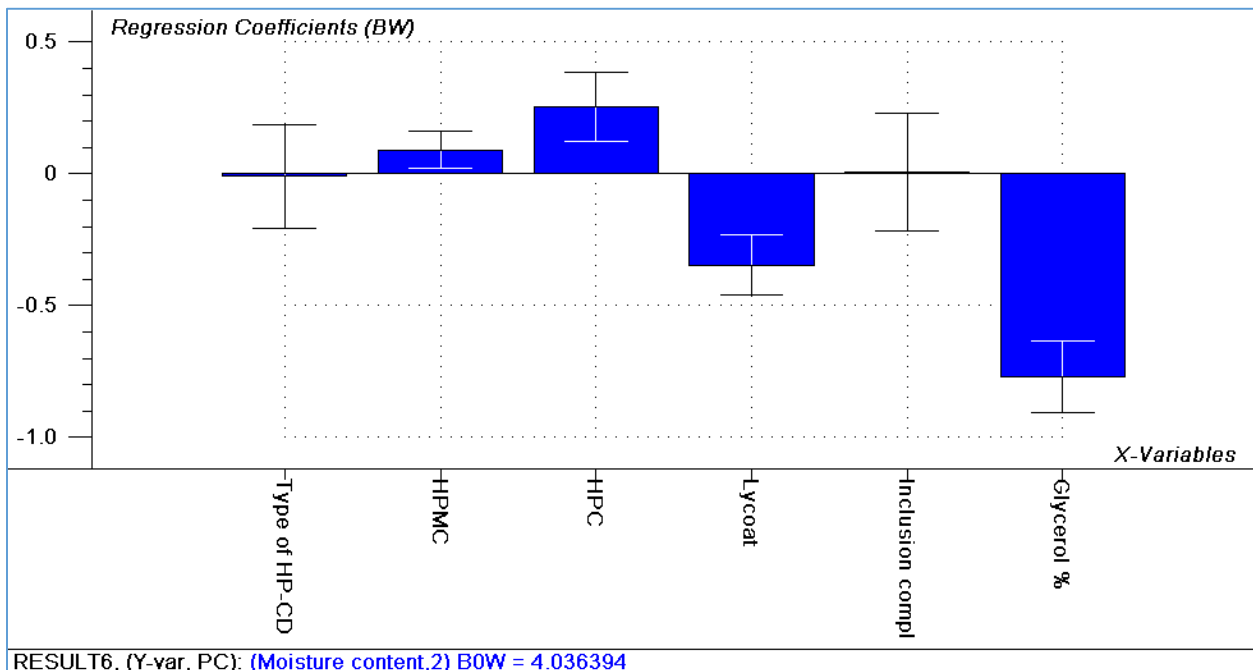
b)
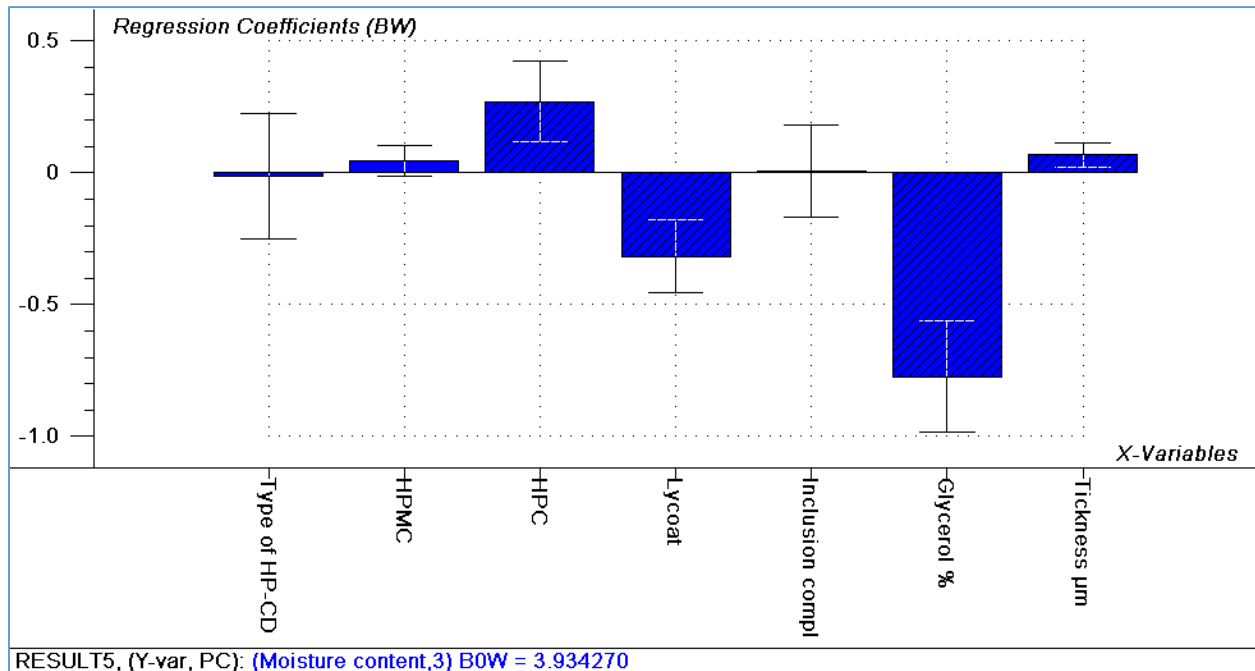
Figure 15: Regression coefficients from PLS of rest moisture content in the films. a) All design variables (2 PC: Expl. X-Var: 40%, Expl. Y-Var. 88%), b) All design variable plus film thickness (2 PC: Expl. X-Var: 50%, Expl. Y-Var. 88%)

4.5   Films as single dose unit - uniformity of dosage unit

Film pieces of 1 x 1 cm were regarded as single dose units, and investigated with respect to uniformity of dosage unit Ph.Eur. 2.9.40. The test of mass variation is applicable to solids in single dose containers, and was performed according to Ph.Eur. 2.9.5. Table 9 summarizes the average mass per dose. All films of all compositions were found to comply with the pharmacopoeia criteria. Furthermore, very low standard deviations were found.

Table 9: Uniformity of dosage units (film pieces of 1 x 1 cm). Mean and standard deviation of mass uniformity, content uniformity (mg) and calculated acceptance value (AV) according to Ph.Eur. 2.9.40, AV limit value (L1) equal 15.

Comp. no.	Design variables				Mass (mg) *		Drug Content (mg)**		(AV)***	
	Type of HP-CD	Type of Polymer	Inclusion complex % w/w	Glycerol % w/w	Mean	SD	Means	SD	Mean	SD
1	β	HPMC	0.1	0.24	58.69	0.18	3.83	0.02	10.20	0.40
2	β	HPMC	1	0.24	59.15	0.52	39.47	0.55	6.74	0.97
3	β	HPMC	0.1	1	78.64	0.23	3.82	0.05	9.29	1.21
4	β	HPMC	1	1	78.44	0.34	39.48	0.53	7.84	1.39
5	β	HPMC	0.55	0.59	68.57	0.32	21.52	0.20	7.03	1.50
6	γ	HPMC	0.1	0.24	58.82	0.19	3.82	0.03	9.56	0.32
7	γ	HPMC	1	0.24	59.12	0.67	39.64	0.36	8.51	2.02
8	γ	HPMC	0.1	1	78.66	0.08	3.83	0.03	9.72	1.23
9	γ	HPMC	1	1	78.77	0.45	39.64	0.77	8.11	1.20
10	γ	HPMC	0.55	0.59	68.87	0.18	21.56	0.14	7.18	1.08
11	β	HPC	0.1	0.24	48.68	0.13	3.83	0.02	11.22	0.76
12	β	HPC	1	0.24	48.79	0.04	41.07	0.52	8.77	1.15
13	β	HPC	0.1	1	68.78	0.26	3.83	0.02	9.15	0.60
14	β	HPC	1	1	68.62	0.13	41.28	0.84	8.63	1.20
15	β	HPC	0.55	0.59	58.75	0.20	21.52	0.14	9.33	1.41
16	γ	HPC	0.1	0.24	48.74	0.16	3.83	0.04	9.74	1.36
17	γ	HPC	1	0.24	48.65	0.03	41.15	0.33	9.37	1.67
18	γ	HPC	0.1	1	68.65	0.20	3.83	0.03	7.53	0.69
19	γ	HPC	1	1	68.81	0.17	41.38	0.67	7.83	3.56
20	γ	HPC	0.55	0.59	58.83	0.14	21.56	8.40	7.49	1.18
21	β	Lycoat	0.1	0.24	38.69	0.19	3.84	0.09	11.22	0.40
22	β	Lycoat	1	0.24	38.86	0.10	41.19	0.06	8.77	0.97
23	β	Lycoat	0.1	1	58.73	0.27	3.83	0.58	9.15	1.21
24	β	Lycoat	1	1	58.55	0.13	41.17	0.06	8.62	1.39
25	β	Lycoat	0.55	0.59	48.75	0.21	21.36	0.95	9.33	1.50
26	γ	Lycoat	0.1	0.24	38.83	0.14	3.82	0.18	9.74	0.32
27	γ	Lycoat	1	0.24	38.69	0.06	40.93	0.03	9.37	2.02
28	γ	Lycoat	0.1	1	58.64	0.34	3.82	0.07	7.53	1.23
29	γ	Lycoat	1	1	58.85	0.10	41.34	0.06	7.83	1.20
30	γ	Lycoat	0.55	0.59	48.72	0.16	21.69	0.63	7.49	1.08

(* n = 60 (mass mg) ** n = 30 (content mg), *** n = 3 (AV value)

The mass of single dose units were correlated to the film thickness as indicated in Figure X ($R^2 = 0.652953$). The thicker the film is the higher mass it will have. Figure 16 shows the same clustering of films depending on the film former used as described earlier: HPMC films have higher masses and higher thickness than HPC films, and Lycoat films.
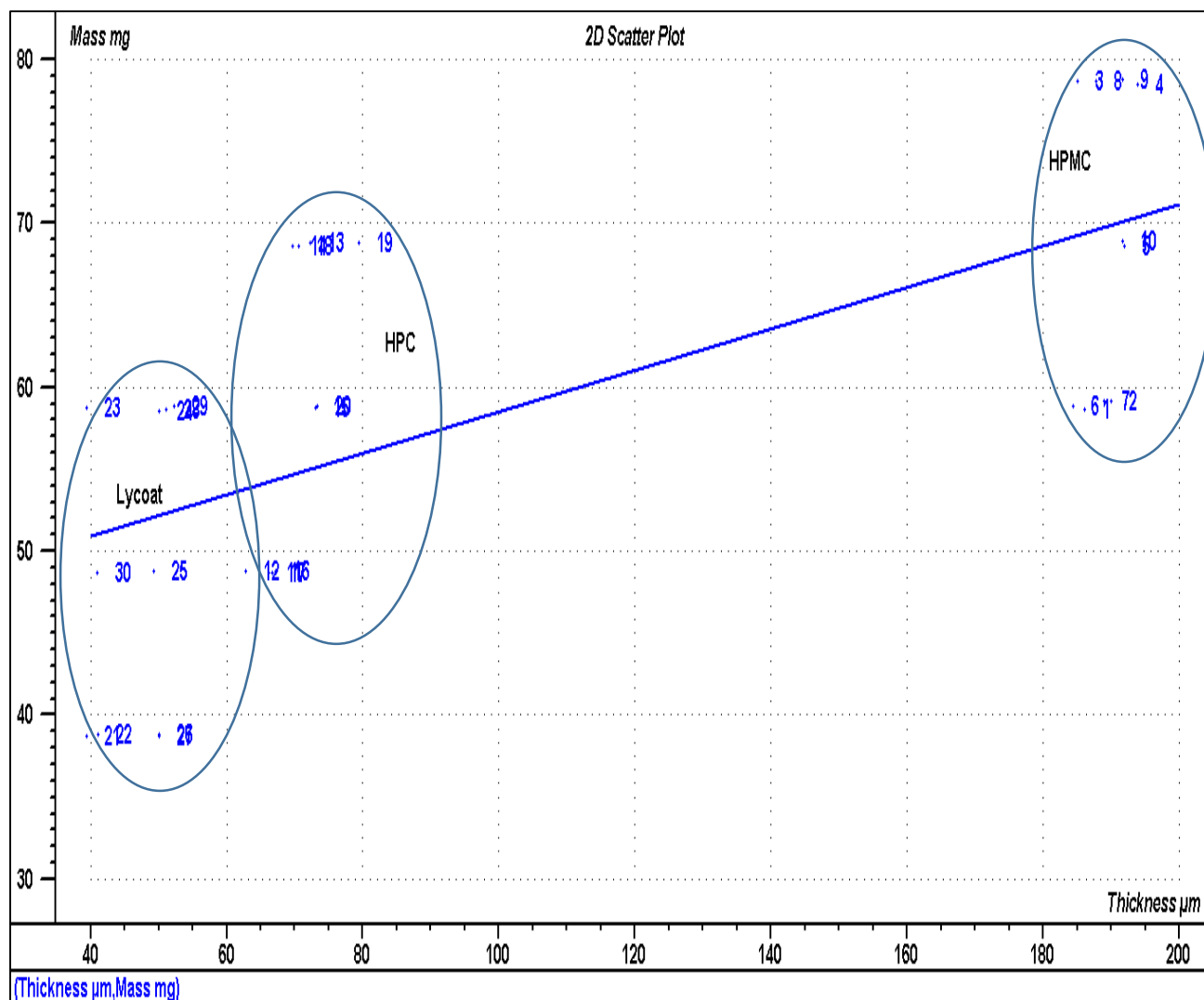
Figure 16: Mass of single dose units (1 x 1 cm) versus film thickness (R2 = 0.652953) Clusters of films from the same film former marked with circles

When it comes to content, the furosemide concentration was determined by the level of inclusion complexes in the formulation; hence three levels were obtained (Table 10). A low level of around 3.8 mg per dose, a medium level (center point) of around 21.5 mg per dose and a high level of around 40-41 mg per dose. For all composition an acceptance value (AV) was calculated according to Ph.Eur. 2.9.40, and the batch complies with the Pharmacopoeia requirements if the value is at or below 15, which is valid for all studied combinations (Table 10). However, there was certain degree of variation, which is illustrated by the plots of Figure 17 a-c where the determined drug content for all films (1 x 1 cm) for each of the compositions are presented. The relative variation

appears to be slightly higher for the films with high drug content (Figure 17c) as compared to those with the low drug content (Figure 17a).

a)
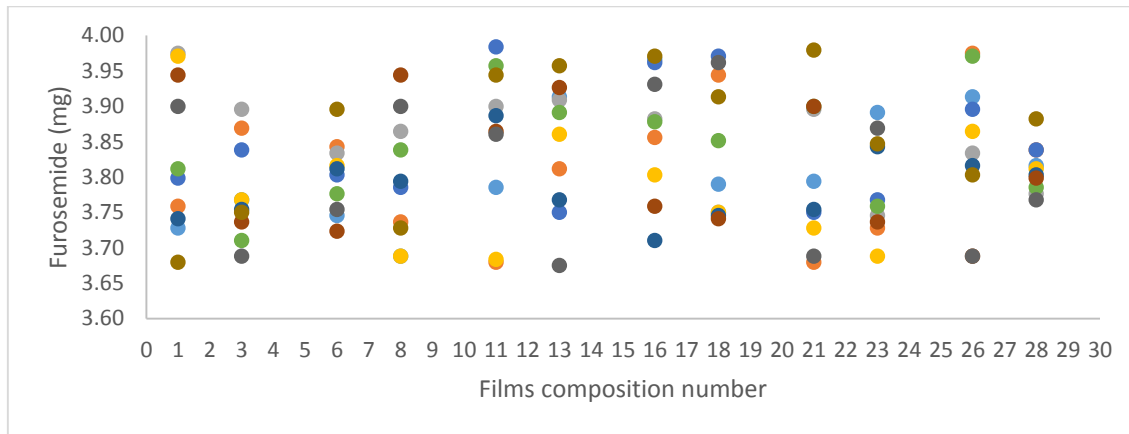
b)
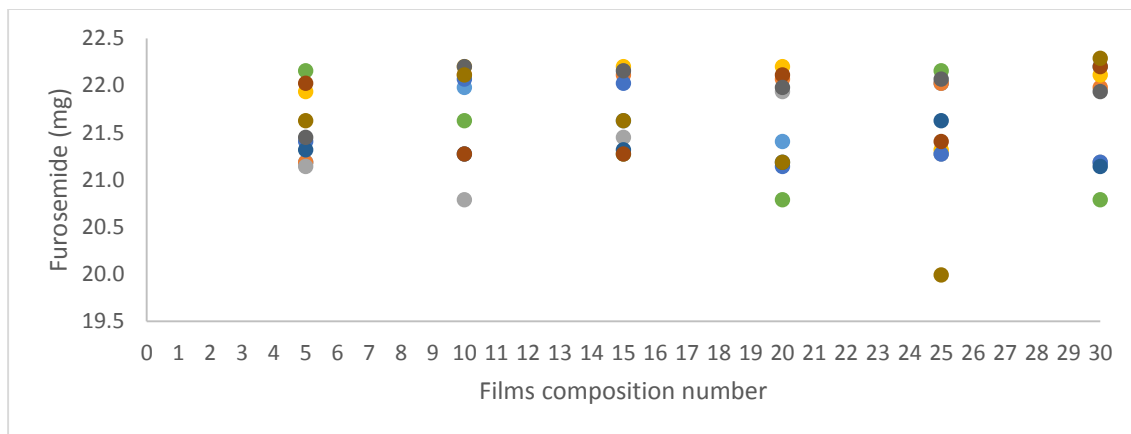
c)
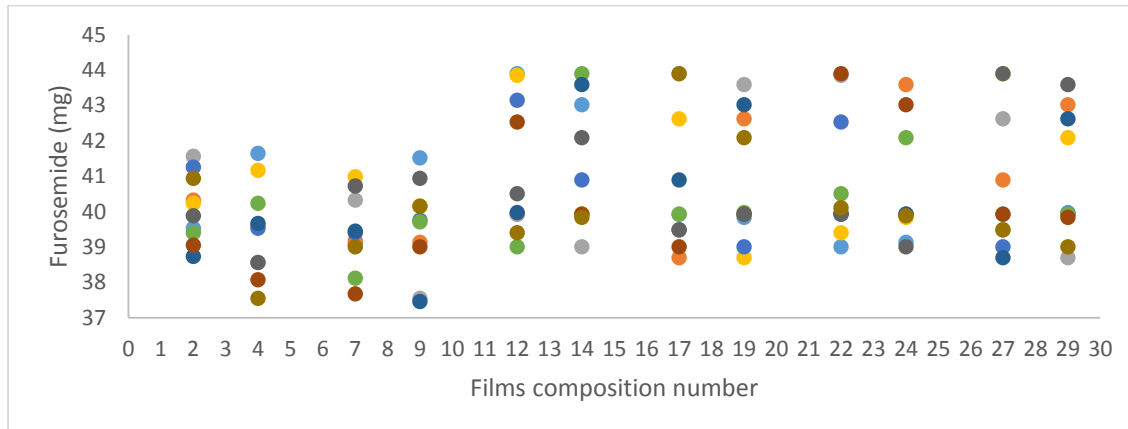
Figure 17: Furosemide content per single unit (Film piece of 1 x 1 cm). Each point represent a single measurement, n = 30 per composition. a) Film compositions with 0.1 % w/w inclusion complexes, b) Film compositions with 0.55 % w/w inclusion complexes, c) Film composition with 1 % w/w inclusion complexes

4.6   Disintegration of films in PBS pH 7.4

Disintegration times of the films were determined by two methods (Table 10). As shown in Figure 17 the two methods are directly correlated ($R2$ = 0.981604), and provides essentially the same information. Therefore only data from one of the methods will be use further in the analysis. The method is the drop method. Figure 18 also show that Lycoat films disintegrate faster than HPC films, which again disintegrates faster than HPMC films.

Table 10: Disintegration time of films in PBS pH 7.4 as measured according to the Petri dish method and the drop method. Mean and standard deviation (SD) (n = 9)

| Comp. no. | Design variables | | | | Disintegration Time (s) | | | |
| | Type of HP-CD | Type of Polymer | Inclusion complex % w/w | Glycerol % w/w | Petri dish Method | | Drop Method | |
					Mean	SD	Mean	SD
1	β	HPMC	0.1	0.24	148.00	1.92	24.56	1.02
2	β	HPMC	1	0.24	146.30	5.61	25.33	1.68
3	β	HPMC	0.1	1	144.00	5.85	24.11	0.77
4	β	HPMC	1	1	146.90	5.00	23.56	2.04
5	β	HPMC	0.55	0.59	145.70	5.67	25.00	2.77
6	γ	HPMC	0.1	0.24	146.90	3.38	25.78	1.17
7	γ	HPMC	1	0.24	148.20	5.81	24.67	0.77
8	γ	HPMC	0.1	1	145.10	5.85	25.67	1.54
9	γ	HPMC	1	1	145.30	4.73	25.11	1.73
10	γ	HPMC	0.55	0.59	145.90	3.52	25.13	1.45
11	β	HPC	0.1	0.24	64.40	3.71	15.78	1.39
12	β	HPC	1	0.24	68.70	1.76	15.22	1.20
13	β	HPC	0.1	1	62.80	1.45	16.44	2.83
14	β	HPC	1	1	70.00	1.68	15.11	0.19
15	β	HPC	0.55	0.59	69.00	3.24	15.73	1.86
16	γ	HPC	0.1	0.24	65.30	3.08	16.11	1.39
17	γ	HPC	1	0.24	70.00	1.35	15.11	3.18
18	γ	HPC	0.1	1	66.10	0.51	14.11	2.03
19	γ	HPC	1	1	72.40	2.03	14.33	1.84
20	γ	HPC	0.55	0.59	70.33	4.05	14.47	1.83
21	β	Lycoat	0.1	0.24	28.70	3.46	6.56	1.35
22	β	Lycoat	1	0.24	32.00	4.51	7.11	0.84
23	β	Lycoat	0.1	1	31.80	1.26	7.44	1.26
24	β	Lycoat	1	1	29.80	2.50	6.67	1.53
25	β	Lycoat	0.55	0.59	29.60	4.75	6.67	1.62
26	γ	Lycoat	0.1	0.24	32.90	4.67	6.67	0.88
27	γ	Lycoat	1	0.24	25.50	4.35	6.67	1.20
28	γ	Lycoat	0.1	1	29.30	4.67	6.44	0.19
29	γ	Lycoat	1	1	32.10	1.17	6.89	1.54
30	γ	Lycoat	0.55	0.59	28.40	2.84	6.13	1.48
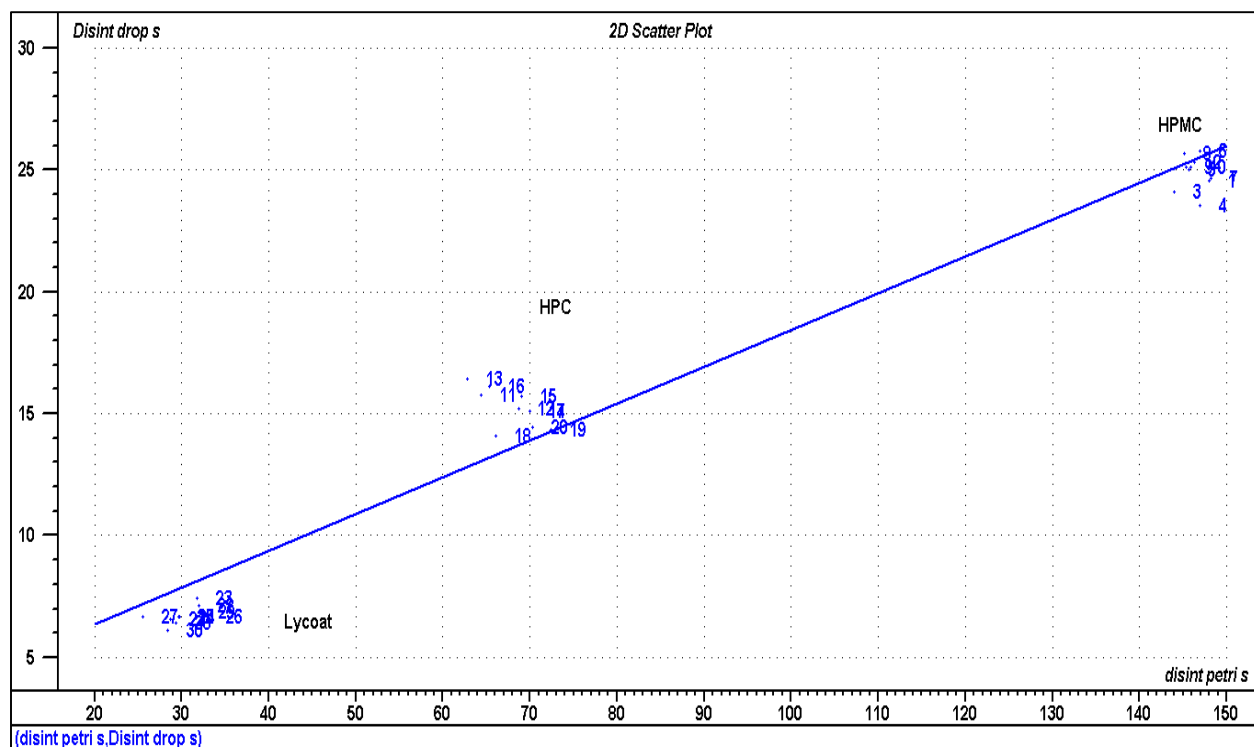
Figure 18: Correlation between the two disintegration methods; disintegration times determined by the Petri dish method versus the drop method (R2 = 0.981604)

To further analyze and quantify the factors that are important for the disintegration time a PLS of all design variables (type of polymer, type of HP-CD, concentration of inclusion complex and concentration of glycerol) plus moisture content and film thickness, was performed. The regression coefficients are shown in Figure 19.

Again, non-significant variables are identified as regression coefficients with error bars crossing zero, meaning that the variable is not important. In Figure 19, it can be observed that the type of Hydroxypropyl cyclodextrin, the content of inclusion complex and the use of HPC as film former do not have an effect on the disintegration time of the film. On the other side, using HPMC and Lycoat as film former, the content of glycerol, the rest moisture content and thickness of the film will have a significant effect on the disintegration time. The size of the regression coefficients indicates that film thickness and polymer type (Lycoat or HPMC) have the strongest influence of the investigated variables. Whether the regression coefficient is positive or negative provides information to which effect it will have on the response (disintegration time). HPMC, high levels of glycerol, high rest moisture content and film thickness have a positive effect on the

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disintegration time, meaning that they will provide longer disintegration times. Using Lycoat as the film former show a negative effect on the disintegration time, i.e. shorter disintegration times, which is favorable for orally disintegrating films. Furthermore, a low glycerol and moisture content and low film thickness would also promote short disintegration times.
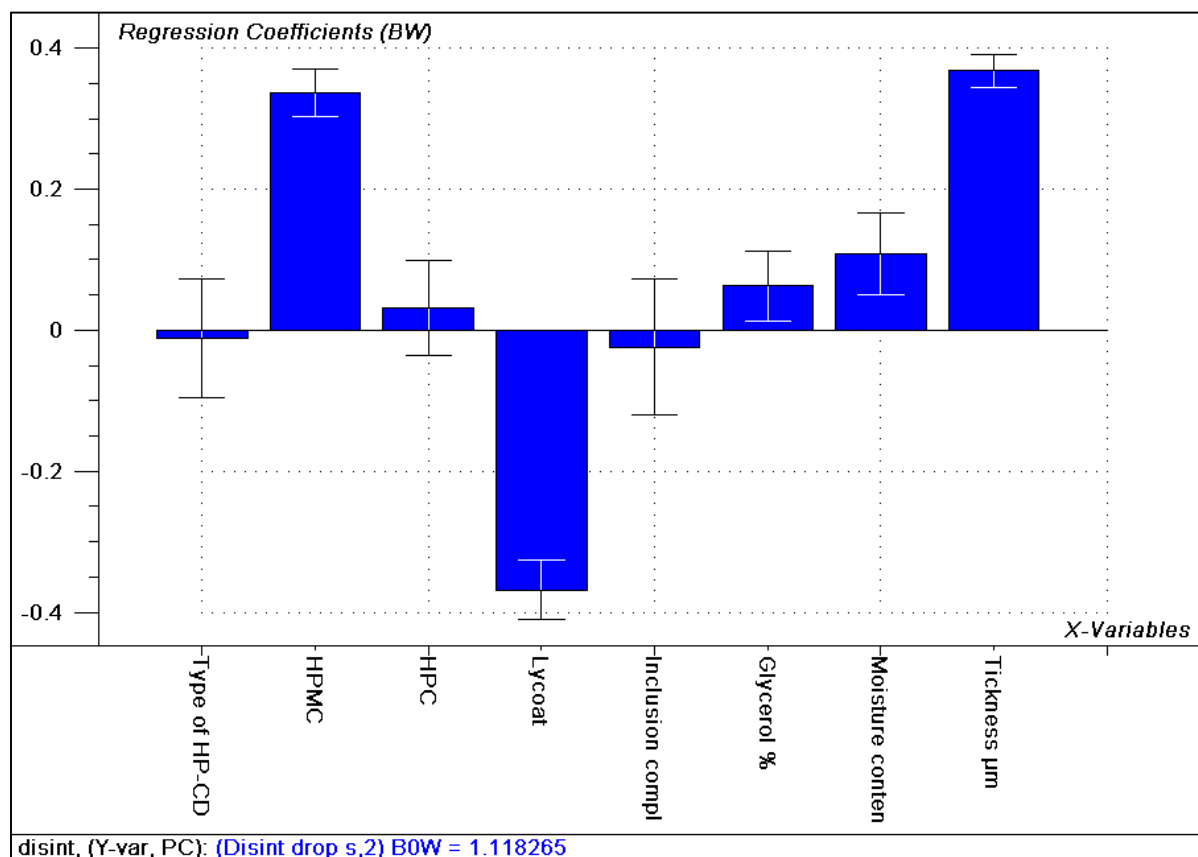
Figure 19: Regression coefficient from a PLS of disintegration time as determined by the drop method, (2 PC: Expl. X-Var: 55%, Expl. Y-Var. 95%) and the model $R2 = 0.959776$

4.7   Mechanical properties of the films

Also when it comes to the mechanical properties of the films, there was a pronounced difference between films prepared with Lycoat as the film former as compared to HPMC and HPC. This is clearly seen in both the puncture strength as well as how long (%) elongation before the film breaks (Table 11). No reliable PLS models could be obtained for the quantification of the factors influencing the mechanical properties; neither for the puncture strength nor for elongation at break.

However, clear grouping of the films from the different polymers was seen in a 3D-scatter plot of puncture strength, film thickness and moisture content (Figure 20).

Table 11: Mechanical properties of the films (2 x 2 cm) determined in the puncture test. Mean and standard deviation (n = 9)

| Comp. no. | Design variables | | | | Puncture strength (N/mm^2) | | Elongation to break % | |
	Type of HP-CD	Type of Polymer	Inclusion complex % w/w	Glycerol % w/w	Mean	SD	Mean	SD
1	β	HPMC	0.1	0.24	0.91	0.22	222	96
2	β	HPMC	1	0.24	0.94	0.09	217	54
3	β	HPMC	0.1	1	0.96	0.09	346	27
4	β	HPMC	1	1	0.69	0.11	165	54
5	β	HPMC	0.1	0.59	0.81	0.08	158	74
6	γ	HPMC	1	0.24	0.90	0.05	190	51
7	γ	HPMC	0.1	0.24	0.91	0.11	151	47
8	γ	HPMC	1	1	1.11	0.17	173	37
9	γ	HPMC	0.1	1	0.89	0.14	185	74
10	γ	HPMC	1	0.59	0.98	0.17	213	111
11	β	HPC	0.1	0.24	1.05	0.07	130	15
12	β	HPC	1	0.24	1.00	0.10	158	85
13	β	HPC	0.1	1	0.94	0.08	178	114
14	β	HPC	1	1	0.86	0.11	153	136
15	β	HPC	0.1	0.59	0.93	0.19	127	38
16	γ	HPC	1	0.24	0.86	0.04	143	86
17	γ	HPC	0.1	0.24	0.85	0.21	131	32
18	γ	HPC	1	1	1.09	0.04	231	136
19	γ	HPC	0.1	1	1.10	0.04	96	19
20	γ	HPC	1	0.59	1.08	0.13	155	92
21	β	Lycoat	0.1	0.24	0.65	0.04	14	4
22	β	Lycoat	1	0.24	0.66	0.01	14	3
23	β	Lycoat	0.1	1	0.66	0.02	33	0
24	β	Lycoat	1	1	0.66	0.00	16	0
25	β	Lycoat	0.1	0.59	0.67	0.01	15	1.
26	γ	Lycoat	1	0.24	0.67	0.03	17	2
27	γ	Lycoat	0.1	0.24	0.65	0.03	15	4
28	γ	Lycoat	1	1	0.72	0.01	23	1.
29	γ	Lycoat	0.1	1	0.65	0.03	16	3
30	γ	Lycoat	1	0.59	0.68	0.03	18.93	3
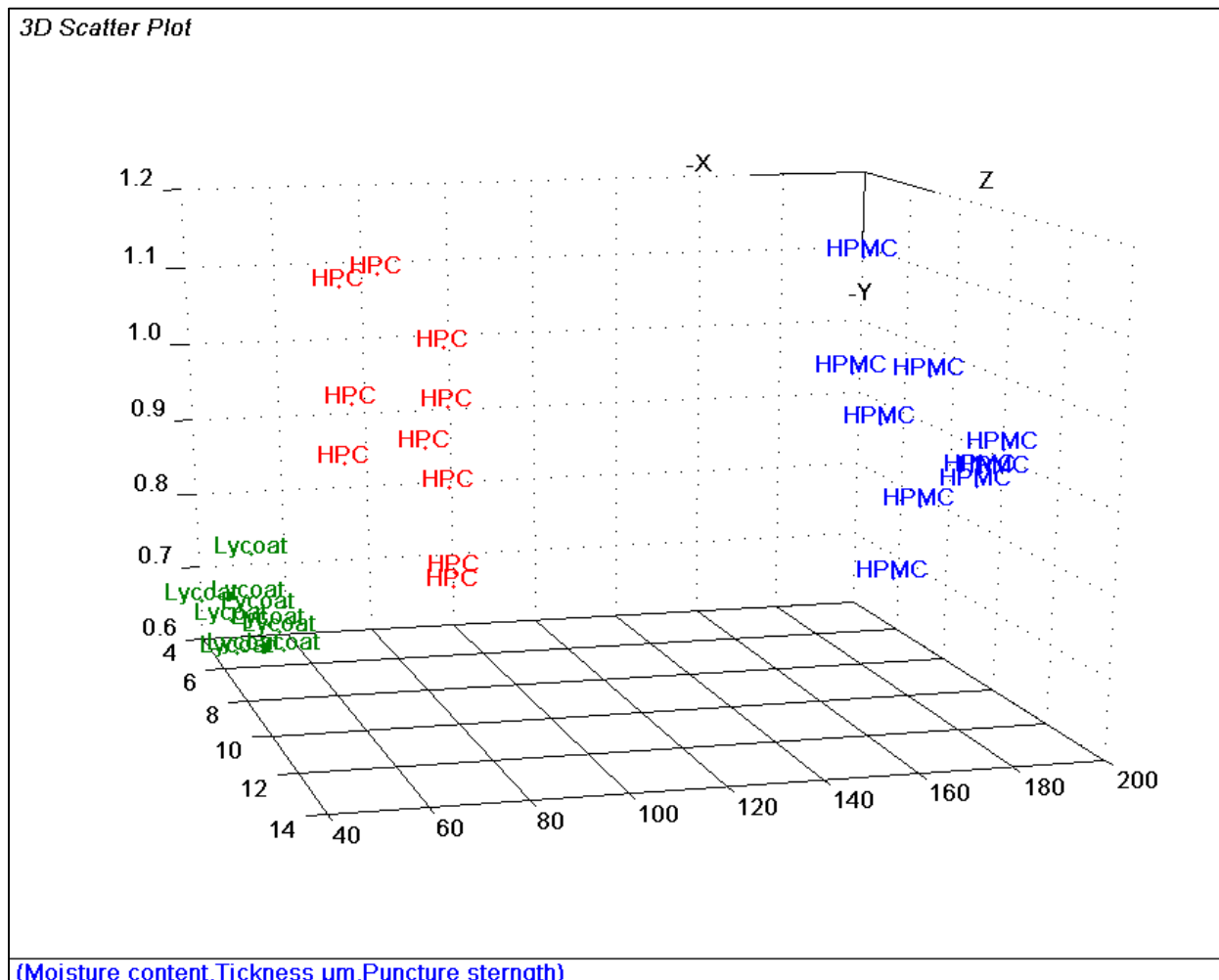
Figure 20: 3D scatter plot of film thickness (X), puncture strength (Y) and moisture content (Z) showing clear grouping of formulations based on the film former.

Figure 21 shows three or maybe four score groupings. The samples grouped in the left side of the plot, and have low values on puncture strength, moisture content, elongation and thickness. Those samples can be identified as the Lycoat films. It means that Lycoat films are thinner, have low moisture content, low puncture strength and low elongation at break. The sample grouping to the right of the figure are HPC films. They have a great influence on moisture content. It means that those sample have higher moisture content.

The sample in the middle of the right of the figure have greater influence on puncture strength. Those samples are HMPC films. It means that those samples have better puncture strength.

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The sample grouping in the bottom in the right of the figure are samples that influence both elongation and thickness. Those samples are a combination of HPMC and HPC films. It means that those films are both thicker and have high elongation.

Puncture strength and elongation are correlated, both variables are high on PC1 while thickness and moisture content are anti-correlated to each other. Both are in opposite side of PC2.

Finally according to the sample groupings polymer type have an effect on mechanical properties.
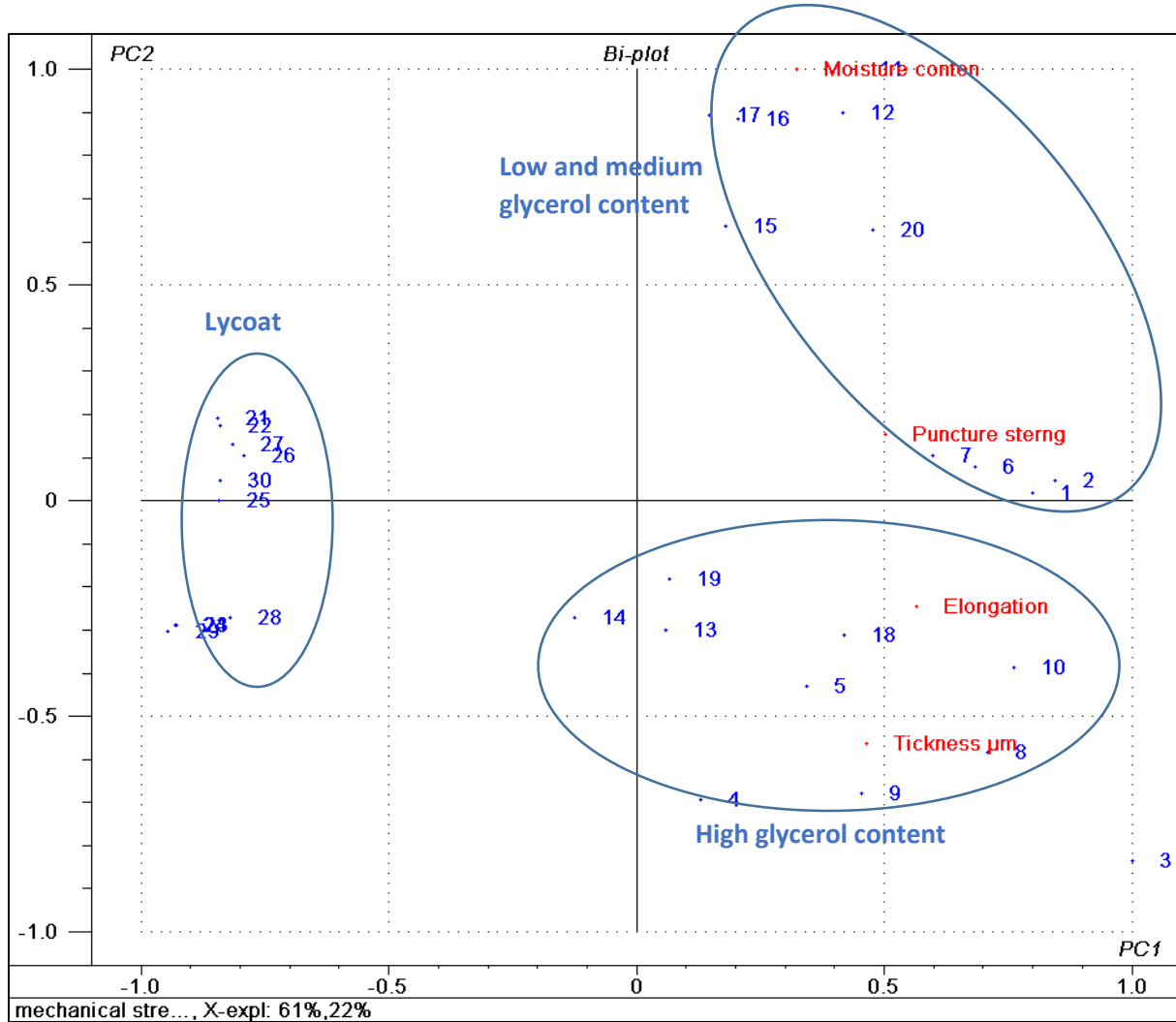
Figure 21: Bi-plot from a PCA of puncture strength, elongation, moisture content and film thickness (83% explained variance on PC1 and PC2). Scores (sample) in blue symbols and loadings (variables) in red. Identified clusters marked with circles.

5 Discussion

5.1 Preliminary tests

A crucial step in the formulation of ODFs prepared by the solvent casting method is identifying an appropriate polymer and plasticizer concentration that is both suitable for the manufacturing process with respect to viscosity and at the same time provides a film with suitable film thickness, flexibility and disintegration properties (46). ODFs should have mechanical properties that allow handling without damage but at the same time they should show rapid disintegration when placed onto the tongue (13). Therefore, to find the suitable polymer and plasticizer concentration for ODFs formulation that is able to accommodate all tasks at once is important.

The preliminary tests indicated that glycerol as plasticizer did not have an effect on the film thickness nor on the disintegration time in the concentration ranging from 0-1% w/w. Films with sufficient flexibility to allow handling were obtained from the investigated film forming polymers HPC, HPMC and Lycoat RS720. Therefore, the glycerol concentration was kept on the low values of 0.2-1 % w/w in the main study. The preliminary tests also showed that polymer type and concentration had an effect both on film thickness and disintegration time. It was decided to use same concentration for all polymers, and a polymer concentration of 4% was used in the main study. This led to the fact that the thickness of the films varied depending on the polymer type. Drying the films at room temperature it took 7 days for the solvent to evaporate, which was considered to be too long with respect to stability concerns. 24 hours was the time required at 40°C in the heating cabinets, and this was considered acceptable for furosemide containing formulations. However, this process might be less suitable for heat labile drugs or drugs prone to hydrolysis.

5.2 Furosemide Hydroxypropyl cyclodextrin inclusion complexes

The preparation of the inclusion complexes with Furosemide with HP-β-CD and with HP- γ-CD was successful, and the kinetic studies showed that equilibrium between inclusion complexes formed and dissolved was reached after 5 days at room temperature for both type of complexes. The respective stability constant were $K_{st} = 3262$ mol^{-1} for FR: HP-β-CD and $K_{st} = 2198$ mol^{-1} for FR: HP- γ-CD. The stability constant is an indication of how easily two reagents form a complex and how stable the complexes are. The higher the stability constant the more towards the right will the complex formation-dissociation equilibrium be. A Comparison of the stability constants of the two different inclusion complexes indicate that the HP-β-CD have a greater ability to form

inclusion complex with FR and the complexes are more stable than with HP- γ-CD. No previous studies were found in the literature reporting K_{st} for FR: HP- γ-CD, so that value can only be compared to the FR: HP-β-CD. A higher stability constant for the FR: HP-β-CD complex implies that the cavity of the HP-β-CD was better suited to accommodate the hydrophobic parts of Furosemide than the HP- γ-CD, meaning that 7 glucopyranose units was more appropriate size; it might be hypothesized that the cavity of the 8-ring CD was too large since compared to the relatively small size of the Furosemide molecule. However, this study showed that HP- γ-CD can also be used to form inclusion complexes with FR.

There are several studies reporting the K_{st} for FR: HP-β-CD (9,10). Vlachou et al reported K_{st} for FR: HP-β-CD of 2110 mol^{-1} at 25°C whereas Sadighi et al reported K_{st} of 335 mol^{-1} in 0.02 M HCl at 35°C (56, 63). Both studies found values that are deviating from the one find in this study, But K_{st} for HP-β-CD reported here is closer to the one reported by Vlachou et al as is the conditions. In addition the study by Vlachou et al. used the shake-flask method whereas Sadighi et al used the rotation method. Vlachou et al used a thermostatically controlled water bath at 25°C, whereas in this study the flasks were shaken at room temperature. That might also influence the results. It is worth noticing that Vlachou et al studied the K_{st} at different temperature, and reported lower stability concstants with increasing temperature (K_{st} = 1112 mol^{-1} at 37°C and K_{st} = 825 mol^{-1} at 47°C) (56). Another point to take into consideration is that other studies reported that equilibrium was reached after 3-4 days for the FR: HP-β-CD complex (56, 57, 63). However, in the current study it was shown that 5 days was need for the complex to reach equilibrium. If equilibrium was not reached the stability constant could be underestimated. The common ground for all studies is that there is a linear relationship between the amount of solubilized drug and the concentration of HP-β-CD in the phase solubility diagrams, meaning that all the studies concluded that stable inclusion complex between HP-β-CD and FR are formed in 1:1 molar ratio (56, 57, 63). The same stoichiometry was also found for the complexation of HP-γ-CD with FR.

5.3   Characterization of the films from the main design

Oral administration and solid dosage forms has many advantages as outlined earlier, however ODFs will be suitable for children that might exhibit swallowing problems and it is age-appropriate for the younger children(less than six months). Enhanced compliance in pediatric patient requires new approaches, and ODFs show great potential for this group of patients. The overall goal of this

study was to investigate ODFs intended for children, and the look into the effects that HP-CD inclusion complexes used as taste masking strategy would have on the quality of the films. The inclusion complexes form particles, probably in the nano-size range (10), which were incorporated in ODFs. This approach of taste masking is frequently used in both the pharmaceutical and Food and Beverage industry as taste masking technique (30, 35). However, this approach of taste masking is more resource and time consuming compare to conventional taste masking strategies like direct addition of sweetener or flavor, but the reliability of the outcome is higher, especially when the traditional methods are not effective, e.g. in cases where the effect of sweetness is overpowered by the bitterness sensation. Another important fact is that this taste masking technique may avoid the use of substances that are considered harmful for the pediatric population, such as come sweeteners. Aspartame is an example of a commonly used sweetener in pharmaceutical formulations, and it is consider as inactive and non-toxic in adults, but can be harmful for children suffering from phenylketonuria (47, 64). Finally, inclusion complex formation of bitter drugs with CD may contribute to reduce the number of excipients needed for taste masking purposes, something that is advantageous for ODFs. In the formulation of ODFs a minimum use of excipient is desired because of the relatively low film loading capacity.

All the film formulations were found to provide films thickness in the range from 39 μm for the thinnest film (Lycoat RS720) to 194 μm for the thickest film (HPMC). But all the films thickness was within the range that has been suggested in the literature (5-200 μm) to be acceptable for ODFs (23). The main characteristic distinguishing films from the three polymers was the film thickness. Hence, it is difficult to really compare the suitability of the polymers for preparation of ODF. A better approach might have been to target the same film thickness for all polymers by selecting different polymer concentrations. This is something to take into consideration for future studies.

Multivariate data analysis (MVA) is a systematic way of studying the influence of independent variables (experimental variables) and their interactions on selected dependent variables (responses variables) (62). A tool use to design experiments that will be analyzed in with MVA.is Design of Experiments (DoE). Investigating variables in a univariate manner, is often not revealing all information, therefore it is useful to study the influence of multiple factors to also capture interactions between variables as well as non-linear behavior. Designed experiments form good basis for revealing latent variables or structures in the data matrix with a PCA or quantifying

significant effects with a PLS regression. In this study A 2^2 full factorial design with one center point was used to systematically investigate the influence of concentration of inclusion complex and concentration of plasticizer (glycerol) on physical and mechanical properties of ODFs. The basic design was repeated several times to also include type of HP-CD inclusion complex (two levels) and type of film forming polymer (three levels) among the design variables. To increase the power of the design, 3 replicates was run for each point and 5 center points, in total 17 films for each type of inclusion complex and 34 films in total. This resulted of a power of 0.8 (the power of the design is between 0-1), and the software (The Unscrambler) defined it as a strong power. A high power of the design ensures that reasonable departures from the null hypothesis are detected. If the design does not have a strong power, experiments are not worth doing (62). So conclusions drawn on this study are based on a number of DoEs with a strong design power.

In the PCA bi-plot in Figure 12, inclusion complex is directly correlated to uniformity of content. That is as expected since FR is determined by the amount of FR-CD inclusion complex is in the film. . All the statistical analysis performed in this study indicated that the amount of inclusion complexes, no matter whether it was of FR: HP-β-CD or FR: HP- γ-CD, did not have an effect on physical or mechanical properties of ODFs. Due to lack of time no test was performed to assess the efficiency of inclusion complex formation as an acceptable taste-masking technique for FR in ODFs. This is something that is left to be addressed in future research.

Dose accuracy is important for all types of single unite dosage form. Dosage accuracy is important to be able to achieve the desired therapeutic concentration to ensure an effective therapy and at the same time minimizing side effects. Achieving dose accuracy requires high level of homogeneity between the single units. Factors that influence the single dose unit homogeneity of films are uniformity of mass, film thickness and uniformity of content. These factors were measured for all films prepared by the three different polymers evaluated in this study. Film pieces of 1 x 1 cm were cut and regarded as single dose units. All the different compositions were found to contain a uniform quantity of the drug in the single units. The weight of the films was within the requirement of the Ph.Eur. This indicates   good reproducibility of the films prepared by solvent casting as manufacturing technique

When it comes to the dose per unit, the low level of inclusion complex (0.1 % w/w) resulted in films of approximately 4 mg per dose of 1 x 1 cm, and the high level (1 % w/w) approximately 40

mg, with the middle level of the center point (0.55 % w/w) in between at approximately 21 mg (Table 9). The different dosing units seem suitable for children, e.g. a child of 10 kg (around 1 year) (65)a Furosemide dose of 5-20 mg is appropriate 2-3 times daily (see Frame 2) and for older children (e.g. above 12 years) the dose is in the range of 20-40 mg. This confirms that even by using cyclodextrines as taste masking strategy reasonable therapeutic doses can be accommodated in ODFs.

Another important characteristic of the ODFs is the Disintegration behavior. ODFs should dissolve or disintegrate rapidly when placed onto the tongue. Short disintegration times are desired for ODFs. Therefore, disintegration behavior can be used to rank the suitability of the different polymers. Based on disintegration time Lycoat RS720 is better suited for ODFs than both HPC and HPMC. However, this does not mean that HPC and HPMC cannot be used for ODFs, because other previous studies have used them successfully for ODFs (46, 66). But the polymers were either used in a lower polymer concentration or a polymer with lower molecular weight resulting in lower viscosity (especially for HMPC), result in thinner films that will disintegrate more rapidly. So these factors have to be taken into consideration when using HPMC for the preparation of ODFs.

Suitable mechanical strength to allow handling is another requirement for ODFs. Literature recommend a puncture strength for at least $0.06 \, \text{N/mm}^2$ to ensure that ODFs resist handling without being damaged (46). All films prepared by the various compositions of the current study showed a puncture strength above that, with HPMC exhibiting higher puncture strength than HPC with Lycoat RS720 having the lowest mechanical properties. However, the puncture strength was influences by the varying film thickness of films from the different polymers, so the ranking should not be given too much weight. So based on that limitation all the polymers study here are suitable for ODFs.

To summarize, based on the findings in this this study Lycoat RS720 seems to be more suitable for preparation of ODFs than HPC and HPMC. Therefore, Lycoat RS720 film composition should the taken further in an optimization design to find the optimum setting that result in desire ODFs for pediatric use.

6 Conclusion

ODFs are a consumer-friendly alternative to oral solid dosage forms like tablets and capsules. Therefore, to take advantages of this new drug delivery technology can help to solve major challenges encountered by pediatric patients when it comes to swallowing conventional solid dosage forms. Masking of the bitter taste of drug is essential, and can be achieved by formation of drug-cyclodextrines inclusion complexes.

Furosemide was found to form 1:1 complexes with both HP-β-CD and HP-γ-CD. Equilibrium was obtained after 5 days of shaking at room temperature using the shake flask method. The

HP-β-CD was found to solubilize the highest amount of furosemide, and produced in the most stable complexes (highest stability constant).

Inclusion complex of FR: HP-β-CD and FR: HP- γ-CD was successfully incorporated in ODFs based on the water-soluble film forming polymers HPC, HPMC and Lycoat RS720 with glycerol as plasticizer. The ODFs were manufactured using solvent-casting technique and dried for 16-24 h at 40°C.

Films with High dose-homogeneity, fast disintegration in PBS pH 7.4 simulating saliva and suitable mechanical strength were produced. Multivariate statistical analysis showed that the incorporation of both types of FR: HP-CD inclusion complexes in ODFs in the investigated range (0.1 -1 % w/w) did not have a significant effect on the quality of ODFs. The choice of polymer and the content of plasticizer were found to be the most important variables determining the film thickness, moisture content, disintegration time and the mechanical strength of the ODFs. Lycoat RS 720 produced the thinnest films with the fastest disintegration time. However, optimization of the film thickness for films based on HPMC and HPC might reduce the disintegration time and make also these polymers well suited for ODF formulations. Based on the results from the current study Lycoat RS 720 appears to be an interesting polymer for further optimization studies, to identify the optimum settings in which they provide desired ODFs.

7 Future perspectives

- Carry out a suitable dissolution test to demonstrate the appropriate release of FR in ODFs

- Taste masking assessment of the efficacy of HP-CD as taste masking agent

- Optimization design with Lycoat RS 720 films to identify the optimum settings in which they provide desired ODFs for pediatric formulations.

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