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ANNA KLUGMAN

Functionality related characterization of pretreated wood lignin, cellulose and polyvinylpyrrolidone for pharmaceutical applications

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Department of Pharmacy, Faculty of Medicine, University of Tartu, Estonia

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Supervisors: Professor Jyrki Heinämäki, Department of Pharmacy, Faculty of Medicine, University of Tartu, Estonia,

Senior Researcher Karin Kogermann, Department of Pharmacy, Faculty of Medicine, University of Tartu, Estonia

Reviewers: Senior Researcher Külli Jaako, Chair of Pharmacology and Toxicology, Faculty of Medicine, University of Tartu, Estonia

Professor Ursel Soomets, Chair of Medical Biochemistry, Faculty of Medicine, University of Tartu, Estonia

Opponent: Associate Professor Ossi Korhonen, School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following publications, which are referred to in the text by their Roman numerals (I–V)

I Penkina A., Hakola M., Paaver U., Vuorinen S., Kirsimäe K., Veski P., Yliruusi J., Repo T., Kogermann K., Heinämäki J. Solid-state properties of softwood lignin and cellulose isolated by a new acid precipitation method. *Int. J. Biol. Macromol.* 2012;51:939– 945.

II Penkina A., Antikainen O., Hakola M., Vuorinen S., Repo T., Yliruusi J., Veski P., Kogermann K., Heinämäki J. Direct Compression of Cellulose and Lignin Isolated by a New Catalytic Treatment. *AAPS PharmSciTech.* 2013;14(3):1129–1136.

III Kogermann K., Penkina A., Predbannikova K., Jeeger K., Veski P., Rantanen J., Naelapää K. Dissolution testing of amorphous solid dispersions. *Int. J. Pharm.* 2013;444:40–46.

IV Penkina A., Semjonov K., Hakola M., Vuorinen S., Repo T., Yliruusi J., Kirsimäe K., Kogermann K., Veski P., Heinämäki J. Towards improved solubility of poorly water-soluble drugs: cryogenic co-grinding of piroxicam with carrier polymers *Drug Dev Ind Pharm.* 2015;1:1–11.

V Penkina A., Parajuli A., Antikainen O., Hakola M., Vuorinen S., Repo T., Yliruusi J., Veski P., Kogermann K., Heinämäki J. Effects of wood lignin on the solid-state, mechanical strength and moisture barrier properties of cellulose ether films. *Manuscript to be submitted for publication* (2015).

Contribution of Anna Klugman (née Penkina) to original publications (I–V):

Publication I: Participation in planning the experiments; performing individually the major part of the experiments (DSC, FTIR, particle size, density, water activity, and bulk powder analyses) and data analysis; participation in writing the paper.

Publication II: Participation in planning the experiments; performing the experiments and data analysis; writing the paper.

Publication III: Participation in planning the experiments; performing part of the experiments (formulation of amorphous solid-dispersions and solid-state analysis) and data analysis; reviewing the manuscript.

Publication IV: Participation in planning the experiments; performing the experiments and data analysis; writing the paper.

Publication V: Participation in planning the experiments; performing part of the experiments and data analysis; writing/co-writing the paper.

ABBREVIATIONS

AFM	Atomic force microscopy
AGU	Anhydroglucopyranose unit
API	Active pharmaceutical ingredient
aPRX	Amorphous PRX
ASD	Amorphous solid dispersion
a_w	Water activity
BC	Bacterial cellulose
BCS	Biopharmaceutics Classification System
CA	Cellulose acetate
CAP	Cellulose acetate phtalate
CPSC	Catalytic pretreated softwood cellulose
CPSL	Catalytic pretreated softwood lignin
CSD	Cambridge Structural Database
DBCP	Dibasic calcium phosphate
DDS	Drug delivery system
DP	Degree of polymerization
DSC	Differential scanning calorimetry
*d*50	Volume median diameter
EC	Ethyl cellulose
EF	Elasticity factor
FRC	Functionality-related characteristics
FTIR	Fourier transform infrared
HPMC	Hydroxypropyl methylcellulose
HPMCAS	Hydroxypropyl methylcellulose acetate/succinate
HPMCP	Hydroxypropyl methylcellulose phtalate
ICH	International Conference on Harmonization
LCC	Lignin-carbohydrate complex
LT	Low temperature
MCC	Microcrystalline cellulose
NC	Nanofibrillar cellulose
PEG	Polyethylene glycol
PEO	Polyethylene oxide
PF	Plasticity factor
Ph. Eur.	European Pharmacopoeia

PM	Physical mixture
PRX	Piroxicam
PRXAH I	Piroxicam anhydrous form I
PRXMH	Piroxicam monohydrate
PVP	Polyvinylpyrrolidone
PVP-CL	Crospovidone
QbD	Quality by Design
QbT	Quality by Testing
RH	Relative humidity
RT	Room temperature
SD	Solid dispersion
SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
Tg	Glass transition temperature
WVP	Water vapour permeation
XRPD	X-ray powder diffraction

1. INTRODUCTION

Pharmaceutical excipients are essential in formulating high-quality pharmaceutical dosage forms and drug delivery systems (DDSs). In recent years, the pharmaceutical industry is increasingly emphasizing the importance of moving from Quality by Testing (QbT) to Quality by Design (QbD). One of the main statements of QbD approach is an importance of science-based understanding of how formulation (including excipients and process) affects the functionality of product. The International Conference on Harmonization (ICH) Q8 guideline on Pharmaceutical Development highlights the importance of the ability of the excipients to provide their intended functionality and performance throughout the intended drug product shelf life. Understanding the physicochemical and biological properties of the excipients is integral, as these characteristics can influence the drug product stability, bioavailability and processability [ICH 2009].

The European Pharmacopoeia (Ph. Eur.) determines the functionality-related characteristics (FRCs) of excipients as controllable physical or chemical characteristics of an excipient that impact on its intended functionality which is directly related to physical and biopharmaceutical properties of final product. Such FRCs can be controlled and attributed to a product-specific quality afford [Ph. Eur. 2010].

Oral pharmaceutical dosage forms such as tablets, capsules, granules and pellets are still the mainstay of the pharmaceutical industry. This is because oral route is much more simple and convenient over the other known routes of drug administration. Direct compression is considered as the most preferred manufacturing technique for orally administered tablets because of its advantages compared to wet granulation (i.e. fewer unit operations, shorter processing time, reduced stability risks for drugs that are sensitive to heat and moisture) [Jivraj *et al*., 2000]. Today, there is an increasing need for new direct compression excipients or co-processed excipients with improved flowability, bulk powder and compression properties [Gonnissen *et al*., 2007; Marwaha *et al*., 2010]. Therefore, the pharmaceutical industry is interested in adopting new excipients with high-functionality to provide an improved performance for their pharmaceutical formulations.

Amorphous solid dispersions (ASDs) have been increasingly used to improve the solubility and bioavailability of the poorly water-soluble active pharmaceutical ingredients (APIs). However, the physical and chemical stability of such systems still remains challenging for the pharmaceutical industry [Newman *et al*., 2012; Serajuddin 1999]. Therefore, the specific focus is to find suitable polymeric excipients that enable to stabilise the amorphous form of the API within ASDs.

All abovementioned challenges explain an increasing interest in the design and synthesis of new biomaterials as excipients with specified functionality in pharmaceutical systems [Jayakumar *et al.,* 2008; Prabaharan 2008; Rinaudo

2008]. Despite of an extensive research, however, virtually only a few new excipients have been introduced over the past twenty years [Baldrick 2000; Crowley and Martini 2007].

Lignocelluloses and lignin are side-products in the paper pulp manufacturing process, and they are readily available and cheap, but have not been investigated as excipients in pharmaceutical applications. Lignin is a polydispersed three-dimensional polymer in which the molecules are slightly cross-linked with each other [Doherty *et al*., 2011]. It is one of the three major polymeric components (like cellulose and hemicellulose) found in the cell walls of higher order plants [Hatakeyama and Hatakeyama 2010]. Natural softwood cellulose is a linear polymer of glucose (with a β-1,4 orientation of the glucosidic bonds) in plant or woody materials [Weil *et al*., 1994]. Application of these biomaterials (or related chemical derivatives) in engineering other polymers could lead to new manufacturing opportunities for a wide range of medical devices and pharmaceutical dosage forms including modern DDSs.

The present research work focuses on the functionality related characterization of catalytic pretreated softwood pine cellulose (CPSC) and lignin (CPSL) and synthetic polymer polyvinylpyrrolidone (PVP) in association with pharmaceutical solid dosage forms.

The major hypothesis was that the present biomaterials are capable of being used in some established pharmaceutical manufacturing processes, and that the respective finished products are stable during storage. Physical and chemical properties of excipients are the major factors affecting the manufacturing and performance of the final dosage form. Therefore, one important aim was to study the physicochemical properties of CPSC and CPSL. Secondly, tablet compression and material behaviour under compression (i.e. consolidation, densification and deformation mechanisms) of CPSC and CPSL was investigated. Thirdly, the applicability of CPSC and PVP as carrier polymers in the ASDs of a poorly water-soluble API, piroxicam (PRX), was investigated. Cryogenic co-grinding (co-milling) of PRX with carrier polymer(s) was used as a technique for preparing the amorphous formulations. The stabilizing properties of the carrier polymers in the ASDs were studied during a short-term storage. Finally, the effects of lignin on the mechanical and solid-state properties of aqueous hydroxypropyl methylcellulose (HPMC) films were investigated. The initial hypothesis was that the film properties of aqueous HPMC could be improved by using lignin in small amounts in the films.

2. LITERATURE REVIEW

2.1 Pharmaceutical excipients

The European Pharmacopoeia (Ph. Eur.) defines an excipient as any constituent of a medicinal product that is not an active substance [Ph. Eur. 2010]. Today, however, pharmaceutical excipients can not anymore be considered as inert or inactive compounds. Excipients play an integral role in the design, development and manufacture of pharmaceutical formulations. It is well-known that the properties and performance of the final pharmaceutical dosage form are highly dependent on the physical and chemical properties of the key excipients and their interactions with an API (or each other). Excipients appearance and role in transforming APIs into a high-quality dosage form is crucial [Crowley and Martini 2007; Rowe *et al.*, 2009].

2.1.1 Design and development of new excipients

The number of raw materials used in the pharmaceutical industry today is up to one thousand [Pifferi *et al*., 1999]. Historically, excipients were taken from the nature or food industry and were employed in the pharmaceutical industry without further modifications on their physicochemical properties [Baldrick 2000; Pifferi *et al*., 1999]. However, it might be expected, that the increased knowledge in modern technologies, innovative systems and novel forms of drug delivery, likewise emphasis on the efficient and cost-effective manufacturing processes would demand for more sophisticated formulation aids that can fulfill the requirements for the manufacturing processes and dosage form performance [Pifferi *et al*., 1999]. Despite of an extensive research, only a few new excipients have been introduced over the past twenty years. The reason for this is that novel excipients need to go through the multiple evaluation processes and regulatory approvements, which are expensive and time consuming for the pharmaceutical companies [Baldrick 2000; Crowley and Martini 2007]. Despite of the abovementioned challenges, pharmaceutical companies are interested in finding new excipient materials and/or modifying the functions of well-known and established excipients that already exist in the pharmaceutical market [Pifferi *et al*., 1999].

By modifying only some of the physicochemical properties (i.e particle size, degree of crystallization, etc) the excipient material with requested qualities for formulation processes can be obtained. *Partially or totally pre-gelatinized starches* are good examples of widely used modified excipients (derivatives) today. The gelatinized starch with enhanced binding and gel forming properties can be obtained by aqueous chemical or mechanical treatment [Pifferi *et al*., 1999; Symecko and Rhodes 1997]. Symecko and Rhodes (1997) indicated that the degree of the pre-gelatinization of the starch impacts the dissolution behaviour of API from the wet-bed granulated tablets, with better dissolution

obtained with tablets containing completely pre-gelatinized starches as a binder. Co-processing is another approach for the development of excipients with enhanced properties and modified functionality. Combination of two established excipients by an appropriate process without altering the chemical structure of the individual components results in the excipient product with added value related to its functionality. Moreover, co-processing of previously accepted excipients aids new excipients to appear on the market without undergoing time-consuming and costly safety testing [Marwaha *et al*., 2010; Patel and Bhavsar 2009]. For instance, *Cellactose* obtained by spray-drying of α-lactose monohydrate with powdered cellulose exhibits better flow characteristics as well as better compactibility properties compared to the physical mixture (PM) of lactose and cellulose [Patel and Bhavsar 2009; Pifferi *et al*., 1999].

By improving the physicochemical characteristics of the excipients at the beginning of drug development process, it is possible to improve the performance of the final drug formulation, which will allow the pharmaceutical industry to move towards faster development processes and easier manufacturing of dosage form formulations [Baldrick 2000; Pifferi *et al*., 1999; Pifferi and Restani 2003].

2.1.2 Excipient source and property variations

Excipients can be classified according to their origin or properties that relate directly to the pharmaceutical dosage form performance. The excipients can be classified according to their origin into four groups: (1) animal origin excipients (e.g. lactose, gelatin, stearic acid); (2) plant origin excipients (e.g. cellulose, starch, arginates, sugars); (3) mineral excipients (e.g. silica, calcium phosphate) and (4) synthetic excipients (e.g. PEGs (polyethylene glycols), povidone, polysorbates) [Pifferi and Restani 2003]. Excipients have different functions in a formulation to assure proper manufacturing and qualified performance of the drug product when administered to patients. The excipients functions are generally classified into three categories whether they are impacting the: (1) stability; (2) bioavailability or (3) manufacturing properties [Pifferi *et al*., 1999]. However, it should be bear in mind that the origin or functionality of the excipient does not necessarily guarantee the quality and intended performance of the dosage form. For including numerous functions in units to meet all quality criteria, new classes of excipients, which are modified from old or derived from new materials (alone or combined with each other), are now becoming available for pharmaceutical manufacturing processes [Baldrick 2000; Pifferi *et al*., 1999; Pifferi and Restani 2003].

2.1.3 Natural *vs* synthetic origin excipients

Pharmaceutical excipients have been originally adopted from the agricultural or food industry, and they have been considered as inert compounds in the drug

formulations [Pifferi *et al*., 1999]. Consequently, as being an extension of the food industry materials, the pharmaceutical excipients have maintained a good safety profile [Marwaha *et al*., 2010]. Natural polymers have been widely investigated in the field of pharmaceutical manufacturing as matrix and film coating formers, binders, drug release modifiers, stabilizers, disintegrants, viscosity enhancers, gelling agents, etc. Due to their low toxicity, readily availability, biodegradability, biocompatibility and renewability, biopolymeric materials serve as promising candidates for pharmaceutical formulations [Beneke *et al*., 2009]. A large number of polysaccharides with different origin (animals, plants), structure and physicochemical properties have been applied as biomaterials in the pharmaceutical formulations, such as controlled DDSs, tissue engineering, wound healing, etc. In recent years, there has been also an increased interest in the biomaterials possessing antimicrobial activity. For instance, chitin, found as a structural component in the exoskeleteon of arthoropods or in the cell wall of fungi and yeast, stimulated a non-specific host resistance against *Escherichia coli* infection in mice [Rinaudo 2008]. However, compounds of natural origin bring several challenges to the pharmaceutical companies: their structure is complex and composition generally depend on the location or season variables, which can results in batch to batch variations and expensive time-consuming isolation and purification processes [Beneke *et al*., 2009].

Synthetic and semi-synthetic excipients, frequently used in the pharmaceutical formulations, offer several advantages over natural polymers. For instance, there is more control over the manufacturing process as well as higher degree of process flexibility with synthetic and semi-synthetic polymers compared to natural origin excipients. Additionally, the variability that exists in natural polymers is excluded. There are also no immunological concerns with synthetic excipients [Russel 2004]. Due to biodegradability and biocompatibility of several synthetic components they have been successfully employed as a domain scaffolding material in the tissue engineering. However, despite of all benefits synthetic polymers still lack the biological signals inherent for many natural polymers, which are crucial for promoting the cell response [Place *et al*., 2009].

After all, pharmaceutical industry will keep on searching for the ways to make the manufacturing processes simpler and drug formulations more efficient. Excipients are just one of the tools to achieve a proper quality of the final product as well as proper efficacy in the manufacturing process itself.

2.1.4 Functionality-related characteristics of excipients

According to Ph. Eur. (2010), the functionality-related characteristics (FRCs) of excipients are controllable physical or chemical characteristics that impact on its intended functionality which is directly related to physical and biopharmaceutical properties of final product [Ph. Eur. 2010]. The variation in the physical and chemical properties of excipient(s) is one of the most important

factor that can impact the manufacturing process, and consequently, the quality of the final dosage form. In addition, the functionality of the excipient itself may depend on the interactions between the components of the formulation and stresses related to the manufacturing process. The European Pharmacopoeia Commission admitted that in order to achieve consistent product quality level, the functionality of the excipients should be evaluated during manufacturing process and in the context of the particular formulation. As a basis for final qualified dosage form formulation a full characterization of the excipient properties and investigation of the functionality of excipients in the concerned formulation must be performed [Kristensen 2007]. The FRC sections in the Ph.Eur. excipient's monographs comprise of the FRCs that have influence(s) on the functionality of the excipient for the stated formulation(s) or in specific uses [Ph. Eur. 2010]. For instance, pharmaceutical excipient Hypromellose can be used as binder, film former, viscosity-increasing polymer and matrix former in prolonged release-tablets. The FRCs section for Hypromellose recommends apparent viscosity and degree of substitution as relevant control parameters for obtaining qualified final medicinal product. In addition to above-mentioned FCRs the following characteristics may be relevant for Hypromellose, when used as matrix former in prolonged release-tablets: molecular mass distribution, particle-size distribution and powder flow [Ph. Eur. 2010].

2.2 Higher plants as a source for pharmaceutical excipients

Plant polymers (cellulose and its derivatives, starch, inulin, pectin, suberin, etc.) have been investigated and widely applied as pharmaceutical excipients in conventional drug products and modern DDSs. These biopolymers exhibit low toxicity, readily availability, biodegradability, biocompatibility and renewability, thus being very promising candidates as pharmaceutical excipients [Beneke *et al*., 2009].

Higher plants provide renewable materials such as cellulose and its derivatives, that are one of the most abundant biopolymers on earth, being widely used in the pharmaceutical industry. Several polysaccharides obtained from the plants such as carrageenan, alginate, gum arabic have been investigated as gel forming agents and carrier materials in matrix type controlled DDSs. Additionally, plants are a source for polysaccharides with good film forming properties. For example, inulin, due to its resistance to digestion in the upper gastrointestinal tract, but biodegradability by colonic microflora, have been used as a novel excipient in the preparation of biodegradable colon-specific films [Beneke *et al*., 2009].

An increasing number of new polysaccharides with different chemical structures and unique properties will form a rich material source for an increasing number of applications of plant-derived polymers in the field of pharmaceutical formulations in the future.

2.2.1 Cellulose, hemicelluloses, lignin

Wood fibers are composed of polymeric materials with cellulose microfibrils being as the major component (45%) covered by an amorphous matrix of lignin (30%) and hemicellulose (25%).

Natural cellulose is a polydisperse linear polymer of glucose in plant or wood materials [Klemm *et al*.,1998; Kamel 2007]. **Figure 1** illustrates the molecular structure of cellulose. Cellulose is composed of repeated D-anhydro-glucopyranose units (AGU), linked together by β-1,4-glucosidic bonds. Two neighboring cellulose AGUs build the dimer or disaccharide called cellobiose. To obtain cellobiose every second AGU ring is rotated 180^{o} in the plane, so the preferred bond angle for creation of acetal oxygen bridges between two neighboring glucopyronosil rings is arranged [Klemm *et al*., 1998; Klemm *et al*., 2005]. Cellulose is organized in a hierarchical fibrillar fashion with the smallest morphological unit denoted as elementary fibril. The glucan chains are bound together by the hydrogen bonds in parallel arrays and assembled into the microfibrils. The microfibrils, in turn, are then aggregated to form larger morphological units called macrofibrils, and further merged into cellulose fibers [Klemm *et al*., 1998].

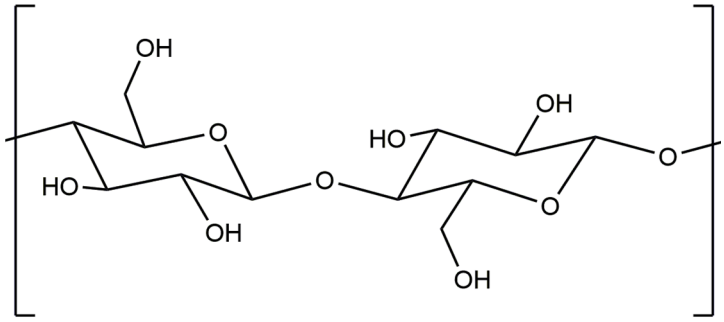

Figure 1. The structrue of cellulose. Dimer (cellobiose) built by cellulose monomers.

Each of the cellulose's AGUs possesses three hydroxyl groups, two primary groups at the positions C2 and C3, and one secondary group at the position C6. These hydroxyl groups along with oxygen atoms of both pyranose ring and glycosidic bond are able to form intra- and intermolecular hydrogen bonds, thereby forming supramolecular semi-crystalline structures of cellulose [Klemm *et al*., 1998]. As a result of the supramolecular structure, cellulose is composed of both high order (crystalline) and low order (amorphous) regions [Klemm *et al*., 2005]. Natural cellulose chains represent a degree of polymerization (DP) of 300 to 10,000, depending on the material source and pretreatment methods of the raw material [Klemm *et al*., 2005]. However, a glucan with the DP value of 30 already represents the structure and properties of cellulose [Klemm *et al*., 1998].

Four different polymorphic crystalline structures of cellulose have been identified in the literature: I, II, III and IV. Cellobiose chains in cellulose I are organized in parallel direction [Gardner and Blackwell 2011], whereas cellulose II is formed by antiparallel cellobiose segments packing. The main intermolecular hydrogen bond for cellulose I is suggested to be between O-6-H and O-3 of neighboring chain. Crystalline cellulose I occurs in two allomorphic modifications Iα and Iβ, where Iα exhibits triclinic unit cells, while Iβ allomorph consists of monoclinic unit cells [Vander and Atalla 1984].

The microfibrils of wood cellulose are typically covered by an amorphous matrix of lignin and hemicelluloses. Hemicelluloses are heteropolysaccharides (composed of both, hexoses and pentoses) that are bound to the surface of cellulose microfibrils, providing a cross-linked matrix. The main hemicelluloses in plants are xyloglucans, arabinoxylans and mannans [Sjöström 1993].

Lignin is considered as structural material to add strength and rigidity to cell walls of the plants, being more resistant than cellulose and other polysaccharides in plants against biological attacks [Akin and Benner 1988]. Lignin is three-dimensional amorphous polymer of phenylpropane units. The higher-order structure of lignin is heterogeneous consisting of three main side chains: guaiacyl (1), syringyl (2) and 4-hydroxyphenyl (3) structures, which differ in the degree of oxygen substitution of the phenyl ring (**Figure 2**). The side chain 4-hydroxyphenyl or H-structure has a single hydroxy or methoxy group, guaiacyl or G-structure has two groups, and syringyl or S-structure has three groups. The significant differences have been found in the content of the phenylpropane precursors among the wood types. Almost all phenylpropane units in the softwoods are guaiacyl type. However, hardwoods contain the mixture of guaiacyl and syringyl units. Phenylpropane units are joined together with both carbon-carbon (C-C) and carbon-oxygen (C-O-C) bonds. The most dominant structural backbone of the lignin consists of β-O-4 linked ether units [Chakar and Ragauskas 2004; Doherty *et al*., 2011; Hatakeyama and Hatakeyama 2010; Sjöström 1993].

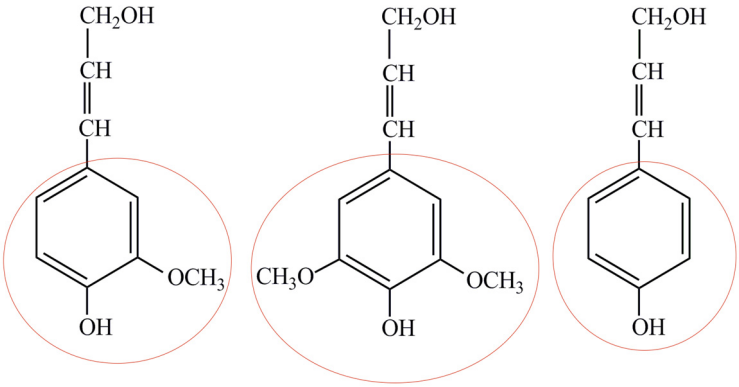

Figure 2. Three main structures of lignin precursors (marked in circles): guaiacyl (1), syringyl (2) and 4-hydroxyphenyl (3) structures.

Lignin polymer chains are formed from a number of characteristic functional groups that can affect lignins reactivity: methoxyl, phenolic hydroxyl, alcoholic hydroxyl and aldehyde groups. Most of the hydroxyl groups, however, are not free, being linked to the neighboring phenylpropane units [Sjöström 1993]. Additionally, lignin is typically linked to other polysaccharides (cellulose, hemicellulose) in the wood through the lignin-carbohydrate covalent bonds. Such aggregates are called "lignin-carbohydrate complexes" (LCC) [Hatakeyama and Hatakeyama 2010; Sjöström 1993].

The polymerization process of the phenylpropanoid monomers is initiated by the enzymatic dehydrogenation of phenolic hydroxyl groups. Lignin biosynthesis is initiated by electron transfer, which results in the formation of phenoxy radicals. The radicals can couple with each other producing dimers and oligomers also referred as lignols [Chakar and Ragauskas 2004; Doherty *et al*., 2011; Hatakeyama and Hatakeyama 2010; Sjöström 1993]. Lignin is a heterogeneous polymer, which exactly defined structure is unknown. The diversity of the lignin's structural combinations is unlimited, since there are numerous variations among the wood species as well as various factors may influence the biosynthesis of the lignin.

Traditionally, lignin has been considered as a by-product of the pulping or bioethanol industries and primarily applicable for a waste stock for energy generation. Recently, however, an increasing interest has been shown in using lignin as a bio-resource for the production of renewable chemical feedstock and new lignin-based products. In spite of a considerable progress, the extraction of the lignin from the lignocellulosic biomass is still remaining challenging. Separation of cellulose from lignin in an economical way is one of the main technical issues that need to be resolved in order to obtain beneficial lignin-based products [Doherty *et al*., 2011].

2.2.2 Pretreatment methods of lignocelluloses

Polymers of a higher plant origin are complex and heterogeneous in their structure, which brings additional challenges to the isolation processes of cellulose. In the plant cell walls lignin along with hemicelluloses form a cross-linked matrix around the cellulose microfibrils. This matrix structure prevents enzymes and acids from accessing the cellulosic biomass for futher practical cellulose conversion processes (e.g. ethanol production). In addition, cellulose's highly ordered, tightly packed semi-crystalline structure itself also challenges further isolation processes. In order to enhance the accessable surface area of cellulosic biomass to the enzymes, which convert carbohydrate polymers into fermentable sugars, matrix modifications step should be performed. This step is called a pretreatment (**Figure 3**).

The pretreament of lignocelluloses consists of the following processes: (1) break down of the lignin seal, (2) solubilisation of hemicelluloses, (3) reduction of the crystallinity of the cellulose and (4) increase of the porosity of ligno-

cellulosic materials [Hsu *et al*., 1980; Kumar *et al*., 2009; Mosier *et al*., 2005; Weil *et al*., 1994]. The conditions or method used to break down the lignin into lower molecular weight fragments can affect the physicochemical properties of the lignin. Consequently, the method of extraction and the source of the lignin most likely have a great influence on the structure and physicochemical properties of isolated lignin [Doherty *et al*., 2011].

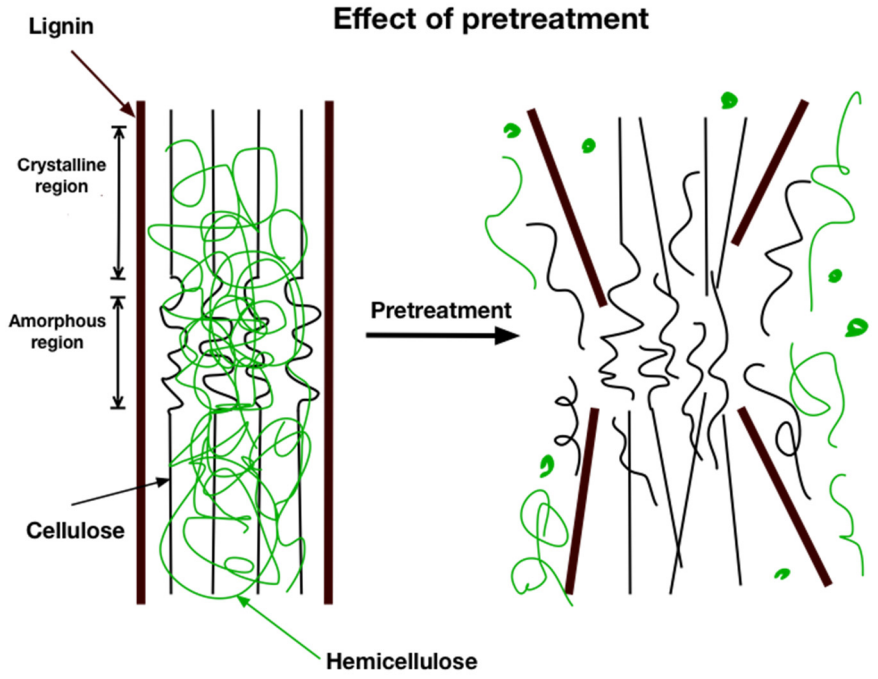

Figure 3. Pretreatment of lignocellulosic biomass (modified from Hsu et al., 1980).

The following six different categories of pretreatment methods for lignocellulosic materials have been described in the literature: physical, physicochemical, chemical, biological, electrical or the combination of any of these [Kumar *et al*., 2009].

Mechanical milling can be applied as a physical pretreatment method to decrease the crystallinity of cellulose to improve the hydrolysis processes [Kumar *et al*., 2009; Sun and Cheng 2002].

Physicochemical pretreatment methods include steam explosion, liquid hot water pretreatment techniques, ammonia freeze explosion and carbon dioxide explosion [Kumar *et al*., 2009; Mosier *et al*., 2005]. Uncatalyzed steam explosion is applied as a pretreatment process in which the lignocellulosic biomass is rapidly heated for a few minutes using high-pressure steam without the addition of any chemical substances, followed by decompression phase. This process resulted in hydrolyzation and degradation of hemicellulose by the acids,

that are released during steam explosion, thus increasing the potential of cellulose hydrolysis [Brownell and Saddler 1984]. Liquid hot water pretreatment utilizes pressurized (to maintain the water in the liquid state at high temperatures) hot water to flow through cellulosic biomass, removing hemicellulose from the lignocellulosic biomass [Sun and Cheng 2002].

Chemical pretreatment methods include organic solvents as well as acids or bases that promote hydrolysis [Brownell and Saddler 1984; Mosier *et al*., 2005]. Kraftpulping is the dominant technology among chemical pretreatment methods [Chakar and Ragauskas 2004]. Kraftpulping method uses sodium hydroxide and sodium sulfite under the strong alkaline conditions and high temperatures and pressure to react with wood chips, resulting in the cleavage of the ether bonds in lignin. Therefore, lignin macromolecules are prone to fragmentation into smaller water or alkali-soluble fragments. Sulfite process is another approach for the delignification of the wood. Sulfite technique involves the reaction of metal sulfite and sulfur dioxide with wood, resulting in the formation of lignosulfonic acid and relatively soluble lignosulfonates and, finally, in the fragmentation of the lignosulfonates. The drawback of these two methods is that the high content of sulfur (5%) in the isolated lignin limits its applications, and so the chemically pretreated lignin is used only for energy generation [Smook 2002]. The organosolv pretreatment strategy uses the mixture of the organic solvents (methanol, ethanol, ethylen glycol, etc.) with inorganic acids (HCl, H_2SO_4) as catalysts for the delignification of the lignocellulosic biomass. Alkaline hydrolysis utilized bases (sodium, potassium, calcium and ammonium hydroxide) to proceed sugar degradation, but comparing with acid pretreatment processes the degradation degree is lower [Kumar *et al*., 2009].

The use of different pretreatment methods is limited by a number of drawbacks, such as the low rate of cellulose hydrolysis (and as a result low final yield), high cost of chemicals, and formation of toxic compounds during the process [Kumar *et al*., 2009]. According to Kumar *et al.* (2009) there is a strong demand for the improved efficient pretreatment technologies. There are four important criteria that pretreatment must meet for achieving beneficially effective yield: the method needs (1) to be cost-effective, (2) to avoid the loss of the hydrolysable carbohydrate products, (3) to avoid the formation of toxic compounds, that can act like inhibitors to the hydrolysis or fermentation processes, and (4) to make cellulose that can be easily hydrolysable and improve fermentation processes [Kumar *et al*., 2009].

2.2.3 Catalytic pretreated softwood pine cellulose and lignin

Recently, Hakola *et al.* (2010) presented a new promising catalytic pretreatment method for the separation of cellulose from lignocellulosic biomass for enzymatic hydrolysis. The present technology (1) provides cellulose that can easily undergo hydrolysis, (2) avoids the loss of hydrolysable carbohydrates, and (3) avoids the formation of toxic compounds. It can be considered also as a cost-

effective and environmentally benign concept based on *in situ* catalysts (copper(II) diimine complexes) and pressurized air or oxygen as the oxidant. The hydrolysis is faster and the degree of hydrolysis notably higher for catalytically pretreated material than for steam-exploded spruce, which represents a state-of-the-art technique for the cellulosic ethanol production [Hakola *et al*., 2010]. Schematic flow chart of the isolation of the catalytic pretreated softwood cellulose (CPSC) and lignin (CPSL) is presented in **Figure 4.** The process description in brief is the following: Pine *(Pinus sylvestris)* chips are first extracted with hexane/acetone mixture for one day to remove the extractives. Efficient catalytic treatment with molecular oxygen (oxygen pressure 6-10 bar) as an oxidant requires strongly alkaline conditions (NaOH, pH>12) and high temperatures (100-120^{o}C). Catalytic oxidation method loosens the wood matrix and liberates cellulose with high yield. After the separation of the solid cellulose fraction from the reaction mixture the filtrate can be acidified with HCl and precipitated lignin is collected with an additional filtration. Using the present technique and by varying the conditions of isolation process, physical material and powder properties as well as the solubility of lignocelluloses and lignin can be modified thus making them very flexible for pharmaceutical applications [Hakola *et al*., 2010]. This pretreatment method was also used in this thesis to produce the lignin and cellulosic biomaterials for futher studies.

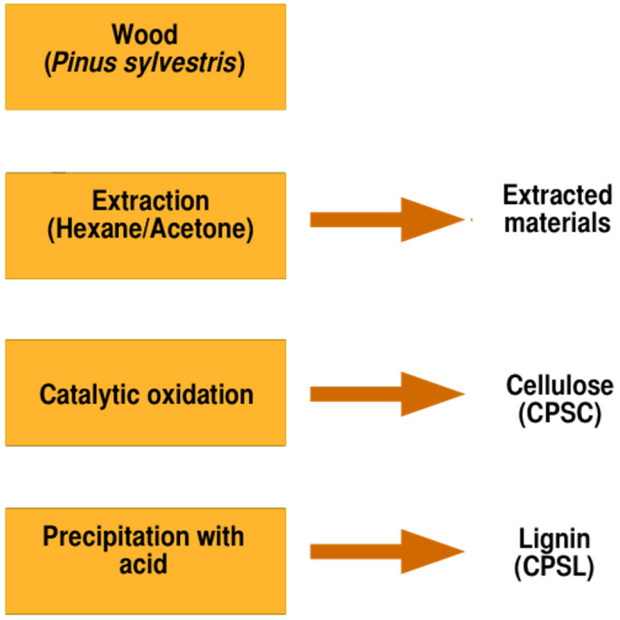

Figure 4. Schematic diagram of the catalytic isolation of the softwood lignocellulose and lignin.

2.2.4 Pharmaceutical applications of cellulose and cellulose derivatives

Cellulose and its derivatives have been widely used as excipients in manufacturing pharmaceutical oral dosage forms and controlled release DDSs, in tissue engineering as well as in blood and water purification [Beneke *et al*., 2009; Chang and Zhang 2011]. Among all cellulose derivatives microcrystalline cellulose (MCC) can be considered as the most frequently used excipient in pharmaceutical formulations. MCC is commonly applied as a tablet binder and diluent in both direct compression and granulation processes [Beneke *et al*., 2009; Hon 1996; Jivraj *et al*., 2000].

Cellulose ethers are commonly used in pharmaceutical film coatings and bioadhesive DDSs [Shokri and Abibkia 2013]. Hydroxypropyl methylcellulose (HPMC) is a cellulose ether derivative applied in a tablet film coating as well as in matrix tablets for extended release of the drug. Karavas *et al*. (2006) formulated HPMC/PVP pulsatile release formulations with enhanced mucoadhesive properties. The enhancement in the mucoadhesive properties was attributed to the complete miscibility of blend, the higher rate of wetting, and the flexibility of the PVP containing matrix [Karavas *et al*., 2006]. Ethyl cellulose (EC) has been used in coated extended-release formulations due to its insolubility in water. The mechanism of action of aqueous coating dispersions of EC, such as Aquacoat® (FMS BioPolymer), is based on the formation of insoluble viscous gel around the tablet resulting in drug release inhibition [Shokri and Abibkia 2013].

Cellulose esters, such as cellulose acetate phtalate (CAP) and and hydroxypropyl methylcellulose phthalate (HPMCP) are intended for the preparation of pH-sensitive enteric coatings for oral site-specific drug delivery. Cellulose acetate (CA) can be used as a semi-permeable film coating material for different types of osmotic DDSs [Shokri and Abibkia 2013].

Additionally, bacterial cellulose (BC) due to its unique structural and mechanical features have been successfully applied for skin tissue repair [Fu *et al*., 2013]. The main characteristic of the BC is the ability to absorb exudate during skin repairing process and to hold the moisture inside the structure, resulting in the painless remover of the BC dressing from a wound surface after the recovery [Czaja *et al*., 2007a]. Furthermore, elastic properties and comfortability of BC allowed to excellent adherence of the biomaterial even to the moving parts of the body [Czaja *et al*., 2007b].

Recently, Kolakovic *et al.* (2012) applied nanofibrillar cellulose (NC) as a matrix former material for sustained release DDSs. The studies showed that the NC film extended the release of poorly soluble drugs up to three months.

2.2.5 Lignin-based formulations

Lignin is very abundant natural polymer and the lignins from different sources have been used for decades in many commercial applications and as a raw material for chemicals [Doherty *et al*., 2011; Gargulak and Lebo 2002]. Lignosulfonates, obtained from the pulping liquors, are used for many production chemistries without any futher modifications and purification. The markets for the lignosulfonates can be divided into two main classes: speciality and commodity [Gargulak and Lebo 2000]. Speciality markets include the production of vanillin, pesticides, dyes and pigments, industrial cleaners, emulsifiers, water treatments etc. Commodity markets in turn consist of such lignin-based products as dust controllers, animal feed pelleting aids, phenol-formaldehyde resins and concrete additives [Gargulak and Lebo 2002].

Due to the fact that lignin can be isolated from different sources and by different extraction methods (thus resulting in the variations of structure and characteristics), it is challenging to develop lignin-based formulations. The diversity of the lignin structure leads to the necessity of the modification methods for deriving the desired substances, materials and products from lignin. However, it is apparent from the past and ongoing studies that there is a strong emphasis on finding more commercial alternative values to lignin [Chowdhury 2014; LigniMATCH 2010]. For example, activate carbon, that is widely used for cleaning environmentally hazardous emissions from air and water, can be produced from lignin with some chemical or physical modifications. Phenols, which can be called as a platform block for the synthesis of many chemicals, can be also derived from lignin. Lignin-based polymers are also widely used to produce different types of plastics [LigniMATCH 2010]. In addition, the lignin-based controlled release matrices for bioactive materials (herbicides, insecticides, pesticides etc) have been widely investigated over the last three decades. However, since the extent of water uptake and matrix swelling depend on the heterogeneous structural properties of the lignin, it is quite challenging to understand and explain the underlying process of the release behaviour of the bioactive materials, which are loaded into the lignin matrices [Chowdhury 2014]. Lignin has been also used as one of the components in the preparation of the hydrogels for different applications. For example, starch based hydrogels containing the lignin are used to remove toxic metal ions. Another example is xanthan/lignin hydrogels that have been investigated as a novel superabsorbants and are promising potential formulations in pharmaceutical and cosmetic industries [Thakur and Thakur 2015].

However, so far lignin has not been broadly investigated in medical or pharmaceutical applications. In this thesis, not only the characterization of the CPSL was performed but its applicability in different pharmaceutical formulations was investigated as well.

2.3 Polyvinylpyrrolidone

2.3.1 Synthesis, chemical structure and physicochemical properties

In 1938, BASF developed polyvinylpyrrolidone (PVP) or Povidone (brand name Kollidon®), with the chemical structure shown at **Figure 5**, by reacting acetylene with pyrrolidone followed by radical polymerization. Particle size of the PVP ranges from 50 μm to 250 μm. PVP is marked under different K-values (e.g. Povidone K-12, Povidone K-25, Povidone K-30, Povidone-90, etc), which are related to the mean molecular weight of the polymer [Folttmann and Quadir 2008; Haaf *et al.,* 1985].

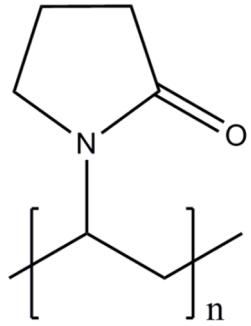

Figure 5. Chemical structure of polyvinylpyrrolidone (Povidone).

One of the main features of the PVP is its universal solubility in hydrophilic and hydrophobic solvents (even at high concentrations) producing slighlty viscous solutions. Povidone possesses the ability to form water-soluble complexes (by hydrogen bonding) with poorly water-soluble APIs, that is widely used in the pharmaceutical industry to improve the release and solubility properties of the drug formulations. Additionally, it is non-toxic, chemically inert, temperature resistent and pH-stable [Folttmann and Quadir 2008; Haaf *et al.,* 1985].

2.3.2 Application in pharmaceutical formulations

Due to its unique properties PVP has been widely used in pharmaceutical applications. The main application for PVP is as a binder in direct tablet compression and in wet and dry granulation. Povidone K 25, K 30 and K 90 are the most widely used binders in wet granulation prior to tableting [Folttmann and Quadir 2008]. Nowadays, Kollidon F 90 is the strongest wet granulation binder on the market, possessing also viscosity modifying properties of liquid dosage forms. Kollidon VA 64 (copovidone with special high binding capacity), is used as a dry binder for direct compression [Fussnegger 2014]. Solid dispersions (SDs) or solid solutions with increased APIs bioavailability can be

formulated using Povidone [Folttmann and Quadir 2008]. For instance, amorphous SDs of PRX with PVP with increased dissolution of the API were obtained by solvent method [Tantishaiyakul *et al*., 1999]. PVP is also known as a disintegrant: Kollidone CL is used as a super-disintegrant and dissolution enhancer [Fussnegger 2014]. Apart from the abovementioned applications of PVP, this polymer can also be used as a solubilizing agent, crystallization inhibitor, suspension and emulsion stabilizer and a film-forming agent. It is still ongoing process of finding different alternative applications for PVP [Folttmann and Quadir 2008].

2.4 Tablet compression

Today, tablet is still considered as the most convenient and most popular oral dosage form (more than 80% of all dosage forms on the market are tablets). The reason is that tablets have numerous advantages compared to other dosage forms, such as accurate dosage, ease of manufacture and administration, good stability properties compared to liquid and semi-solid formulations, they are suitable for large scale production and easy in packaging and shipping. Additionally, the possibility of special release profiles and good acceptance by patients make them essential products in the industrial scale [Jivraj *et al*., 2000].

2.4.1 Tablet formulation

Tablet formulation represents the process of combining several components (APIs, excipients) with different properties and functions. The tableting materials can be pre-granulated or they can be directly compressed into tablets. The main advantage of a granulation stage prior to tableting is that the poor compressibility and flowability properties of the materials can be significantly improved resulting in better manufacturability (tabletability) of the powder. Today, however, direct compression is considered as a convenient technique for tablet manufacturing because of a number of advantages compared with wet granulation: fewer unit operations, shorter processing time, a need of less number of excipients in a tablet formulation, fewer stability risks for drugs that are sensitive to heat or moisture, and faster dissolution of the final product [Jivraj *et al*., 2000].

2.4.2 Compression and compaction behaviour of materials

Compression of powder is defined as a reduction in the volume of powdered mixture as a result of applied stress. All materials differ from each other in their mechanical and compaction properties during tableting [Alderborn and Nyström 1996]. The fundamental knowledge about the compression of pharmaceutical powders is essential for the improvement of the quality of the final tablets and for the development of the compaction process.

When powder is compressed, solid powder particles can deform elastically, plastically or they can fragment (**Figure 6**). Elastic deformation is reversible, plastic deformation is irreversible and fragmentation can be considered as breaking particles into smaller, discrete parts [Duberg and Nyström 1982; Nyström *et al*., 1993]. The deformation mechanism of the material strongly depends on the particle size and structure of the powdered material, as well as on the applied forces and compression speed [Rasenack and Müller 2002]. During tablet compression, powder undergoes the following transformation phases: (1) *Rearrangement* (when the punch starts penetrating into the die the densification of the powder takes place; during this stage small particles start to occupy voids between the larger particles); (2) *Deformation* (when there is no more space for the movements of the particles the material starts to deform elastically); (3) *Compaction* (when the specific elastic limit of the particles is exceeded the material starts to deform plastically or fragment; in this stage the materials do not regain their original shape even when the applied force is released), and (4) *Relaxation or decompression* (in this stage the applied compression force is removed and the compacted mixture will undergo relaxation) [Jivraj *et al*., 2000, Rubinstein 1988].

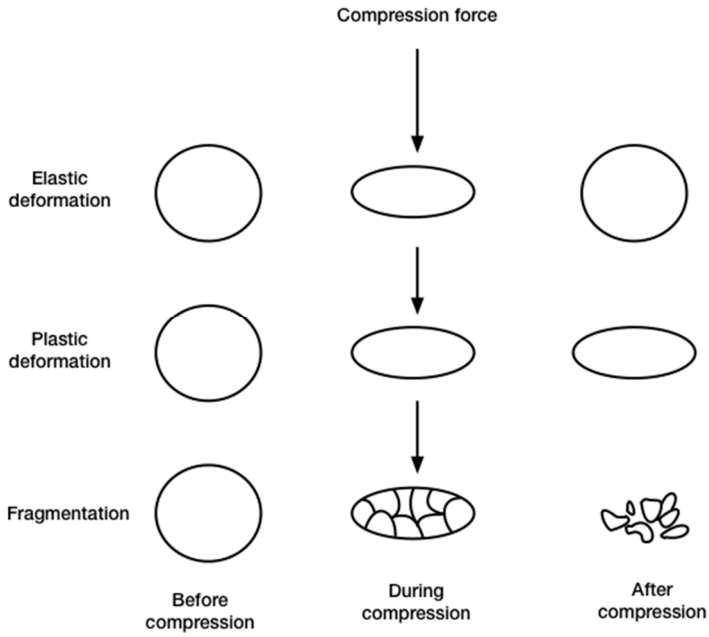

Figure 6. Powder deformation during compression (modified from Ragnarsson 1996).

2.4.3 Analysis of tablet compaction data

Nowadays, there are several mathematical analyses to investigate the compaction profiles of the materials. The following compression parameters can be evaluated to characterize the compaction properties of pharmaceutical powders: a ratio of breaking strength as a function of compression pressure, plasticity (PF) and elasticity (EF) factors of the materials, stress relaxation measurements and pressure-porosity equations. Additionally, different types of pressure cycle plots (force-time and force-displacement compression profiles) aided in evaluating the compaction and consolidation mechanisms of pharmaceutical powders [Antikainen 2003].

The Heckel analysis is probably the most widely used method to evaluate the consolidation and compaction behaviour of the powder. The Heckel equation (1) determines the decrease in the porosity of the powder under the compression pressure during tableting:

$$\ln [1/(1-D)] = KP + A \quad (1)$$

where D is relative density of the tablet, 1-D is porosity, P is pressure, K and A are constants derived from the slope and intercept of the Heckel plot (ln [1/(1-D)] vs. P), respectively. Slope K characterizes the plasticity of the compressed materials, and constant A indicates the processes related to the volume reduction [Paronen and Ilkka 1995].

However, differences in the instrumentation of the tableting machines and technical setups make it challenging to compare the compression profile data between different authors [Antikainen 2003].

In 2003, Antikainen and Yliruusi introduced a new method for the evaluation of the compression behaviour of the materials using the information derived from compression force-punch displacement curves [Antikainen and Yliruusi 2003]. This method has been exploited in analysing for example the deformation and compaction properties of native amino polysaccharides chitin and chitosan [Garcia Mir *et al.*, 2008]. The present method was also used in this thesis to classify the compaction behaviour of lignin and cellulosic biomaterials.

In recent years, much attention has been paid on the development of new excipients and/or modification of established excipients for direct tablet compression [Gonnissen *et al.*, 2007]. The major reasons for this interest is due to an increasing interest in the direct compression process, increasing speed capabilities of modern tableting machines, technological and end-user limitations of existing excipients and increased performance expectations of excipients [Patel and Bhavsa 2009].

One of the aims of the present thesis was to investigate the direct compression properties and deformation mechanisms (elasticity, plasticity or fragmentation) of CPSC and CPSL when compressed into tablets.

2.4.4 Characterization of final tablet properties and quality

The Ph. Eur. describes several final tablet characterization and qualitcy tests including Uniformity of mass, Uniformity of content, Uniformity of dosage units, Disintegration time, Dissolution, Mechanical strength (tensile strength) and Friability (hardness). Readers are referred to the corresponding pharmacopoeia [Ph. Eur. 2010] for details on the test procedures and requirements.

2.5 Amorphous solid dispersions

Bioavailability enhancement of poorly-water soluble drugs is considered as one of the most challenging goal for the pharmaceutical industry. Formulation of the API in the amorphous form provides a solution to overcome the limitation of poor water-solubility and dissolution rate, and as a consequence poor bioavailability of the drugs [Newman *et al*., 2012; Serajuddin, 1999]. Unlike crystalline solids, amorphous solids do not possess a long-range order in their molecular packing. Furthermore, in comparison to the crystalline solids, amorphous solids possess higher free energy, thus offering enhanced apparent solubility, higher dissolution rate and thus bioavailability [Teja *et al*., 2013; Zografi and Newman 2015] Together with higher energy, however, amorphous state is thermodynamically very unstable and tends to crystallise out over time. Therefore, amorphous drug alone is rarely developed into drug formulation [Teja *et al*., 2013]. Amorphous solid dispersions (ASDs) can be considered as systems where poorly water-soluble API is molecularly dispersed in the hydrophilic carrier polymer producing a single-phase completely miscible amorphous mixture [Padden *et al*., 2011]. Therefore, ASDs offer more stabilized system, where the amorphous state and its solubility advantages are retained.

2.5.1 Preparation of amorphous solid dispersions

There are several techniques for obtaining ASDs, spray drying and hot melt extrusion being two of the leading formulation methods nowdays [Dobry *et al*., 2015].

Spray drying method involves the preparation of the solution, suspension or emulsion of the solubilized drug and carrier polymer followed by futher evaporation of the solvent [Dobry *et al*., 2015].

Hot-melt extrusion method consists of solubilization step of the drug in the molten polymer followed by the extrusion step. Melt agglomeration and Meltrex™ are another two types of melting methods where the drug and the stabilizer are heated and mixed together [Brown *et al*., 2014; Vasconcelos *et al*., 2007].

Grinding (milling) is one other possible approach for obtaining ASDs. Grinding process is a combination of strain field formation and relaxation, wherein the first phenomena leading to the disruption of the crystalline lattice of the drug. With more energy applied to the system the defects on the crystalline

surface lead to the complete amorphization of the system. Ball-grinding at extremely low temperature (cryo-grinding/milling) prevents the occurence of thermal damage and undesirable chemical reactions between phases [Colombo *et al*., 2009]. However, physical and chemical stability of the amorphous form of the API remain still challenging in cryo-ground formulations [Adrjanowicz *et al*., 2011]. Therefore, to inhibit the recrystallization of amorphous form of API the drug can be co-ground with the stabilizing polymer [Brown *et al*., 2014]. The specific aim of this thesis was to obtain ASDs using cryogenic co-grinding technique.

2.5.2 Physicochemical and biopharmaceutical properties of amorphous solid dispersions

In order to successfully apply ASDs for solubility and bioavailability enhancement of poorly water-soluble API, it is necessary to maintain the amorphous form of the API during the manufacturing process as well as during the shelf life of the drug formulation [Taylor 2015]. Hence, ASDs provide a formulation-based approach to stabilise the amorphous form of an API by adding the crystallization inhibitor to reduce the molecular mobility and the rate of crystallization of amorphous state, thus providing long-term stability [Zografi and Newman 2015].

There are numerous polymers that can be used in ASDs as crystallization inhibitors. The selection of the proper polymer to prepare ASDs with good stability properties depends on several performance characteristics of the polymer: (1) structural compatibility with an API; (2) thermal stability and high glass transition temperature (Tg); (3) good solubility in organic solvents; (4) and good solubilizing properties of the API in ASDs [Narayan *et al.,* 2015].

Carrier polymers with high Tg are preferred, since they increase the Tg of the system compared to the Tg of API alone. As a consequence, the high Tg of the ASD system lowers the mobility of the compound's molecules inside the system, preventing the system from phase separation, when stored at a particular temperature, and acting like a recrystallization inhibitor [Brown *et al*., 2014].

Additionally, drug-polymer miscibility is essential to maintain a long-term physical stability of the ASDs. This is due to the fact, that in most ASDs the API is supersaturated beyond it's solubility limit. The supersaturated system is often thermodynamically unstable tending to phase separation and API recrystallization. The supersaturation of the API in ASDs is considered as the main driving force inducing the solution-mediated nucleation and crystalline growth. The supersaturation of the system is maintained through the miscibility of two phases. If the API and the polymer are not homogeniously miscible the phase separation occurs leading to the recrystallization of the amorphous drug [Brown *et al*., 2014; Newman *et al*., 2012]. Polymers commonly used in the preparation of the ASDs are: (1) polyglycols (PEG), polyethylene oxide (PEO)); (2) polyvinyl polymers (PVP, crospovidone (PVP-CL)); (3) cellulosic polymers (HPMC, HPMCP, hydroxypropylmethyl cellulose acetate/succinate

(HPMCAS)) and (4) methacrylate/methacrylic acid copolymers [Narayan *et al.,* 2015]. This thesis aimed to investigate the stabilizing properties of the CPSC and PVP25 during short-term storage of the ASDs prepared with poorly water-soluble API during cryogenic co-grinding.

By incorporating a hydrophobic drug into a hydrophilic polymer the enhanced dissolution of the API can be achieved. Increased dissolution behaviour is attributed to several aspects such as: (1) separation of the drug particles by polymer particles (reducing the aggregation of the particles); (2) improved wettability of the drug particles by carrier polymer particles and (3) prevention of solvent-mediated recrystallization of the amorphous API (maintaining the supersaturation state of the API in the solution) when contacting aqueous media by a carrier polymer [Brown *et al*., 2014; Zografi and Newman 2015].

2.6 Film coating of tablets

2.6.1 Pharmaceutical applications

Aqueous polymeric film coatings are widely used for the formulation of pharmaceutical oral solid dosage forms. The major application areas of polymeric coatings include taste masking, protecting pharmaceutical formulations from environmental conditions (e.g to control the moisture permeability), and controlled drug release. Today, aqueous coating polymer dispersions are preferred over organic solvents, since they offer several advantages from the pharmaceutical industry point of view, such as non-toxic and environmentally safe materials, high solid loadings, low viscosity values, and improved fluid properties (resulting in higher spray rates and short coating times) [Carlin *et al*., 2008].

2.6.2 Film coating formulation

There are two main groups of aqueous polymer dispersions differing from each other in the preparation method: latexes and pseudolatexes. Latex-type coating dispersions are formed by the polymerization of the monomer in an emulsified state. Pseudolatex dispersions are prepared by emulsification of already existing polymer. Pseudolatexes are typically prepared by first dissolving the polymer in a solvent followed by emulsifying step of the polymer solution into water. Homogenization is performed in order to decrease the particle size of the polymer droplets. Removing the solvent by distillation generally leads to a 30% w/w solid dispersion [Harris and Chebre-Sellassie, 2008]. Additionally, plasticizer is commonly applied to improve the polymer chain mobility and flexibility and thereby to reduce the brittleness of the films.

Film coating is a dynamic process consisting of different steps leading to the formation of acceptable layer of film on the substrate [Mehta 2008]. Firstly, the coating droplets are formed from a nozzle system followed by the subsequent contact of the droplets with substrate. For adequate coating, the coating material

needs to be spread, adhered and coalesced with the surface of the substrate. The final film coating is formed after the solvent has been evaporated. The film coating can be performed by using conventional pans, perforated pans and fluid-bed processors [Mehta 2008].

HPMC is one of the most widely used primary coating material for pharmaceutical tablets. HPMC forms very flexible and tough films from aqueous solutions. HPMCAS, enteric polymer that has been derived from HPMC, has been widely used for coating pH-dependent delayed-release dosage forms [Obara and Kokubu 2008].

2.6.3 Characterization of film properties

The film forming and tablet film coating properties are evaluated with free films and with the films applied to tablets. Free films are commonly used in an early-stage of pharmaceutical film formulation development, and free films can be prepared by a solvent casting method or spraying method. The film properties essential for the final qualified film-coated tablet product include appearance, mechanical properties, thermal behaviour, permeability (moisture, oxygen and UV light) and dissolution [Obara and Kokubu 2008].

The appearance of polymeric films and film coating defects can be studied by using visual inspection or different microscopic techniques (e.g. stereo microscopy, scanning electron microscopy (SEM), atomic force microscopy (AFM)). The surface topography and morphology are commonly investigated with free films.

Tensile testing is one of the most widely used mechanical tests for assessing the elasticity and mechanical strength of free films [Felton *et al*., 2008] The key parameters generated from the stress-strain profile include: (1) tensile strength (the maximum stress that can be applied before the material will break), (2) strain (reflects the flexibility of the film; under applied stress the film will stretch or elongate with lower value of elongation employing more brittle film structure), (3) work of failure (the work required to break the film representing the toughness of the film), and (4) Young's modulus (measure of stiffness of the film; it is calculated as the ratio of the applied stress and appropriate strain at the region of linear elastic deformation).

Thermal analysis of free films is an approach to investigate the interactions between the film forming polymer, plastcizer and/or other coating experiments. Glass transition temperature (Tg) is the temperature at which the mechanical behaviour of the film is changing: below Tg the material is glassy and brittle, above the Tg the polymer is in a softer, ruberry state. Plasticizers are used to decrease the Tg of the polymers. A strong interaction between the plasticizer and polymer results in greater lowering in Tg of the polymer [Felton *et al*., 2008].

The present work aims to investigate the effects of lignification of aqueous HPMC films on the mechanical and solid-state properties of films, as well as water vapour permeation (WVP) properties of the films**.**

3. AIMS OF THE STUDY

The main aim of this study was to investigate and gain understanding of the functionality related characteristics and the pharmaceutical applicability of two natural wood-origin biopolymers (CPSC and CPSL) and one synthetic polymer (PVP).

The specific objectives were:

1. To investigate the physicochemical material properties (i.e., solid-state properties, particle and powder properties, powder flowing and physical incompatibilities) of CPSC and CPSL relevant to pharmaceutical solid dosage form manufacturing **(I)**
2. To study tablet compaction behaviour (i.e. consolidation, densification and deformation mechanisms) of CPSC and CPSL and to estimate their value as direct compression excipients **(II)**
3. To investigate the applicability of CPSC and PVP in cryogenic co-grinding for preparing ASDs and to enhance the solubility of poorly water-soluble drugs. Moreover, the stabilizing properties of the carrier polymers and the recrystallization behaviour of ASDs during storage were evaluated **(III, IV)**
4. To evaluate and explain the effects of lignification (CPSL) on the mechanical and solid-state properties of aqueous HPMC films intended for tablet coating **(V)**

4. EXPERIMENTAL

Complete details of the materials and methods used in this work can be found in the original publications (**I–V**).

4.1 Materials

4.1.1 Catalytic pre-treated softwood cellulose and lignin (I, II, IV, V)

CPSC and CPSL were isolated from pine soft wood (*Pinus sylvestris*) by using a catalytic oxidation and subsequent acid precipitation treatments described by Hakola *et al.* (2010) with some modification.

4.1.2 Polyvinylpyrrolidone (III, IV)

Two different grades of PVP, Kollidon® K25 (PVP25) (**III, IV**) and Kollidon® K90 (PVP90) (**III**) from BASF SE (Ludwigshafen, Germany), were used for cryogenic co-grinding.

4.1.3 Other excipients (I, II, V)

Industrial softwood kraft lignin (Indulin AT) (Sigma Aldrich, USA) (**I, II, V**), and hardwood lignin (PC-1369) (MeadWestvaco, USA) **(I, II)** were used as reference materials for investigating the physicochemical (**I**) and compression (**II**) properties of the materials.

Commercial microcrystalline cellulose, MCC (Avicel® PH 101, FMC Biopolymer, USA) (**I, II**), lactose monohydrate (Pharmatose® 80 M; DFE Pharma, Germany) **(II)** and dibasic calcium phosphate, DBCP (Emcompress®, E. Mendell, NY, USA) **(II)** were used as reference tableting materials (**II**). Magnesium stearate (Ph.Eur.) and acetone (E. Merck, Germany) were used for preparing a lubricant suspension for tablet compression (**II**).

Hydroxypropyl methylcellulose, HPMC (Methocel E5, Colorcon Ltd., UK) (**V**) was used as a cellulosic film-forming polymer and polyethylene glycol, PEG 400 (Macrogol 400) (**V**) was used as a plasticizer for preparing the free isolated films (**V**). Purified water was used as a solvent (**V**).

4.1.4 Active pharmaceutical ingredients (III, IV)

Piroxicam anhydrous form I (PRXAH I, USP grade, 99.4%) was purchased from Lianyuangang Ruidong International Co., Ltd. (China) **(III)** and from Letco Medical, Inc. (USA) **(IV).** The crystallization of PRXAH I from a hot aqueous solution was used to obtain piroxicam monohydrate (PRXMH) **(III).**

4.2 Methods

4.2.1 Preparation of catalytic pretreated softwood lignin and cellulose (I, II, IV, V)

Isolation of lignin and lignocelluloses from pine soft wood (*Pinus sylvestris*) was carried out according to the method described by Hakola *et al.* 2010 with some modification. Schematic diagram of the isolation process is shown in **Figure 4**. For the pretreatment, the pine chips were first extracted with hexane for two days and with acetone for one day to remove the extractives. The alkaline water solution was prepared by mixing 5.2 g (49.0 mmol) Na_2CO_3 to 200 ml of water, and it was added together with 10 g of extractive free pine chips (dry weight) to a preheated autoclave equipped with magnetic stirrer and oil bath heating. The reaction was carried out for 20 h at elevated temperature (120 °C) and with oxygen pressure (10 bar). After pretreatment, the solution was filtered. The solid cellulose fraction (CPSC) was dried at room temperature (RT) (approx. 22 °C) and subjected to size reduction (ball grinding/milling) using a Planetary Mono Mill pulverisette 6 (Fritsch GmbH, Germany) at 400 rpm for 3 min. The filtrate, which contained the solubilised lignin, was acidified with HCl and the precipitated lignin (CPSL) was collected with additional filtration. The obtained solid lignin was vacuum dried and used without further size reduction.

4.2.2 Tablet compression (II)

The materials were stored and dried under controlled temperature and low humidity conditions (21 °C / 35% RH (relative humidity)) for at least one week before testing. Another additional set of compaction tests was preformed with non-dried CPSC and milled fractions of it. Prior to tablet compression, the materials were kept (as thin layers) under controlled RT and humidity conditions at 21 °C and 50% RH for at least 48 h.

Tablets were compressed with an instrumented Korsch EK-0 eccentric tableting machine (Erweka Apparatebau, Germany) equipped with 9-mm flat-faced punches. The upper punch was first placed to its lower position and the position of lower punch was adjusted by using a 3-mm calibration plate between the punches. For compression studies, excipients were individually weighed out and poured into a pre-lubricated die (lubrication was made with a 5-% w/w magnesium stearate dispersion in acetone). The operating speed of tablet machine (36 rpm) and the height of tablets (3.0 mm) were kept constant in all compactions.

4.2.3 Preparation of amorphous solid dispersions (III, IV)

The binary physical mixtures (PM) of PRXAH I and polymers (with ratios 1:1 (**III**), 1:2 (**III**), 1:3 (**IV**)) were prepared manually by gently mixing with spatula. For preparing the cryogenic co-ground SDs, CPSC was first pre-milled separately for 30 min with a laboratory-scale Retsch MM 400 Mixer Mill (Retsch GmbH, Germany) to obtain more homogenous and reduced size of the particles. Co-grinding (co-milling) of PRXAH I with a carrier polymer was performed with the same laboratory-scale Retsch MM 400 Mixer Mill. The samples were placed in a 25-ml volume stainless steel milling jar containing one 12-mm diameter stainless steel ball, and milled at 28 Hz frequency for 180 min either at room (RT) (**III**) or at low temperature (LT) (4 °C) (**IV**).

4.2.4 Preparation of aqueous free films (V)

The aqueous film coating solution consisted of HPMC polymer 10% (w/w) and PEG 400 20% (w/w) of the polymer weight. Different amounts of lignin were dissolved in PEG 400 by using a gentle heating before the final mixing with aqueous HPMC polymer solution (see **Table 1**).

Table 1. Concentration levels of catalytic pre-treated softwood lignin (CPSL) and industrial softwood kraft lignin (Indulin AT) containing free films.

Material	**Concentration of lignin (calculated as a % w/w of plasticizer PEG 400 weight)**
CPSL	0.1
	0.5
	1.0
	5.0
	10.0
Indulin AT	0.1
	0.5
	1.0
	5.0
	10.0
Reference	0

Free films were prepared by a casting/solvent evaporation method. For preparing isolated free films, 10.0 g of plasticized and lignified HPMC solution (10% w/w) was carefully poured into polytetrafluoroethylene (Teflon®) molds. After filling, the molded solutions were allowed to dry for at least 24 h at a

controlled RT (21 ± 2 °C) and RH (50%). The films were gently removed with a surgeon knife for further testing. Plasticized HPMC polymer films without lignin (0%) were used as reference films.

4.2.5 Physical material characterization (I, II)

4.2.5.1 Particle size, shape and surface morphology (II)

Particle size, shape, surface morphology and microstructure of the biomaterials were studied by using a high-resolution scanning electron microscopy, SEM (Quanta FEG 250, FEI Company, USA). The samples were mounted on aluminium stubs with a double-sided tape and then coated with 3 nm platinum layer (Quorum Q150TS, turbomolecular-pumped high-resolution coater, Quorum Technologies, UK) in an argon atmosphere prior to microscopy.

The particle size and size distribution of the biomaterials were determined by using a 3D-surface image analysis method (Flashsizer FS3D, Intelligent Pharmaceutics Ltd, Helsinki, Finland) described by Soppela *et al.* (2011). The FS3D image analysis setup consists of a camera connected to a computer and glass cuvette towards the camera. The size of the measurement field was 1280 × 960 pixels, i.e. 15.1 × 11.3mm. The particle size was recorded in triplicate and eight images were taken per batch. The number of particles per image measured varied from 200 to 5,600.

4.2.5.2 Moisture content and water activity (I)

Moisture content of biomaterials was determined by using a Sartorius MA 100 moisture analyzer (Sartorius AG, Germany). The water-activity (a_w) measurements were carried out with an AquaLab (Series 3TE, Sweden) water-activity meter.

4.2.5.3 Fourier transform infrared spectroscopy (I)

Fourier transform infrared (FTIR) spectra of biomaterials were obtained using a IRPrestige-21 Spectrophotometer (Shimadzu Corp., Japan) and Specac Golden Gate Single Reflection ATR crystal (Specac Ltd., U.K.). Spectra were collected from 4000 to 600 cm^{-1}.

4.2.5.4 X-ray powder diffraction (I)

X-ray powder diffraction (XRPD) patterns on CPSL and CPSC powders were obtained by using a X-ray diffractometer (D8 Advance Bruker AXS GmbH, Germany). The XRPD experiments were carried out in symmetrical reflection mode (Bragg-Brentano geometry) with CuK_α radiation (1.54 Å).

4.2.5.5 Differential scanning calorimetry (I)

The glass transition temperature range (T_g) of the biomaterials was investigated using differential scanning calorimetry, DSC (DSC 4000, Perkin Elmer Ltd, Shelton, CT, USA). The DSC system was calibrated for temperature and enthalpy using indium as a standard. Samples of 2–3 mg were sealed in an aluminium pan with 2 pinholes in a lid. A nitrogen purge with a flow rate of 20 ml/min was used in the furnace. The scans were obtained by heating from 30 °C to 220°C at a rate of 20°C/min. Each run was performed in triplicate.

4.2.5.6 Physical bulk powder and consolidation properties (I)

Bulk, tapped and true (absolute) densities of the powders were determined by the standard method described in the Ph. Eur. (2010). A standardized tapped density tester (Erweka SVM1, Erweka GmbH, Heusenstamm, Germany) was employed. Each sample was measured in triplicate. The true (absolute) density of materials was measured using a helium pycnometer (Micromeritics, Model 1305, Norcross, GA, USA). Carr's index and Hausner ratio were calculated from the bulk, tapped and true densities [Ph. Eur. 2010].

Flow rate of powders was measured by using a laboratory FlowPro flow meter (SAY Group, Helsinki, Finland). This flow meter measures the mass of a powder that flows through a hopper assisted by vertical oscillations, which break the cohesive forces [Wells and Aulton 1998]. Five parallel measurements were performed under controlled room conditions (21± 2°C / 50% RH).

4.2.6 Characterization of tablet compression and tablets (II)

4.2.6.1 Plasticity and elasticity factors (II)

The plasticity and elasticity of the materials under compression were determined by the method described by Antikainen and Yliruusi (2003). In this method, the ratio of two areas of work, W1 and W2, obtained from the force-distance curve near the maximum force, were calculated. The plasticity factor (PF) determines the extent of plastic flow at a certain compression force level and gives a comparable numerical value (Eq. 1):

$$PF = \frac{W1}{W1 + W2} \times 100\ \% \qquad [1]$$

The elasticity factor (EF) was calculated with Eq. (2):

$$EF = \frac{H - H_{min}}{H_{min}} \times 100\ \% \qquad [2]$$

where H is the height of the tablet after removing it from the die, H_{min} is the height of the tablet during maximum compression force.

4.2.6.2 Final tablet properties (II)

The produced tablets were characterized in terms of their thickness, mass, and mechanical properties. Immediately after compression, the height of each tablet was measured with a digital micrometer (Sony DZ 521, Tokyo, Japan). The mass of each tablet was measured with an analytical balance (Sartorious CP 2245, Raute, Goettingen, Germany). The crushing strength of tablets was determined using a tablet hardness tester (Schleuniger 2E, Dr. Schleuniger Pharmatorn AG, Solothurn, Switzerland).

4.2.7 Characterization of solid dispersions (III, IV)

4.2.7.1 Physical solid-state stability (III, IV)

XRPD was used to determine the solid-state changes during cryogenic co-grinding (loss of crystallinity) and upon storage (details about X-ray diffractometer can be found in original publications **III** and **IV**). Samples were taken during grinding after specified time-periods: 30, 60, 90, 120, 150 and 180 min. All determinations were performed at least in triplicate.

The effect of time on the physical stability and recrystallization behaviour of the prepared formulations was investigated by storing the samples in desiccators over sodium silicate (silica gel) at RT (**III, IV**). All samples were analyzed immediately after the preparation and upon storage under specified conditions. Solid-state analyses were performed by means of XRPD (**III, IV**), FTIR (**IV**), DSC (**IV**) and Raman spectroscopy (**III, IV**). The details of the present analytical devices are given in previous sections and in original publications.

4.2.7.2 Dissolution studies *in vitro* (III, IV)

Complete details of the dissolution testing can be found in the original publications (**III, IV**).

The recrystallization of amorphous PRX (aPRX) from ASDs as well as from respective PMs was monitored in a simulated gastric fluid (SGF) in-line by using Raman spectroscopy (details can be found in publication **III**). The amount of aPRX in the experiments was approximately 1 mg and the volume of SGF was 2.5 ml. A magnetic stirrer was used to obtain movement in wet slurry.

Conventional dissolution testing: the *in-vitro* dissolution testing of ASDs and corresponding PMs were performed according to the USP (A) basket (with hard gelatin capsules) (**III**) and (B) paddle methods (**IV**).

(A) Hard gelatin capsules filled with an amount of formulation corresponding to 20 mg of PRX (mesh size 140 μm, n = 3) were used for dissolution

testing (Erweka DT 70, Erweka GmbH, Heusenstamm, Germany) in SGF. A 5-ml sample (replaced) was withdrawn at 3 min interval. The amount of dissolved PRX was detected by UV spectrophotometer (Evolution 300, Thermo Fisher Scientific, Waltham, MA, USA) at 353 nm (**III**).

(B) The dissolution behaviour was investigated in an automated dissolution system (Termostat-Sotax AT7, Sotax AG, Switzerland). The samples (n = 5–6) containing 40 mg of PRX were analysed at regular intervals over 8 h using an UV-VIS spectrophotometer (Ultrospec III, Biochorm Ltd., UK) at 354 nm. The dissolution media was 900 ml of distilled water at 37.0 ± 0.5 °C. The paddle rotation speed was set at 100 rpm. All samples were tested immediately after the preparation. Three separate parallel dissolution tests were performed for each formulation at the same dissolution conditions (**IV**).

Small volume dissolution testing: a commercial small volume dissolution testing instrument (μDiss Profiler, Pion, MA, USA) was used to investigate the dissolution behaviour of ASDs and respective PMs in SGF from powder (n = 3). 20 ml of SGF was used as dissolution medium and the rotation speed was set to 200 rpm. The amount of formulations tested corresponded to 1 mg or 5 mg of aPRX/PRXAH I/PRXMH. Different concentrations were investigated in order to study the influence of saturation on the dissolution performance (**III**).

Experimental determination of solubility: the experimental determination of solubility of aPRX:polymer ASDs, respective PMs and pure crystalline forms were conducted using a range of dispersion concentrations from 50 μg/ml up to 1500 μg/ml (**III**). The solubility was determined at 37 °C by weighing formulation into a 30 ml screw-capped glass vial and adding the precise amount of SGF. The vials where mechanically shaken for 5 m in a shaker bath (Julabo SW 23, Seelbach, Germany) at 200 rpm. The solution samples were filtered through a 0.22 μm syringe filter, diluted with additional SGF to prevent the crystallization and analyzed by UV spectrophotometer to determine the PRX concentration in solution (**III**).

4.2.8 Characterization of free films (V)

4.2.8.1 Surface topography and morphology (V)

The surface topography and microstructure of free films were investigated with a high-resolution SEM (Quanta FEG 250, FEI Company, USA). The film samples were mounted on aluminium stubs with a doublesided tape and then sputter coated with 3 nm platinum layer (Quorum Q150TS, turbomolecular-pumped high-resolution coater, Quorcum Technologies, UK) in an argon atmosphere prior to microscopy.

4.2.8.2 Glass transition temperature (V)

The Tg of free films was determined by using a differential scanning calorimetry, DSC (Mettler Toledo DSC 823e, Mettler-Toledo AG, Analytical, Switzerland).

Before DSC experiments, small pieces of film samples were stored in a desiccator (0% RH) for 4 days. The dried film samples (3-6 mg) were sealed in an aluminum pan with 2 pinholes in a lid. A nitrogen purge with a flow rate of 20 ml/min was used in the furnace. The scans were obtained by heating from 30 °C to 220 °C at a rate of 20 °C/min. Each run was performed in triplicate.

4.2.8.3 Mechanical stress-strain properties (V)

The mechanical strength and strain properties of the plasticized free films were studied by using a Lloyd LRX materials tester (Lloyd Instruments Ltd, UK). Before testing, the isolated films were cut into strips with the length of 10 cm and width of 2 cm. The thickness of each film sample was measured at three different points by using a Sony digital micrometer (Sony Digital Indicator U30-F, Sony, Japan). The stress-strain measurements were performed using a 100-N load cell, initial gauge length of 7.5 cm and a cross speed of 5 mm/min. Tensile strength, elongation (strain) at break, modulus of elasticity (Young's modulus) and work done were calculated from stress-strain curve. At least five parallel measurements were made.

4.2.8.3 Water vapour permeation (V)

The WVP of films was determined using the free films (150–200 μm) cut to a suitable size, fixed onto the anhydrous calcium chloride ($CaCl_2$) containing glass vials and immediately tightly sealed with a thin elastic band and Parafilm® M barrier film. The glass vials were held at 23 ± 2 °C and 80 ± 2% RH, and the increase in weight was measured at regular intervals.

5. RESULTS AND DISCUSSION

5.1 Physical material properties of pretreated wood lignin and cellulose (I, II)

5.1.1 Chemical structure analysis

Cellulose is a linear polymer of glucose with a β-1,4-orientation of the glucosidic bonds [Weil *et al*., 1994]. Lignin is a partially cross-linked heterogeneous polymer, composing of the C3 chain and the aromatic part with three main moieties: guaiacyl, syringyl and 4-hydroxyphenyl, which are conjugated to produce a typical three-dimensional structure of lignin polymer [Doherty *et al*., 2011; Hatakeyama and Hatakeyama 2010]. In addition, isolated lignin is composed of fragments of carbohydrate because of the presence of LCC [Sjöström 1993; Hatakeyama and Hatakeyama 2010]. Thus lignin does not have a regular structure like cellulose.

The FTIR spectra of CPSC, CPSCL and reference lignins are shown in **Figure 7**. In these spectra methylene and methyl C-H stretch (2800–3000 cm^{-1}), ether C-O (1194 cm^{-1}), primary alcoholic C-O (1040 cm^{-1}) and secondary alcoholic C-O (1128 cm^{-1}) can be identified. The broad bands in the range of 3100 to 3500 cm^{-1} are assigned to OH-stretching vibration region. The FTIR spectra of all lignin samples showed bands at 1420–1430, 1505–1515, 1595–1603 cm^{-1} corresponding to aromatic (C=C stretch) ring vibrations of phenylpropane skeleton (**Figure 7**).

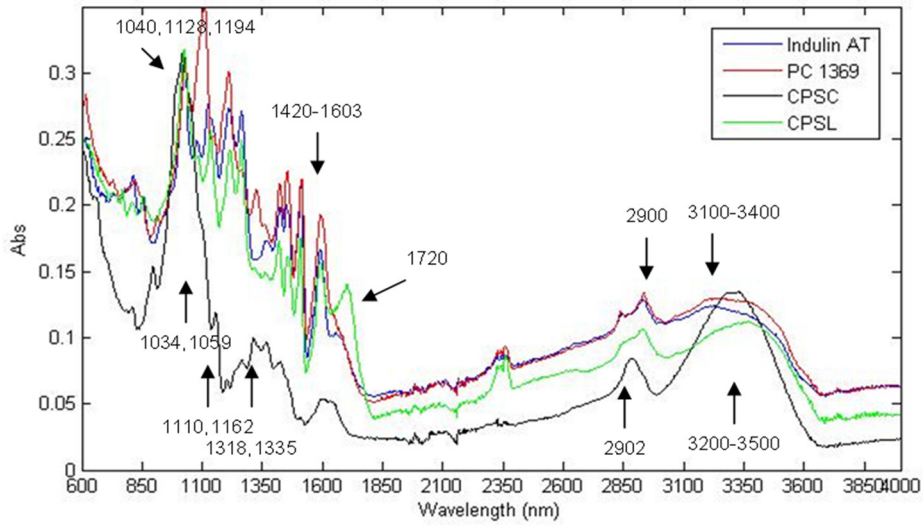

Figure 7. Fourier transform infrared (FTIR) spectra of industrial reference Indulin AT and PC-1369, softwood pine cellulose (CPSC) and pine lignin (CPSL).

A wide absorption band appeared in the range of 3100–3400 cm^{-1} assigned to the aromatic and aliphatic alcoholic –OH stretch. Comparing the FTIR spectrum of CPSL with those of industrial reference lignins, Indulin AT and PC-1369, suggests that the spectra are quite similar. The only difference observed is the appearance of increased absorption band at the wavelength range of 1700–1740 cm^{-1}. This peak obviously corresponds to the unconjugated C=O stretch.

5.1.2 Particle size, shape and surface morphology

Particle size, particle size distribution, shape and surface morphology of CPSC and CPSL were investigated and compared to those of reference materials: MCC (Avicel PH 101), industrial softwood kraft lignin (Indulin AT) and industrial hardwood kraft lignin (PC-1369). Scanning electron micrographs of the biomaterials are shown in **Figures 8** and **9.** Both the *pre-milled* CPSC and MCC consisted of particles with elongated or fiber-like shape (**Figure 8**). The shape of CPSL particles was flaky and sharp-edged, while reference softwood and hardwood industrial kraft lignins presented almost round granular particles **(Figure 9)**.

The FS3D particle size distributions are shown in **Table 2**. The FS3D results for CPSC showed a particle size ranging from 125 to 1400 μm with a volume median diameter (*d*50) 774 μm. The *d*50 for MCC was 151 μm and 90% of the particles were smaller than 240 μm. The FS3D *d*50 value for CPSL was 141 μm (*d*90 = 265 μm). The corresponding values for the industrial reference lignin powders (softwood Indulin AT and hardwood PC 1369) were *d*50 = 363 μm (*d*90 = 567 μm) and *d*50 = 471 μm (*d*90 = 749 μm), respectively (**Table 2**).

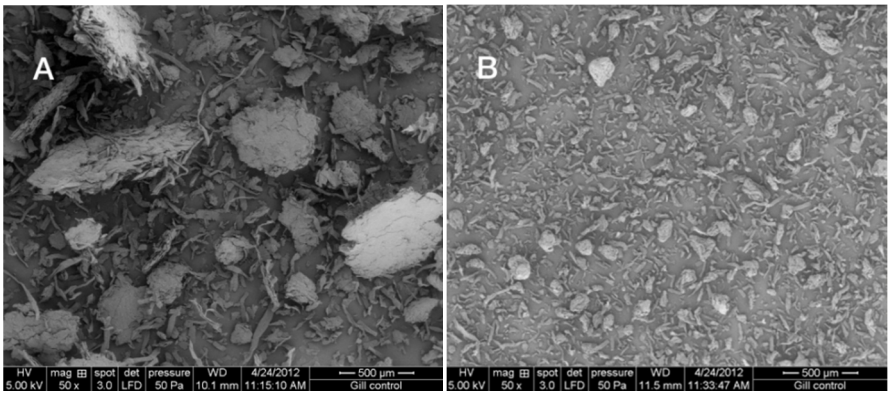

Figure 8. Scanning electron microscopy (SEM) images of catalytic pretreated softwood cellulose, CPSC (A) and microcrystalline cellulose, MCC (B) with ×50 magnification.

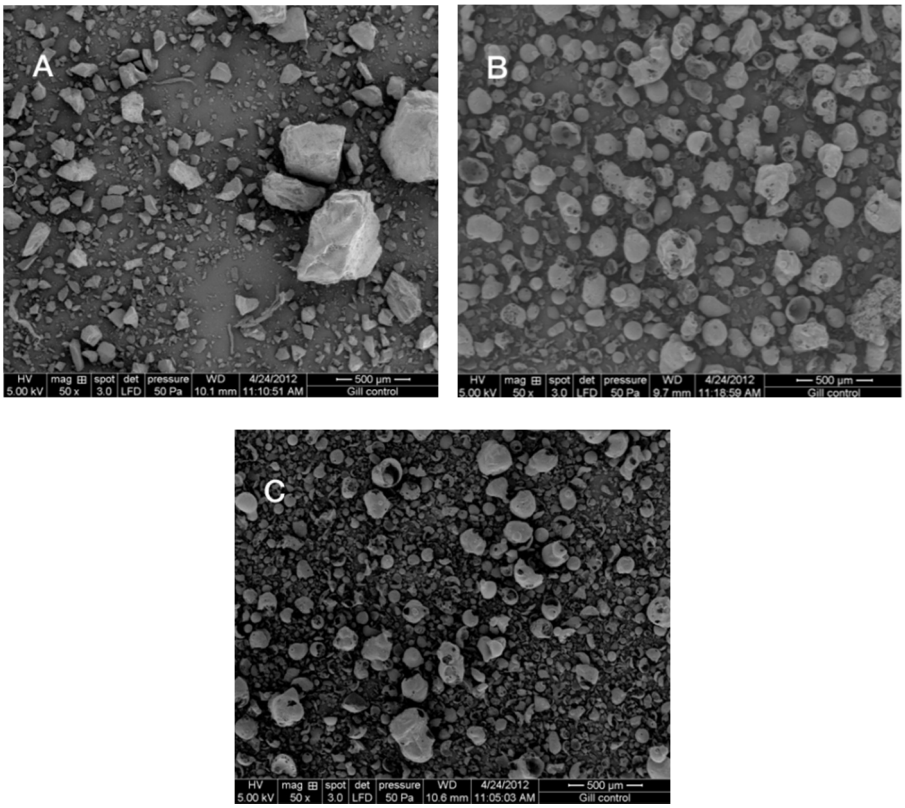

Figure 9. Scanning electron microscopy (SEM) images of catalytic pretreated softwood lignin, CPSL (A), industrial softwood kraft lignin, Indulin AT (B) and industrial hardwood kraft lignin, PC 1369 (C) with ×50 magnification.

Table 2. FS3D (μm) particle size distribution of pre-milled catalytic pretreated softwood cellulose (CPSC) and lignin (CPSC), microcrystalline cellulose, MCC (Avicel® PH 101), kraft softwood lignin (Indulin AT) and hardwood lignin (PC-1369).

Sample	d_{10}	d_{50}	d_{90}
CPSC	368	774	1215
CPSL	72	141	265
MCC	84	151	239
Indulin AT	193	363	567
PC 1369	242	471	749

5.1.3 Solid-state and thermal properties

Many pharmaceutical excipients today exist in a fully or partially amorphous form [Yu 2000]. The XRPD patterns of softwood cellulose (CPSC) and MCC (Avicel PH 101), showed semi-crystalline structure, with MCC XRPD reflections being somewhat narrower than those of the CPSC (**Figure 10**). This agrees with the earlier studies on wood celluloses suggesting that cellulose crystallites in MCC are thicker than those in wood crystalline cellulose [Andersson *et al*., 2004]. Additionally, typical crystal lattice of cellulose I was present for both celluloses since no doublet in the intensity of the main reflection was observed. As regards with lignins, XRPD pattern revealed amorphous structure for both CPSL and reference lignins (Indulin AT and PC-1369), since typical amorphous XRPD halo patterns were observed for all these lignin materials studied (**Figure 10**).

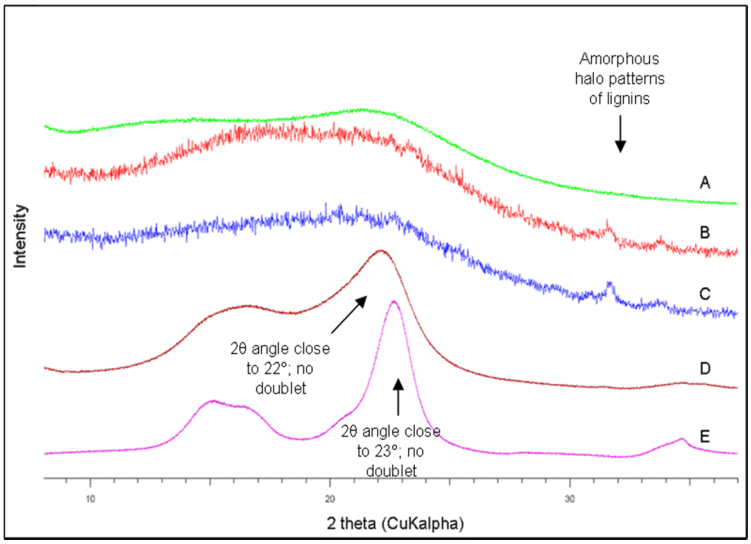

Figure 10. Representative X-ray powder diffraction (XRPD) patterns for the biomaterials studied: catalytic pretreated softwood lignin, CPSL (A) vs. industrial reference kraft softwood lignin, Indulin AT (B) and hardwood lignin, PC-1369 (C), and catalytic pretreated softwood cellulose, CPSC (D) vs. microcrystalline cellulose, MCC (E).

Since moisture content can greatly affect the physicochemical as well as processing properties of the materials, the state of water in biomaterials was evaluated (**Table 3**). Water activity (a_w) reflects the active part of moisture content that can be exchanged between the product and its environment [Snider 2007]. CPSL exhibited the highest average moisture uptake (10.7% w/w) and water activity (a_w = 0.653). As regards with celluloses, the average moisture content and water activity of both cellulosic materials were lower than those obtained for lignin. The average moisture uptake for CPSC was 4.5% (w/w) and a_w of 0.272, and for commercial cellulose MCC 4.2% (w/w) and 0.400, respectively.

Table 3. Moisture content and water activity (a_w) of catalytic pretreated softwood lignin (CPSL) and cellulose (CPSC) and microcrystalline cellulose, MCC (Avicel® PH 101) (n = 3)

Sample	Moisture content mean ± SD (%)	Water activity, a_w	Temperature (°C)
Catalytic pretreated softwood lignin, CPSL	10.7±1.1	0.653	24.9
Catalytic pretreated softwood cellulose, CPSC	4.5±5.1	0.272	24.8
Microcrystalline cellulose, MCC	4.2±0.1	0.400	24.6

The T_g values for CPSC were in a range of 169–171 °C, which was higher than the corresponding range obtained for the reference MCC (131–143 °C) (**Table 4**). The T_g values for CPSL were in a range of 168–171 °C that was significantly higher compared to that measured for the reference kraft softwood lignin, Indulin AT (135–142 °C) and hardwood lignin, PC-1369 (136–139 °C) (**Table 4**). Characteristic rigid groups in the main polymer chain of lignin and slight cross-linking restrict molecular motion could explain the relative high values of T_g for the lignin samples. Thermal characterization of CPSL and CPSC suggested that the catalytic pretreatment method increases the range of T_g of lignin and cellulose compared to the other isolation techniques, and thus the catalytic pretreatment could produce biomaterial with an increased thermal stability.

Table 4. Glass transition temperature range (T_g) of catalytic pretreated softwood cellulose (CPSC) and lignin (CPSL), microcrystalline cellulose, MCC (Avicel® PH 101), kraft softwood lignin (Indulin AT) and hardwood lignin (PC-1369).

Sample	T_g (°C)
Catalytic pretreated softwood cellulose, CPSC	169–171
Microcrystalline cellulose, MCC	131–142
Catalytic pretreated softwood lignin, CPSL	168–171
Kraft softwood lignin, Indulin AT	135–142
Hardwood lignin, PC–1369	136–139

5.1.4 Bulk powder properties

Bulk properties of excipient powders are of great importance in many pharmaceutical manufacturing processes. As seen in **Table 5**, the bulk and tapped density of CPSL were approximately two times higher compared to those obtained with celluloses (CPSC and MCC), suggesting that CPSL can be considered as a potential excipient for e.g. direct-compression tablets.

Additionally, the flow properties of the biomaterials were determined by using both Carr's index and Hausner ratio (**Table 5**). The Carr's compressibility index of CPSL was 10.1% suggesting that the material is free flowing (Carr's compressibility index below 16 % indicates good flowability). The low average value for the Hausner ratio (1.12) also confirmed the good powder flow properties. CPSC was classified as a poor flowing powder with the calculated values of 26.3% and 1.36 for the Carr's compressibility index and the Hausner ratio, respectively. The flow rate values (FlowPro) of biomaterials are in line with the results on the Carr's compressibility index and the Hausner ratio. The average flow rate of CPSL (20.2 mg/s) was approximately four times higher than the flow rate obtained with CPSC (4.9 mg/s).

Table 5. Summary table of bulk and powder properties of catalytic pretreated softwood lignin (CPSL) and cellulose (CPSC), and microcrystalline cellulose, MCC (Avicel® PH 101) (n = 3–5)

Sample	Bulk density (g/cm^3)	Tapped density (g/cm^3)	True density (g/cm^3)	Carr's index (%)	Hausner ratio	FlowPro flow rate (mg/s)	Powder flow
CPSL	0.617	0.687	1.402	10.1	1.12	20.2	Good
CPSC	0.317	0.430	1.557	26.3	1.36	4.9	Poor
MCC	0.273	0.333	1.546	18.0	1.22	14.7	Moderate-to-good

5.2 Functionality-related characteristics of pretreated wood lignin and cellulose, and polyvinylpyrrolidone (II–V)

5.2.1 Tablet compression behaviour of pretreated wood lignin and cellulose (II)

5.2.1.1 Densification and deformation under compression

The tendency of the biomaterial for plastic deformation, fragmentation or elasticity was investigated with a method described by Antikainen and Yliruusi (2003). The plasticity and elasticity under compression were evaluated by using a force-displacement treatment and by determining the characteristic plasticity (PF) and elasticity (EF) factors for each material studied.

With all biomaterials studied, the PF under compression decreased exponentially as the compression force increased (**Figure 11**). Both celluloses (MCC and CPSC) had a clear tendency for plastic deformation, and MCC was the most plastic material studied. CPSL presented very similar compression behaviour

(plasticity) with reference materials industrial softwood kraft lignin (Indulin AT) and hardwood lignin (PC-1369). Interestingly, two softwood lignins (CPSL, Indulin AT) presented almost identical compression behaviour in a range of upper punch compression forces 7–10 kN. At lower compression forces, however, CPSL exhibited more plastic deformation than Indulin AT. Hardwood lignin (PC-1369) was found to be more plastic material than the two softwood lignins under compression with the PF values closed to MCC.

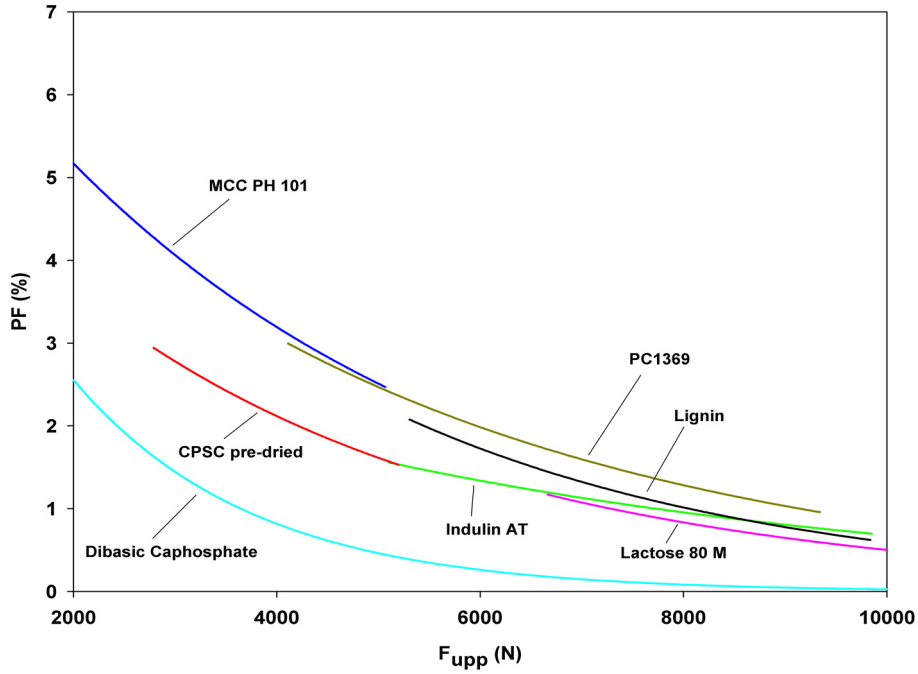

Figure 11. Effect of compression force on the plasticity (PF) factor of biomaterials.

The results for the elasticity properties are presented in **Figure 12.** CPSC was clearly the most elastic material with the highest EF value of 18%. Interestingly, the compression force applied in tableting did not significantly affect the elasticity of CPSC and MCC while the EF values for other materials studied increased as compression force increased. The EF values for CPSL ranged from 12 to 15%, which were close to the EF values obtained with lactose monohydrate (11–14%). Reference hardwood and softwood lignins showed almost identical elastic behaviour with EF slightly lower than those obtained with CPSL.

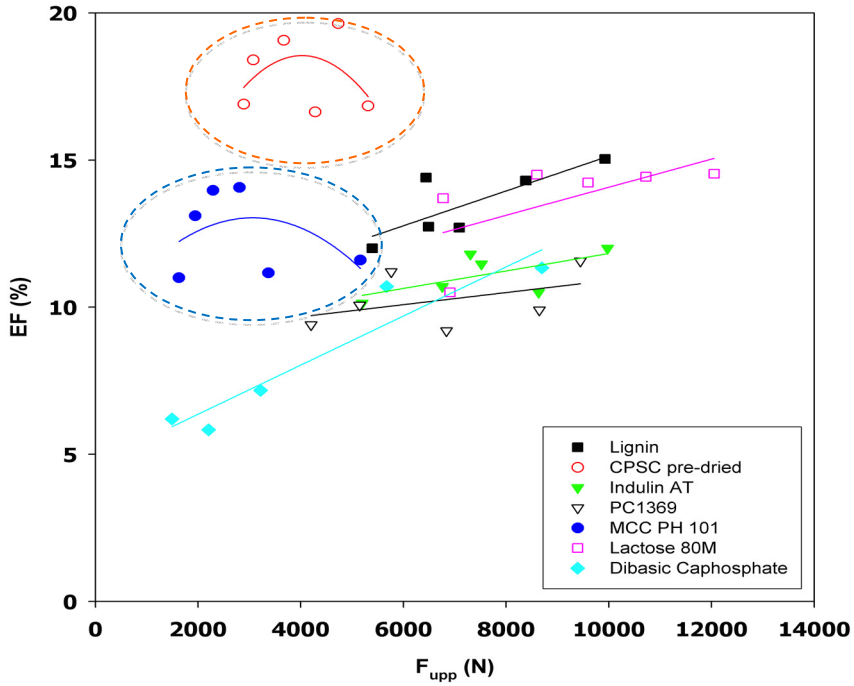

Figure 12. Effect of compression force on the elasticity (EF) factor of biomaterials. The values for EF obtained with celluloses are circulated (B).

5.2.1.2 Compaction properties

The compactibility of the biomaterials was evaluated by determining the relationship between upper punch compression force (F_{upp}) and tablet crushing strength (**Figure 13**). Both celluloses exhibited clearly the highest tablet crushing (breaking) strength values *versus* compression force (i.e. the highest slope of the curve). Industrial reference lignins (Indulin AT and PC-1369) showed almost linear compression force breaking strength profile being more compressible than CPSL. CPSL presented clearly the lowest tablet breaking strength values (the lowest slope of the curve).

As seen in **Figure 13**, industrial hardwood kraft lignin (PC 1369) presented surprisingly good compaction properties without any signs of tableting defects. The compaction properties of CPSL were close to that obtained with lactose monohydrate (with the low slope of a compression force–crushing strength curve). Some capping, however, was observed with the tablets prepared from CPSL at higher compression forces. DBCP tablets presented capping even at the lowest compression forces used, and consequently, the poorest compaction behaviour among the materials studied.

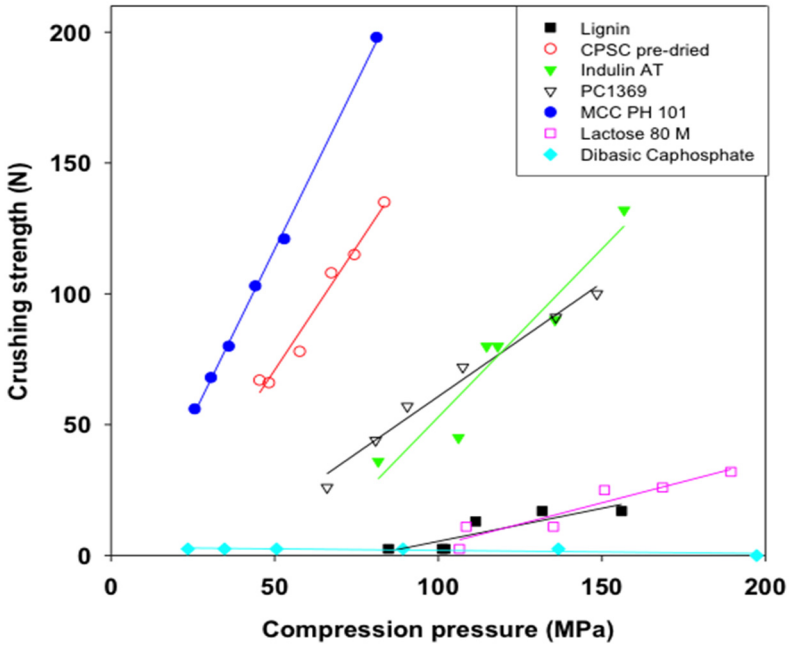

Figure 13. Effect of compression force on crushing strength of biomaterials.

5.2.1.3 Effects of drying and grinding on tablet properties

The effect of particle size and moisture content of CPSC on the mechanical strength of the tablets was also investigated (**Figure 14**). Interestingly, that non-dried CPSCs (*non-milled*, 0.5 h and 3 h milled) exhibited the same tablet breaking strength values as MCC, thus confirming the particle-size independent plastic behaviour of CPSC under compression. Moisture content of CPSC enhance the tablet strength even in a range of relatively low upper punch compression forces, suggesting moisture dependency of tablet compression when CPSC is used as a filler.

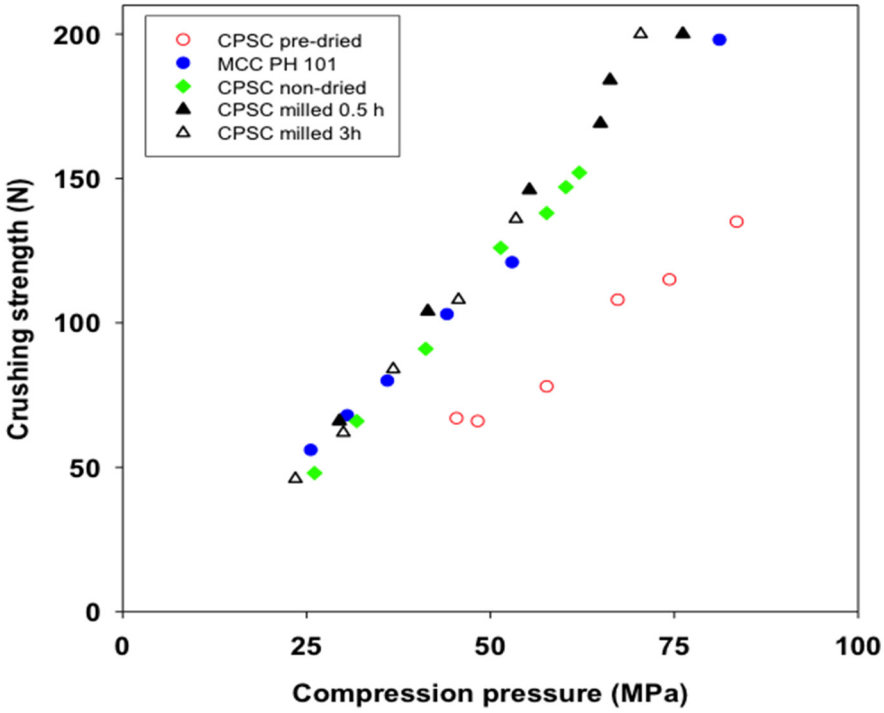

Figure 14. Effects of drying and grinding (particle size) of CPSC on the crushing strength of tablets.

5.2.2 Functionality-related characteristics of carrier polymers in amorphous solid dispersions (III, IV)

Piroxicam (PRX) is a poorly water-soluble, non-steroidal anti-inflammatory drug, which belongs to Class II (high permeability, low solubility) in the Biopharmaceutics Classification System (BCS). For highly permeable and low soluble drugs, the limiting factor for oral absorption is the dissolution rate of drug and formulation [Sheth *et al*., 2004].

ASDs of poorly water-soluble PRX with carrier polymers were prepared using ball-grinding at room (**III**) and low temperature (LT) (**IV**). The main focus was on detecting and monitoring the solid-state transitions throughout processing and the effect of storage conditions, time and carrier polymers (CPSC, PVP) on the recrystallization behaviour of aPRX by using different characterization methods.

5.2.2.1 Particle and powder properties

Crystalline PRXAH I powder consisted of white prisms with a particle size ranging from 5 μm to 35 μm (**Figure 15A**). Particle and powder properties of the CPSC were discussed in previous section (5.1. Physical material properties of pretreated wood lignin and cellulose). Prior to preparation of ASDs CPSC was pre-milled for 30 min resulting in a reduced particle size ranging from 10 μm to 100 μm (**Figure 15B**). PVP25 powder consisted of particles with round shape with a particle size ranging from 10 to 100 μm (**Figure 15C**).

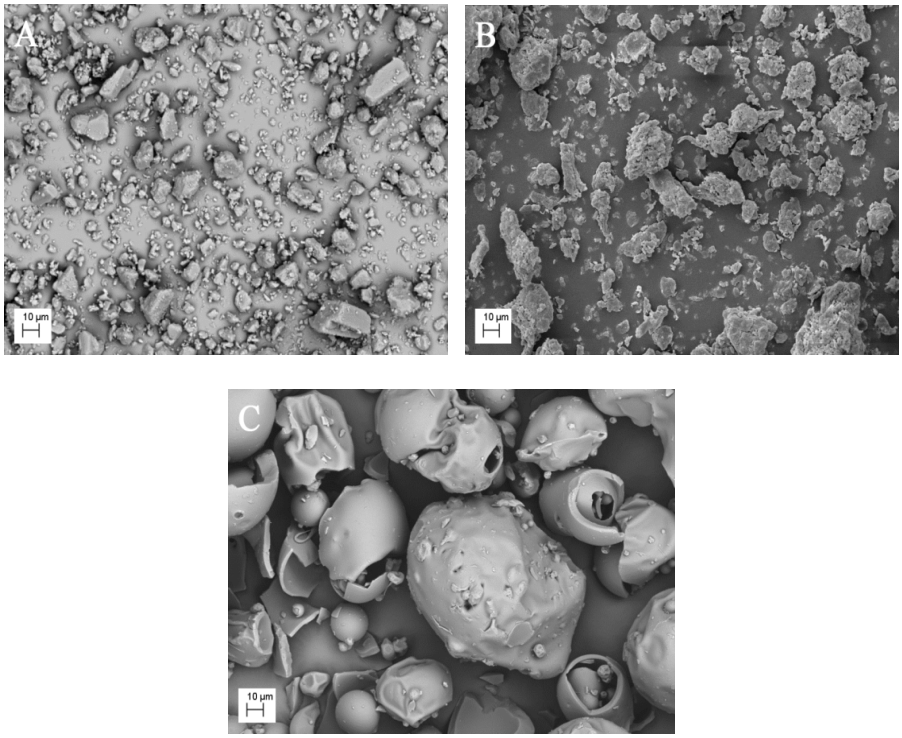

Figure 15. Scanning electron micrographs (SEMs) of (A) piroxicam anhydrous form I, PRXAH I; (B) catalytically pretreated softwood cellulose, CPSC (pre-milled); (C) polyvinylpyrrolidone, PVP 25. Magnification ×1000.

5.2.2.2 Solid-state changes of piroxicam during preparation and storage of solid dispersions

The XRPD patterns of PRXAH I, *pre-milled* CPSC and PVP25 are shown in **Figure 16**. The XRPD pattern of PRXAH I was in a good agreement with the calculated theoretical pattern obtained from Cambridge Structural Database (CSD) and with previously published data [Kogermann *et al*., 2011; Naelapää *et al*., 2012]. The XRPD pattern of the *pre-milled* CPSC revealed that vibrational ball grinding for 30 min resulted in amorphous CPSC.

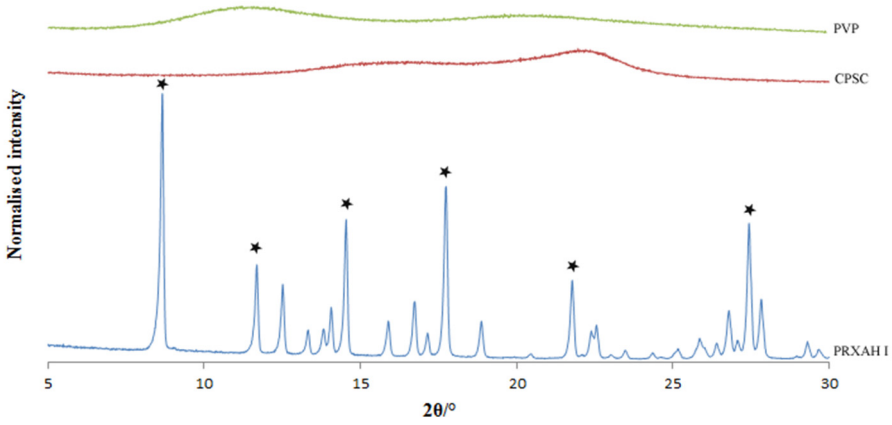

Figure 16. X-ray powder diffraction (XRPD) patterns of piroxicam anhydrous form I (PRXAH I), catalytically pretreated softwood cellulose (CPSC) (pre-milled) and polyvinylpyrrolidone (PVP 25). The characteristic reflections of PRXAH I are indicated with asterisks (*) and monitored in the subsequent cryogenic co-grinding experiments.

Co-grinding of PRXAH I with PVP25 and PVP90 in the ratios 1:2 and 1:3 at RT for 180 min resulted in amorphous PRX (data not shown) (**III**).

In case of cryogenic co-grinding both carrier polymers (CPSC and PVP25) improved the amorphisation of a poorly water-soluble PRX (**IV**). After cryogenic co-grinding for 180 min, the amorphous form of PRXAH I was achieved with PVP25 (in the drug:polymer ratio 1:3) (**Figure 17B**). However, with *pre-milled* CPSC a sign of very low-intensity characteristic crystalline reflection of PRXAH I at 8.6° 2θ could be observed in the XRPD pattern presumably indicating still some degree of crystallinity (**Figure 17A**). When *non-milled* CPSC was used for preparing the cryogenic co-ground SDs, only partially XRPD amorphous PRX was obtained after 180 min of cryogenic co-grinding (data not shown). This could be explained by the fact that the reduced particle size of CPSC increased the specific surface area of the material, hence maximizing the number of contact points and the amount of possible hydrogen bonds sites available for interaction with PRX.

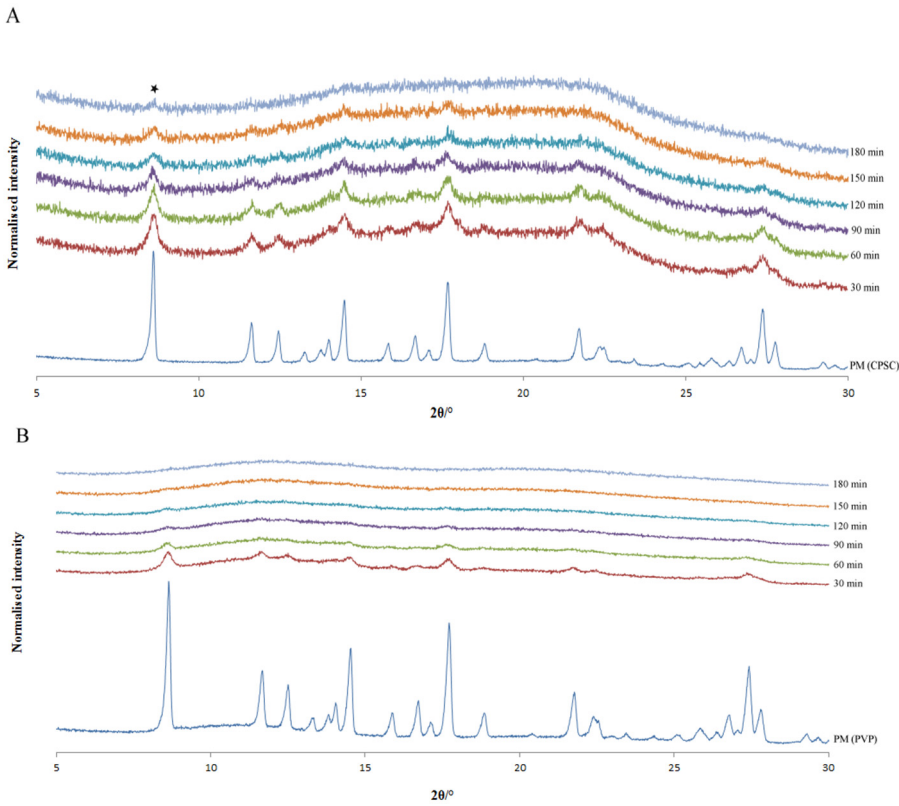

Figure 17. The effects of cryogenic co-grinding on the solid state of piroxicam anhydrous form I (PRXAH I). The carrier polymers studied were (A) pre-milled catalytic pretreated softwood cellulose (CPSC) and (B) polyvinylpyrrolidone (PVP). X-ray powder diffraction (XRPD) patterns from down to top: Physical mixture (PM) of PRXAH I and a carrier polymer (1:3 w/w), and the corresponding cryogenic co-ground solid dispersions (SDs) sampled at 30, 60, 90, 120, 150 and 180 min during a cryogenic co-grinding.

Physical stability investigations of the co-ground ASDs with PVP25 and PVP90 in the drug:polymer ratios 1:1 and 1:2 revealed that ASDs were physically stable up to 6 months and one year, respectively (**III**).

The effects of a short-term aging on the physical solid-state stability of cryogenic co-ground SDs were investigated (**IV**). In case of using CPSC as a carrier polymer amorphisation of PRX was found to be only temporary. A phase transformation appeared and crystalline peaks were detected after 24 h of storage at an ambient controlled RT (25°C / 20% RH) (**Figure 18A**). Cryogenic co-ground mixtures of PRX and PVP (1:3) remained in an amorphous state over a one-week storage period (**Figure 18B**). PRX structure contains O-H and one N-H functional groups, which probably form hydrogen bonding with a carbonyl group of PVP. Controversially, CPSC acts as a physical matrix, where PRX is located during cryogenic co-grinding at a particle level between cellulose microfibers.

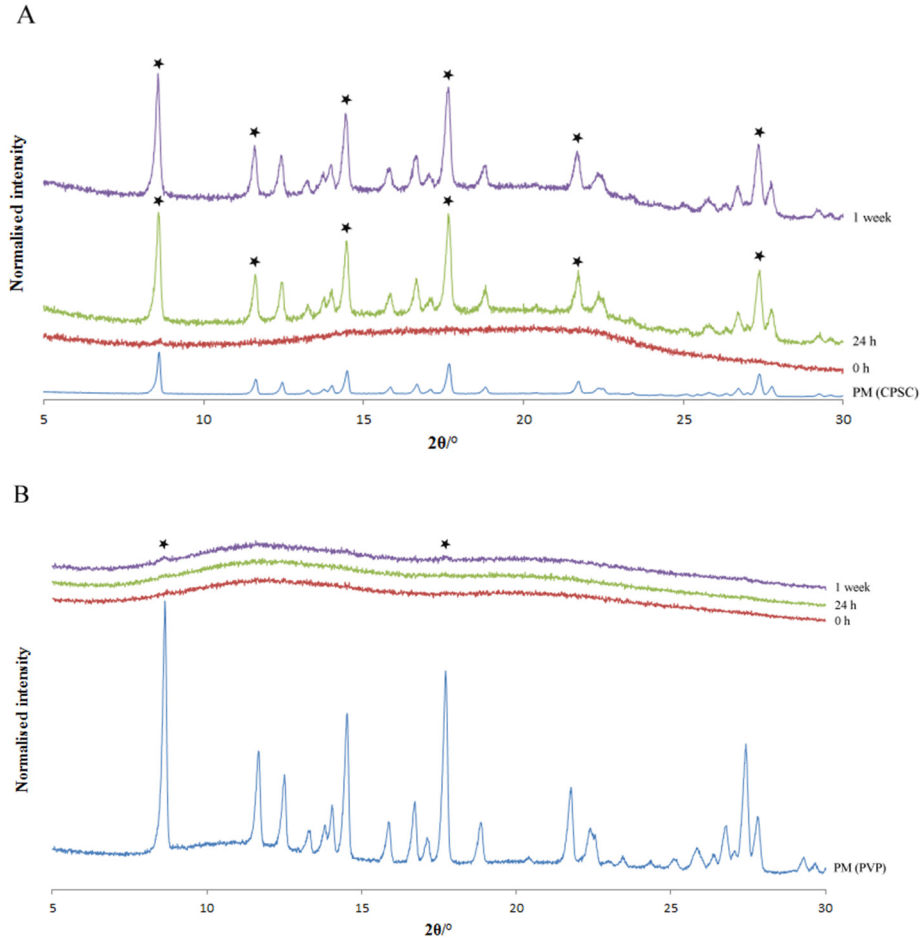

Figure 18. The effects of short-term aging on the solid state of cryogenic co-ground solid dispersions (SDs) of piroxicam anhydrous form I (PRXAH I) and carrier polymer: (A) pre-milled catalytic pretreated softwood cellulose (CPSC) and (B) poly-vinylpyrrolidone (PVP).

5.2.2.3 Dissolution of solid dispersions

In-line Raman spectroscopic monitoring was performed in order to investigate the stability and solid-state changes of co-ground ASDs and corresponding PMs during dissolution in SGF (**III**). Conversion of aPRX to PRXMH was monitored with all co-ground ASDs (**Figure 19A, C**). In case of PM no solid-state changes with PRXAH I occurred during wet slurry experiments (**Figure 19B, D**). The difference in the solvent mediated transformations between PMs and ASDs is due low physical stability of aPRX.

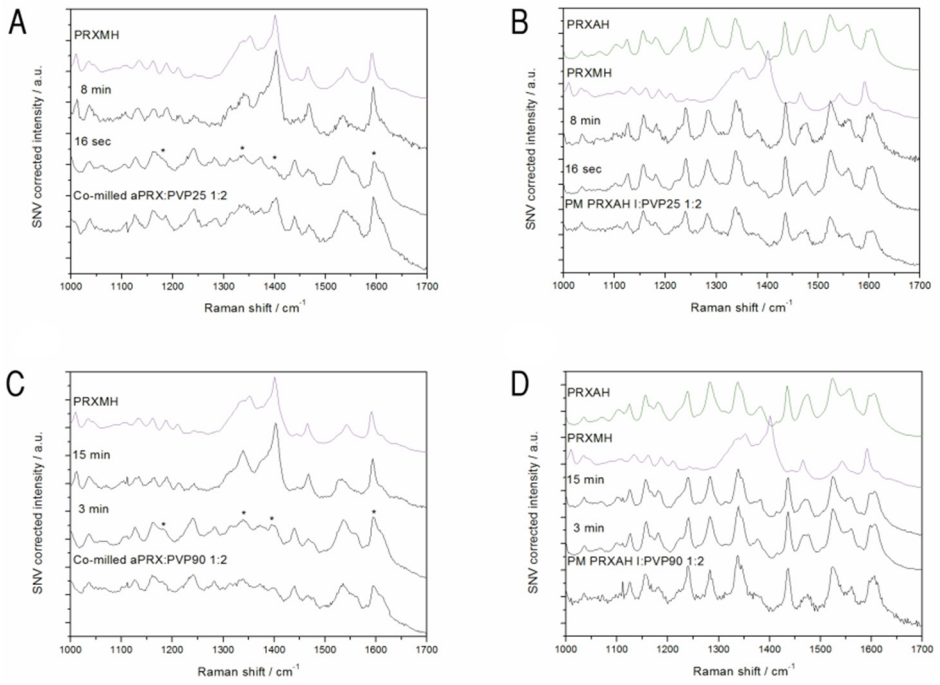

Figure 19. Recrystallisation behaviour of PRX in simulated gastric fluid (SGF) from co-ground amorphous solid dispersions (ASDs) and physical mixtures (PMs), monitored with in-line Raman spectroscopy (n = 3) compared to crystalline PRXAH I and PRXMH spectra (A) aPRX:PVP25 (1:2), co-ground; (B) PRXAH I:PVP25 (1:2), PM; (C) aPRX:PVP90 (1:2), co-ground; (D) PRXAH I:PVP90 (1:2). First changes in amorphous PRX (aPRX) spectrum are denoted with asterisk (*).

The dissolution behaviour of ASDs was compared to the dissolution of respective PMs in order to investigate whether the combination of API and polymer in ASD can enhance the dissolution rate and the amount of dissolved API (**III**). **Figure 20** indicated that dissolution rate of aPRX from ASDs was lower compared to PRXAH I, which was again due to fast recrystallisation of aPRX to PRXMH. However, the results confirmed that the addition of polymer increased the amount of dissolved PRX, as after 2h of dissolution higher amount of PRX was dissolved from ASDs and PMs compared to PRXAH I.

Additionally, the molecular weight of the polymer was influencing the dissolution performance of aPRX from both ASDs and PMs. In case of ASDs, PRX was released faster from the system containing PVP90 as a carrier polymer (approx. 100% released after 60 min) (**Figure 20B**), than from the PRX: PVP25 ASD (100% release of PRX was achieved after 100 min) (**Figure 20A**). However, when evaluating the dissolution profiles of PMs the higher dissolution rate of PRX was obtained with the system containing PVP25 (**Figure 20A**). This may be explained by the formation of different diffusion layers around the particles. It is evident that PVP90 forms thicker diffusion layer compared to

PVP25, thereby inhibiting the dissolution of drug particles. In case of co-ground ASDs, the thicker diffusion layer had an opposite effect on the dissolution of the PRX. As a result, lower penetration of the dissolution media through the layer occurred, inhibiting the transformation of aPRX into less soluble PRXMH.

Commercial small volume dissolution testing was performed to investigate the dissolution performance of the ASDs without using hard gelatin capsules (**Figure 21**). The commercial small volume dissolution tester gave more insight into the dissolution behaviour at the beginning of the dissolution process, allowing the concentration measurements of dissolved PRX every 5 s. The results for both ASDs, containing 1 and 5 mg of PRX, showed similar trends in the dissolution behaviour of the PRX, with higher dissolution rate for aPRX:PVP90 formulation compared to aPRX:PVP25. As shown in **Figure 21**, within the first minute of dissolution testing, the maximum amount of PRX was dissolved from the ASDs (containing 5 mg of PRX), and no changes in the concentration of dissolved PRX were observed. Therefore, it became possible to evaluate the saturation concentration for aPRX. In case of aPRX:PVP25 approximately 48% (120 µg/ml) of the PRX dissolved whereas for aPRX:PVP90 the amount of PRX dissolved was approximately 58% (145 µg/ml).

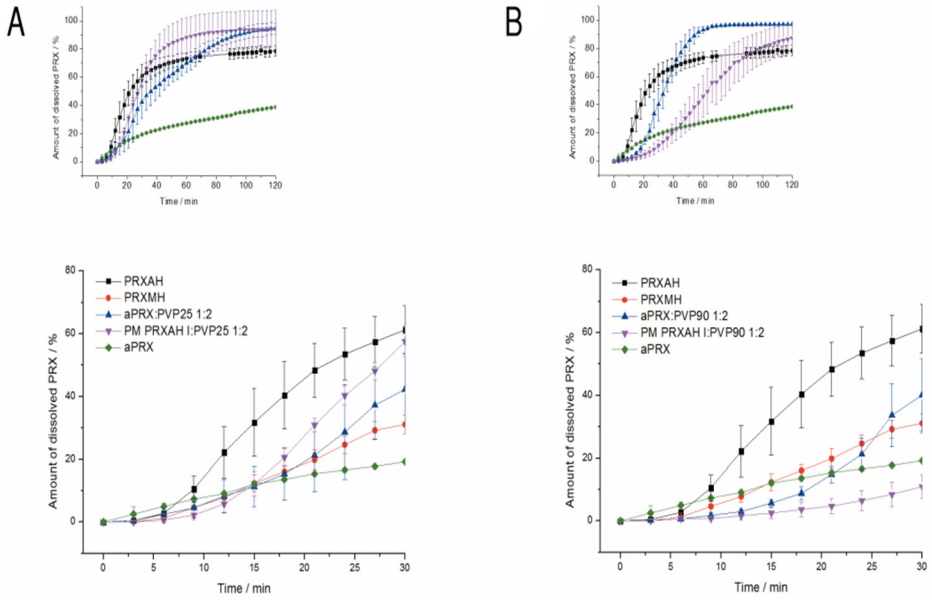

Figure 20. Dissolution behaviour of PRX from co-ground amorphous solid dispersions (ASDs) and physical mixtures (PMs) compared to crystalline PRXAH I and PRXMH during conventional dissolution testing in 900 ml simulated gastric fluid (SGF) (a) aPRX:PVP25 (1:2) and (b) aPRX:PVP90 (1:2). Y-error bars indicate the standard deviation.

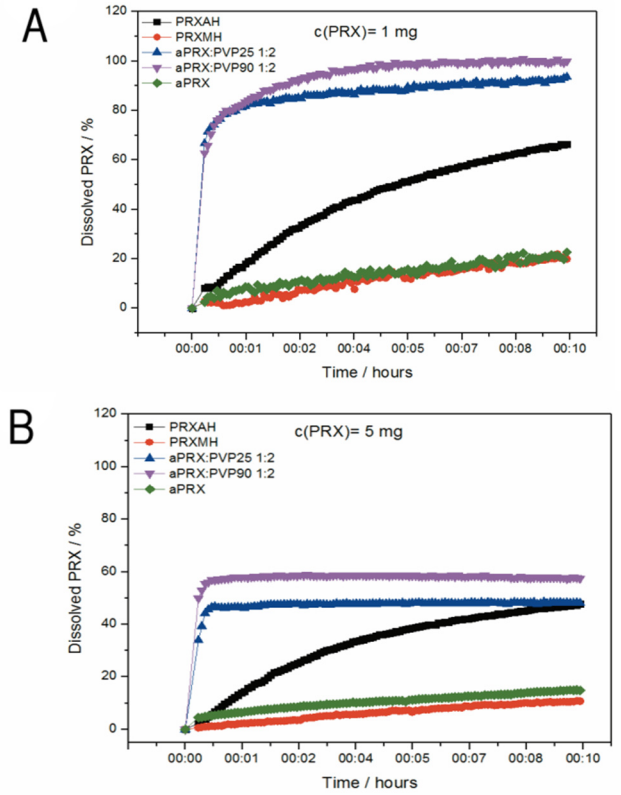

Figure 21. Dissolution performance of PRX:polymer amorphous solid dispersions (ASDs), amorphous PRX (aPRX), crystalline PRXAH I and PRXMH during commercial small volume dissolution testing in 20 ml of simulated gastric fluid (SGF), the amount of PRX corresponds to (A) 1 mg and (B) 5 mg.

From the commercial small volume dissolution testing it became evident that there exists a limit of how much PRX will be dissolved from aPRX:polymer ASDs. Therefore, experimental solubility determinations were performed to further investigate, how much of PRX from aPRX:polymer ASDs actually dissolves. According to the results, higher amounts of PRX were dissolved from co-ground ASDs with PVP90 compared to formulations containing PVP25, however this was valid up to 250 µg/ml of aPRX above what the situation changed (**Figure 22**). The variation in the saturation concentrations when using different amount of aPRX:polymer is determined by the polymer itself and it's molecular weight. At low amount of aPRX:polymer ASDs the particles can move around freely and the dissolution of PRX is dependent only on the polymers' molecular weight (the thickness of diffusion layers). By increasing the amount of aPRX:polymer the amount of free water decreases and changed dispersion properties starts to influence the dissolution of PRX. In these conditions polymer particles could interact with each other and the formation of more complex polymer chain networks may have influenced the dissolution of PRX.

This could explain the change in the dissolution performance of aPRX:polymer ASDs at concentrations above 250 µg/ml.

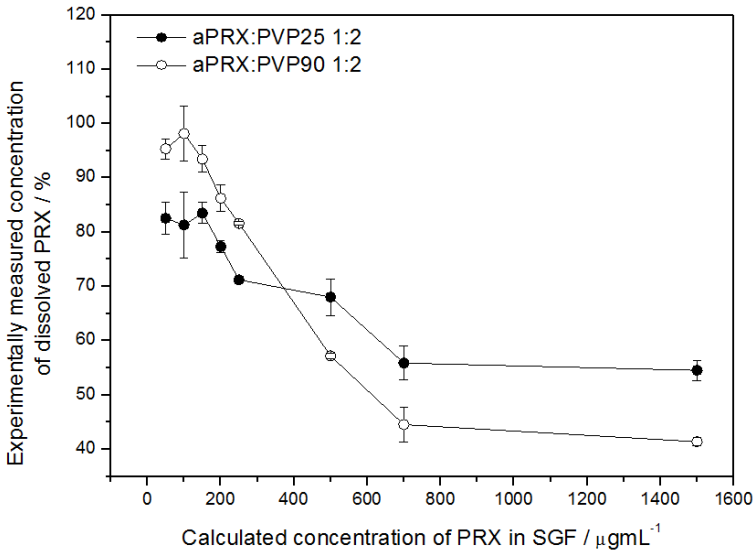

Figure 22. Determination of experimental solubility of PRX in simulated gastric fluid (SGF). X-axis – calculated theoretical concentration of the PRX in solution when 100% dissolved, Y-axis – the experimentally measured percentage of PRX dissolved relative to 100%.

The effects of cryogenic co-grinding and carrier polymer on the dissolution behaviour of poorly water-soluble PRXAH I were studied in purified water (corresponding PMs were used as a reference) and presented in **Figure 23** (**IV**). With PMs, the presence of carrier polymer (CPSC or PVP25) increased the dissolution rate of PRX compared to the dissolution rate of pure API. The enhanced dissolution of PRXAH I from the PMs was obviously due to the surface covering of polymer particles with more fine API particles, thereby increasing the effective surface area that is in contact with a dissolution medium. Additionally, it has been reported previously, that carrier polymer can inhibit the transformation of PRXAH I to a less-soluble PRXMH during dissolution testing, and consequently, increase the dissolution rate of PRX [Paaver *et al*., 2012].

The cryogenic co-ground SDs prepared from PVP25 enhanced the dissolution rate of PRX (**Figure 23**). The complete dissolution of PRX in purified water while using PVP25 as carrier polymer was achieved within 10 min. The use of CPSC as a carrier polymer resulted in the sustained release behaviour of PRX, that can be explained by the recrystallisation of aPRX after preparation of cryogenic co-ground SDs followed by the hydrate formation during dissolution (some vague XRPD crystalline reflections were still observed after the preparation of cryogenic co-ground SDs). According to the literature, absorbed water can induce the disruption of drug-polymer interactions in ASDs leading

to phase separation of the formulation [Rumondor *et al*., 2009]. Even though CPSC is a hydrophobic polymer, it contains a relatively high amount of residual water (4.5% w/w), which can induce the separation of cryogenic co-ground SD into amorphous drug-rich (PRX) and polymer-rich (CPSC) domains followed by recrystallisation of aPRX, thereby decreasing the dissolution rate of PRX.

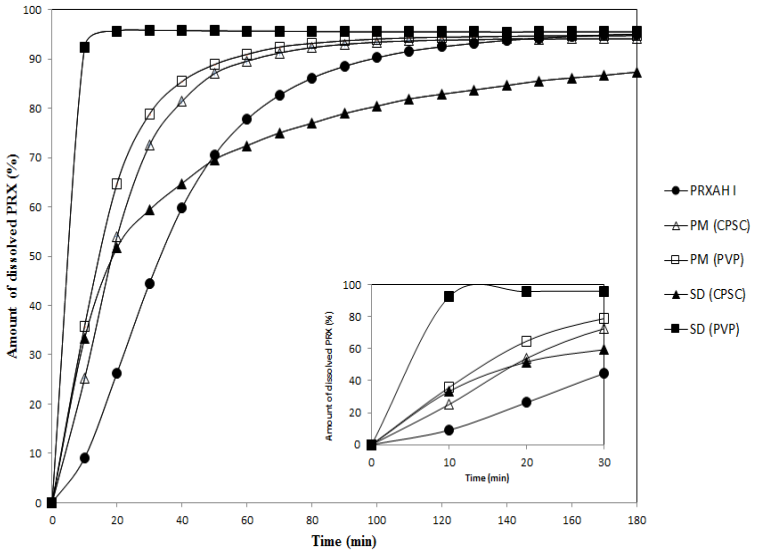

Figure 23. Dissolution profiles (n = 5–6) of physical mixtures (PM) and cryogenic co-ground solid dispersions (SD) of piroxicam anhydrous form I (PRXAH I) and carrier polymer

5.2.3 Effects of lignification on the film properties of cellulose ether (V)

Since lignin is primarily a structural material in plants to add strength and rigidity to cell walls, it would be expected that it could also improve the mechanical properties of pharmaceutical cellulosic films and coatings. In addition, lignin has a good film forming ability by itself [Doherty *et al*., 2011].

5.2.3.1 Surface morphology and physical appearance

As seen in **Figure 24,** the lignified HPMC films were transparent, slightly coloured (yellowish to brown), smooth and continuous. Lignification of the HPMC films slightly increased the dry thickness of the plasticized HPMC films after film formation (**Table 6**). The differences in film thickness were statistically significant ($p < 0.05$) between the lignified films and non-lignified reference film when the concentration of lignin was 1% (w/w) or more in the film composition (calculated as a % w/w of a plasticizer weight).

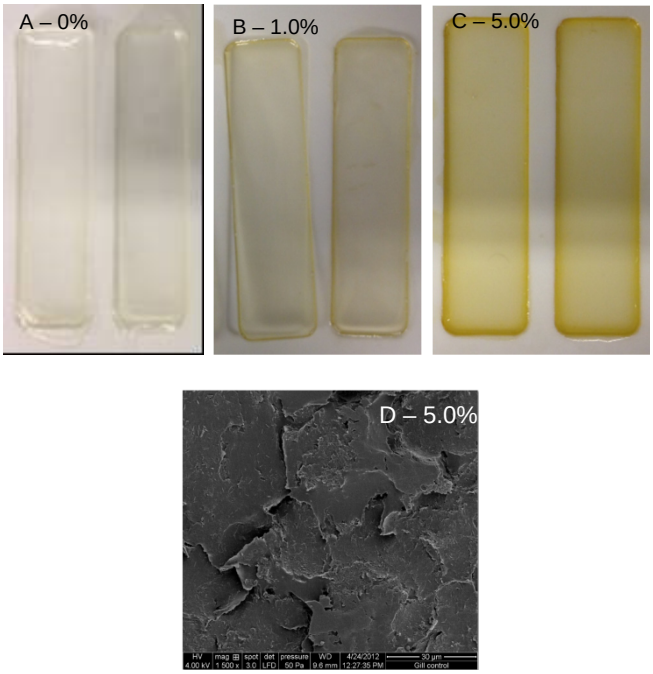

Figure 24. Visual appearance and morphology of the CPSL lignified hydroxypropyl methylcellulose (HPMC) films.

Table 6. Concentration levels and dry thickness of catalytic pre-treated softwood lignin (CPSL) and industrial softwood kraft lignin (Indulin AT) containing free films.

Material	**Concentration of lignin (calculated as a % w/w of plasticizer PEG 400 weight)**	**Thickness (μm) (mean ± SD)**
CPSL	0.1	168.6 ± 5.4
	0.5	187.0 ± 8.1
	1.0	187.4 ±7.5
	5.0	194.4 ±6.1
	10.0	186.3 ±6.1
Indulin AT	0.1	190.1 ±10.9
	0.5	195.0 ±3.7
	1.0	192.7 ±4.1
	5.0	200.6 ±2.4
	10.0	225.3 ±12.2
Reference	0	175.5 ±5.5

5.2.3.2 Solid-state and thermal properties

Many polymers used in film coating possess brittle properties at ambient temperature and humidity conditions, and the addition of a plasticizer is necessary for obtaining effective coatings without cracks or splitting defects. Plasticizers main functionality is to increase the workability or flexibility of the polymer and to improve the flow, to increase toughness and reduce brittleness of the films by decreasing the Tg of the film [Harris and Chebre-Sellassie 2008]. DSC has been extensively used to investigate the miscibility in polymer blends (including films). A single composition-dependent Tg is indicative of blend miscibility [Bourara *et al*., 2014]. Additionally, an increase in Tg generally indicates a restriction in mobility of the film polymer chains or an increase in the crystallinity of the polymer, which could be also related to interactions with film excipients [Felton 2007].

A slight increase of Tg was observed as the concentration of lignin in the films was increased (**Figure 25**). The Tg increased by 20°C for CPSL films when the concentration of lignin in the films was 10%. With industrial lignin, however, the increase was only by 13°C. It can be supposed that wood lignin at higher concentrations increase the stiffness or rigidity of HPMC polymer matrix due to the hindered mobility of polymer chains in the presence of the partially undissolved lignin residues.

However, when low concentrations of the lignin were used (0.5% and 1%), the values for Tg decreased, thus indicating the induced plasticization of the films.

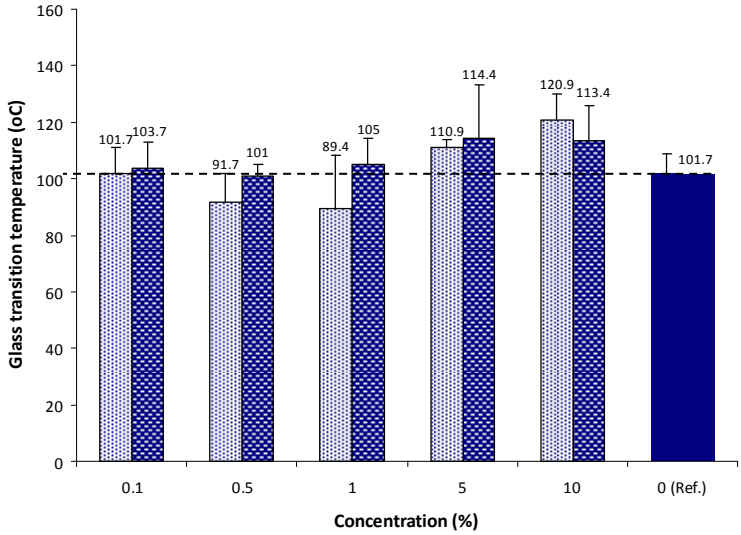

Figure 25. Glass transition temperature (T_g) of the lignified HPMC films (mean ± SD, n = 3-4). The HPMC films are lignified at a concentration of 0.1%, 0.5%, 1.0%, 5.0% and 10% (calculated as a % w/w of a plasticizer PEG 400 weight). The reference film ("0 Ref.) is unlignified plasticized HPMC film. Key ( ) catalytic pretreated softwood lignin (CPSL), ( ) industrial softwood kraft indulin (Indulin AT) and ( ) unlignified HPMC reference film "0 (Ref.)".

5.2.3.3 Mechanical stress-strain properties

A desirable pharmaceutical film coat should be hard and tough without being brittle and is characterized by high tensile strength, moderate elongation at break and a high Young's modulus [Cole *et al*., 1995].

Analysis of tensile strength provided evidence that Indulin AT lignified HPMC films had higher tensile strength compared to the films lignified with CPSL (**Figure 26A**). Higher tensile strength (compared to reference films) was obtained for films containing higher concentration of lignin and Indulin AT, i.e. 5% for Indulin AT and 10% for CPSL.

The mean elongation of both lignified and non-lignified reference films was between 4–8% **(Figure 26B).** Elongation at break reflects flexibility of films. A lower value of elongation implies a more brittle film structure. From the obtained results it is seen that for lower concentrations of CPSL and Indulin AT the mean elongation was found to be almost similar to reference, whereas for higher, 5 and 10% concentrations, Indulin AT containing films had higher elongation percentage than the reference films.

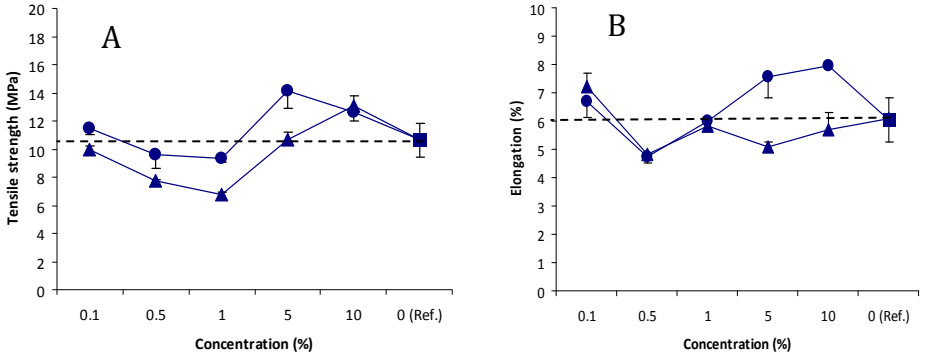

Figure 26. Tensile strength (A) and elongation (B) of lignified HPMC films (mean ± SD, n = 3–6). Key: (▲) Catalytic pretreated softwood lignin (CPSL), (●) Industrial softwood kraft indulin (Indulin AT) and (■) Unlignified plasticized HPMC reference film "0 (Ref.)"

The Young's modulus (or the modulus of elasticity) is a measure of the "stiffness" of the polymeric film or the ability of the film to withstand a high stress while undergoing little elastic deformation [Felton 2007]. Higher concentration of both lignins increased the value of Young's modulus compared to the reference films **(Figure 27)**. Interestingly, the films with low concentration of CPSL have higher Young's modulus, than those containing higher concentrations of CPSL. However, for films containing Indulin AT, Young's modulus seems to rise as the concentration of Indulin AT increases.

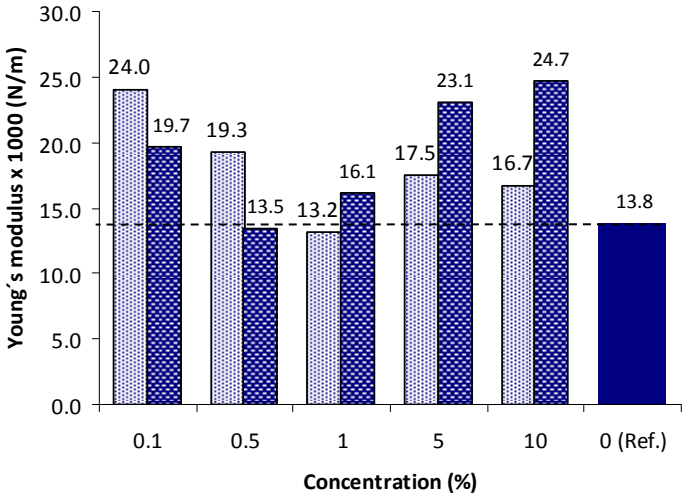

Figure 27. Young's modulus of the lignified HPMC films (mean ± SD, n = 3–6). The HPMC films are lignified at a concentration of 0.1%, 0.5%, 1.0%, 5.0% and 10% (calculated as a % w/w of a plasticizer PEG 400 weight). The reference film ("0 Ref.) is unlignified plasticized HPMC film. Key: ( ) catalytic pretreated softwood lignin (CPSL), ( ) industrial softwood kraft indulin (Indulin AT) and ( ) unlignified HPMC reference film "0 (Ref.)"

5.2.3.4. Water vapour permeation properties

The calculated average values of WVP are presented in **Table 7.** The addition of wood lignin in the HPMC film composition did not significantly affect the moisture barrier properties of HPMC films. The WVP values of the films lignified at a low concentration of 1.0% (calculated as a % w/w of a plasticizer weight) were somewhat lower than those of the films lignified at a higher concentration of 5.0%. Indulin AT wood lignin appeared to improve more effectively the moisture barrier properties of plasticized HPMC films than CPSL but this finding could not be verified, since the difference of the WVP values was not statistically significant. It is evident that the macromolecular, slightly cross-linked and hydrophobic nature of lignin can attribute the WVP barrier properties of HPMC films. However, the increase of lignin concentration in the films did not improve the moisture barrier properties of cellulose ether films suggesting a limited film formation and uneven film structure associated with a high concentration of lignin.

Table 7. Water vapour permeability (WVP) of plasticized lignified hydroxypropyl methylcellulose (HPMC) films (n = 6–10).

HPMC film composition*	**Water vapor permeability (WVP) [×10–7g//mm2h) × mm/Pa] (mean** ± SD
HPMC + PEG 400 (ref.)	0.46 ±0.25
HPMC + CPSL (1%) + PEG 400	0.53 ±0.11
HPMC + CPSL (5%) + PEG 400	0.73 ±0.12
HPMC + Indulin (1%) + PEG 400	0.46 ±0.35
HPMC + Indulin (5%) + PEG 400	0.58 ±0.14

6. SUMMARY AND CONCLUSIONS

Catalytic pretreated softwood cellulose (CPSC) and lignin (CPSL) obtained from pine softwood (*Pinus sylvestris*) were investigated as new excipients in pharmaceutical solid dosage manufacturing. Physical material properties and functionality-related characteristics (FRCs) of these biomaterials relevant to preparation and performance of oral solid dosage forms were studied and compared with those of some established pharmaceutical excipients. CPSC and CPSL were characterized in terms of solid-state and thermal properties, particle size and shape, material densities, and flowing properties. Thermal characterization of CPSL and CPSC showed the T_g values of the biomaterials being higher compared to that measured for the reference kraft softwood lignin (Indulin AT), hardwood lignin (PC-1369) and microcrystalline cellulose (MCC), suggesting increased thermal stability. Additionally, CPSL confirmed the good powder flow properties, with flow rate being approximately four times higher compared to the flow rate obtained with CPSC.

Tablets made of CPSC and CPSL were successfully prepared by direct compression method. MCC and CPSC were found to have a clear tendency for plastic deformation, and consequently, they showed better compression behaviour than the lignins. CPSC confirmed particle-size independent plastic behaviour under compression. However, moisture content of CPSC affected the tablet strength. Compression properties of CPSL were not as good as those of celluloses, and the tablets compressed from CPSL presented clearly the lowest tablet breaking strength values.

Amorphous solid dispersions (ASDs) were prepared to investigate polyvinylpyrrolidone (PVP) and CPSC as carrier polymers in cryogenic co-grinding (amorphisation) of poorly water-soluble piroxicam (PRX). The results suggest that both CPSC and PVP improved the cryogenic co-grinding (amorphisation) of a poorly water-soluble PRX. After cryogenic co-grinding for 180 min, the amorphous form of PRX was achieved with PVP used as a carrier polymer. However, with *pre-milled* CPSC a sign of very low-intensity characteristic crystalline peak of PRXAH I was still observed in XRPD pattern indicating some degree of crystallinity. Additionally, the solid-state stabilization of ASDs was dependent on carrier/stabilizing polymer used. Cryogenic co-ground mixtures of PRX and PVP (1:3) remained in an amorphous state over a one-week storage period. However, in case of using CPSC as a carrier polymer, the amorphisation of PRX was found to be only temporary: phase transformation appeared and crystalline reflections were detected after 24 h of storage at an ambient controlled room temperature (25°C / 20% RH). The present results suggest that CPSC acts as a physical matrix, where drug particles are located between cellulose microfibers.

More in depth in-line Raman spectroscopic monitoring was performed in order to investigate the stability and solid-state changes of ASDs during the dissolution in SGF. It was found that in case of all ASDs the conversion of

amorphous PRX (aPRX) to PRX monohydrate (PRXMH) occurred. Effect of carrier polymers on the dissolution behaviour of PRX in ASDs was also investigated. With PMs, the presence of carrier polymer (CPSC or PVP) increased the dissolution rate of PRX compared to the dissolution rate of pure API. In case of ASDs the use of CPSC as a carrier polymer resulted in the sustained-release behaviour of PRX, which can be explained by the recrystallization of aPRX in cryogenic co-ground SDs followed by a hydrate formation during dissolution. In case of PVP, the molecular weight of polymer affects the dissolution performance of aPRX from both ASDs and PMs.

The effect of lignin on the mechanical stress-strain properties and water vapor permeation (WVP) of hydroxypropylmethyl cellulose (HPMC) films was investigated with free films. The results suggest that the film properties of aqueous HPMC films can be modified by the inclusion of a wood lignin in the film composition. Lignification of HPMC films with Indulin AT at a concentration of 5% w/w (calculated from the plasticizer weight) resulted in mechanically strongest and elongated films.

As a summary, the present thesis represents the very first steps on a way of exploring the functionality-related characteristics (FRCs) of new biomaterials CPSC and CPSL as well as their applications in the formulation of pharmaceutical dosage forms. Further studies are needed in order to find out their actual utilization in the pharmaceutical manufacturing processes and final formulations.

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8. SUMMARY IN ESTONIAN

Abiainete – eeltöödeldud lignotselluloosi, ligniini ja polüvinüülpürrolidooni – funktsionaalsusega seotud omaduste iseloomustamine farmatseutiliste ravimvormide valmistamiseks

SISSEJUHATUS

Euroopa Farmakopöa (Ph. Eur. 2010) definitsiooni järgi on abiained mistahes ravimi koostisained, või ravimi tootmisel kasutatavad ained, mis sisalduvad ravimis ja ei ole raviained. Täna aga ei saa enam abiaineid pidada lihtsalt inertseteks ja mitteaktiivseteks ravimi koostisosadeks. Abiained mängivad olulist rolli ravimpreparaatide disainimisel, arendamisel ja valmistamisel. Lõpliku ravimvormi omadused ja kvaliteet on otseselt seotud abiainete füüsikaliste ja keemiliste omadustega ning samuti nii abiainete omavahelise kui ka abiainete ja raviainete vahelise sobivusega. Abiainete roll on väga oluline, kuna nad võimaldavad raviaine viia manustamiseks mõeldud sobivasse ravimvormi. Abiaine funktsiooniks on luua raviaine(te) organismi viimiseks sobiv keskkond ja sellega parandada näiteks ravimi stabiilsust, biofarmatseutilisi omadusi, välimust või lihtsustada ravimi tootmist [Crowley and Martini 2007; Rowe *et al.*, 2009]. Euroopa Farmakopöa (Ph. Eur. 2010) defineerib abiainete funktsionaalsusega seotud omadusi kui kontrollitavaid abiainete füüsikalisi või keemilisi omadusi, mis mõjutavad oluliselt lõpp-produkti – ravimpreparaadi omadusi ja toimet.

Suukaudselt manustatavad tahked ravimvormid (tabletid, kapslid ja graanulid) on kõige efektiivsemad ja ohutumad ning tänapäeval ka kõige sagedamini kasutatavad ravimvormid. Tablettide otsepressimist peetakse kõige eelistatumaks suukaudselt manustatava ravimvormi tootmisetehnoloogiaks, kuna tal on palju eeliseid võrreldes teiste ravimvormi tootmistehnoloogiatega, nt märggranuleerimisega (vähem operatsiooniprotsesse, lühem tootmisaeg, sobilik temperatuuri- ja niiskustundlikele raviainetele) [Jivraj *et al.*, 2000]. Seega, vajadus uute otsepressimist võimaldavate abiainete järele farmaatsiatööstuses tänapäeval järjest kasvab.

Lahustuvus ja lahustumiskiirus on ühed olulisemad raviainete omadused, millest sõltub ravimpreparaadi biosaadavus ja toime organismis. Halb vesilahustuvus ning seega ka aeglane imendumine organismis on üheks suuremaks probleemiks ravimpreparaatide disainimisel. Paljud raviained võivad eksisteerida ravimpreparaadis erinevates tahketes vormides (kristalliline, amorfne, solvaat/hüdraat), omades erinevaid füsikokeemilisi omadusi. Püütakse leida erinevaid meetodeid, kuidas muuta raviaine omadusi ning parandada tema vesilahustuvust ning seeläbi tema lahustumiskiirust ja biosaadavust. Amorfne raviaine omab kõige paremat lahustuvust ja suuremat lahustumiskiirust. Samas on amorfne vorm väga ebastabiilne ja võib kergesti rekristalliseeruda, mille

tagajärjel võib oluliselt muutuda raviaine lahustumine ning biosaadavus organismis. Samuti on teada, et polümeerid suudavad stabiliseerida amorfseid raviaineid hoides ära nende rekristalliseerumist. Näiteks, polüvinüülpürrolidoon (PVP) on sünteetiline polümeer, mida tavaliselt kasutatakse raviaine amorfse vormi stabiliseerimiseks. Amorfne tahke dispersioon on süsteem, kus amorfne raviaine on ühtlaselt segatud ja dispergeeritud koos polümeeriga. Polümeeri peamine roll on stabiliseerida raviaine amorfset vormi, pärssides seega raviaine rekristalliseerimist säilitamisel ja dissolutsiooni käigus, ning suurendades seejuures raviaine lahustumiskiirust [Newman *et al.*, 2012; Serajuddin 1999]. Et aga disainida stabiilne ja toimiv ravimvorm, on vaja juba preformulatsiooni käigus välja selgitada, kuidas erinevad abiained mõjutavad ja muudavad raviaine käitumist ja kas on võimalik abiainetega inhibeerida raviainega toimuvaid muutusi.

Kõik eelnimetatud faktid on suurendanud veelgi farmaatsiatööstuse huvi leida uusi biomaterjale, mida oleks võimalik abiainetena (tablettide koostisosana) kasutada. Uute abiainete leidmine ja nende väljatöötamine annab farmaatsiatööstusele hea võimaluse ravimpreparaatide (ravimvormide) kvaliteedi parandamiseks ja biosaadavuse suurendamiseks. Lignotselluloos ja ligniin on paberitootmise jääkproduktid, mis on kergesti kättesaadavad ja odavad. Neid pole varem farmaatsiatööstuses abiainetena uuritud. Ligniin on üks kolmest (koos tselluloosi ja hemitseluloosiga) polümeersest komponendist, mida võib taimede rakuseinas leida [Doherty *et al*., 2011]. Viis aastat tagasi Hakola *et al*. (2010) töötasid välja uue meetodi, kuidas eraldada lignotselluloos ja ligniin puu biomassist. Antud meetod omab palju eeliseid võrreldes teiste seni kasutusel olevate meetoditega: (i) on võimalik muuta lignotselluloosi ja ligniini füsikokeemilisi omadusi; (ii) saadud tselluloosi saab kergesti hüdrolüüsida; (iii) meetod väldib hüdrolüüsitavate süsivesinike kaotust ja mürgiste ühendite teket. Lisaks on antud uus meetod keskkonnasõbralik, kuna kasutatakse *in situ* valmistatud katalüsaatoreid ja oksüdeerijatena kasutatakse hapnikku või suruõhku. Kuna katalüütiliselt eeltöödeldud lignotselluloos (CPSC) ja ligniin (CPSL) on üsna uued võimalikud abiained farmaatsias, siis nende füsikokeemilistest ja võimalikest tehnoloogiliselt olulistest omadustest on veel väga vähe teada.

Selle uurimistöö peamiseks eesmärgiks oli biomaterjalide CPSC, CPSL ja sünteetilise PVP füsikokeemiline iseloomustamine ja nende funktsionaalsusega seotud omaduste uurimine.

TÖÖ EESMÄRGID

Töö eesmärgiks oli uurida abiainete – katalüütiliselt eeltöödetud lignotselluloosi (CPSC), ligniini (CPSL) ja sünteetilise polüvinüülpürrolidooni (PVP) – olulisi funktsionaalsusega seotud omadusi farmatseutiliste ravimvormide valmistamiseks. Täpsemad töö eesmärgid olid:

1. analüüsida CPSC-i ja CPSL-i järgmisi füsikokeemilisi ja ravimvormi valmistamisega seotud omadusi: struktuuriomadusi (kristallilisust/amorfsust), veesisaldust, pulbri tihedust, poorsust, voolavust, adsorptsioonivõimet ja lahustuvust;
2. hinnata CPSC-i ja CPSL-i tehnoloogiliselt olulisi omadusi tablettide otsepressimisel;
3. hinnata CPSC-i ja PVP-i kasutamisvõimalust amorfsetes tahketes dispersioonides vees halvasti lahustuvate raviainete omaduste modifitseerimiseks ja raviaine amorfse vormi stabiilsuse parandamiseks;
4. hinnata CPSL-i mõju ravimvormi katete mehaanilistele ja füsikokeemilistele omadustele ning katete vastupidavusele vee suhtes.

MATERJALID JA MEETODID

Uuringus kasutati kolme erinevat abiainet: CPSC, CPSL ja erineva molekulmassiga sünteetilist polüvinüülpürrolidooni (PVP, PVP25 ja PVP90). Mudelraviainena kasutati vees halvasti lahustavat piroksikaami, PRX (nii veevaba piroksikaami vorm I, PRXAH I, kui amorfset PRX).

Uuringus kasutatud meetodite hulka kuuluvad:

1. Uuritavate biomaterjalide (CPSC, CPSL) füsikokeemiliste omaduste uurimine: osakeste suurus ja suuruse jaotus, osakeste kuju, tahke aine poorsus, tihedus ja voolavus, pulbri pinnaomadused ja adsorptsioonivõime. Ainete füsikokeemilisi omadusi ja vormide vahelisi muutusi analüüsiti kasutades erinevaid analüütilisi meetodeid: Ramani ja infrapunaspektroskoopia (FTIR), pulber-röntgendifraktomeetria (XRPD), termilised meetodid (diferentsiaalne skaneeriv kalorimeetria (DSC)). Osakeste suuruse ja kuju uurimiseks kasutati skaneerivat elektronmikroskoopiat (SEM).
2. CPSC-i ja CPSL-i tehnoloogiliselt oluliste omaduste määramiseks tablettide otsepressimisel kasutati tableteerimismasinat. Lisaks selgitati välja nende biomaterjalide deformatsiooni, tihendamise ja kompaktsuse omadused ja võrreldi neid teiste tuntud abiainetega farmaatsiatööstuses nagu mikrokristalliline tselluloos (MCC), laktoos, tärklis ja kaltsiumfosfaat. Tableteerimisomaduste analüüsimiseks kasutati kokkusurumisjõu – kauguse kõverat. Biomaterjalide kompaktsust aga analüüsiti graafiku põhjal, mille x-teljel oli kokkusurumisjõud ja y-teljel tableti murdetugevus.
3. Mehaanilise jõu (krüopeenestamine ja kuulpeenestamine toatemperatuuril) tagajärjel toimuvaid tahke raviaine (PRX) transformatsioonide (amorfse vormi saamise) uurimine; polümeeride mõju uurimine amorfse PRX-i stabiilsusele ja lahustumiskiirusele. Polümeeriks kasutati PVP-t (PVP25, PVP90) ja CPSC-t vahekorras 1:1, 1:2 ja 1:3. Saadud amorfse PRX-i stabiilsust uuriti kasutades XRPD. Stabiilsuse uuringuanalüüside kogumisepunktideks olid: 24 tundi, 48 tundi, 72 tundi, 96 tundi ja 1 nädal. Tahke aine vormi muutuste ja abiainete mõju raviaine käitumisele hinnati *in vitro* dissolutsioonitestiga

korvikeste meetodil. Raviaine tahke vorm ja tahke vormi muutused tehti kindlaks kasutades *in situ* Raman spektroskoopiat. Lahustunud raviaine kontsentratsiooni määramiseks kasutati UV spektrofotomeetrit.

4. CPSL-i mõju uurimine hüdroksüpropüülmetüültselluloosi (HPMC) katete mehaanilistele omadustele ning katete stabiilsusele niisketes säilitustingimustes. Plastifikaatoriks kasutati polüetüleenglükooli (PEG), mida lisati 20% polümeeri massist. CPSL-i kontsentratsioonide vahemik oli 0.1, 0.5, 1, 5, ja 10% plastifikaatori massist. Katete mehaanilist tugevust uuriti Lloyd LRX masina abil. Katete stabiilsusuuringud viidi läbi hoides katteid kõrge niiskuse tingimustes ja analüüsiti katete massi muutust ajas.

UURINGU PÕHITULEMUSED

1. Esimeseks sammuks kvaliteetsete ravimvormide disainimisel on uurida võimalike abiainete füsikokeemilisi omadusi. CPSC-i osakesed olid piklikud ja kiutaolise kujuga, suurusega 125–1400 μm. CPSL-i osakesed olid helbelised ja teravate servadega. Biomaterjalide klaasistumistemperatuurid olid vahemikus 168–171 ^{o}C, mis on üsna kõrged ja sobivad hästi farmaatsias kasutamiseks. Biomaterjalide struktuurianalüüsid näitasid, et CPSL-l on keeruline amorfne struktuur, mis koosneb omavahel osaliselt ristseotud heterogeensetest polümeerimolekulidest, samas kui CPSC-l tehti kindlaks poolkristalliline struktuur.

2. Farmaatsiatööstusele on tablettide otsepressimise-alased uurimistööd väga olulise tähtsusega. Baasteadmised farmatseutiliste abiainete käitumisest tablettide otsepressimisel aitavad parandada tablettide kvalitatiivseid omadusi ning võimaldavad arendada otsepressimise protsessi ning parendada lõpliku ravimvormi (tablettide) disainimist. Kokkusurumisjõu suurendamisega kõikide biomaterjalide plastilisus vähenes. Eeltöödeldud CPSC-i plastilise deformatsiooni ning kokkupressimisomadused olid sama head kui MCC-il. MCC on tuntud abiaine, mida laialdaselt farmaatsiatööstuses tablettide valmistamiseks kasutatakse. CPSL-i plastilisus oli sarnane tööstuslike ligniinidega, kuid plastilisusefaktori väärtus oli madalam kui CPSC-l. CPSC oli kõige elastsem materjal võrreldes teiste testitud biomaterjalidega. CPSC-i elastusfaktor oli 18%. CPSL-i elastsusfaktori väärtused olid vahemikus 12–15%.

Baseerudes CPSC-i ja CPSL-i olulistele mehaanilistele omadustele, võib järeldada, et antud biomaterjalide kompaktsus ja tableteerimisomadused sobivad tablettide otsepressimiseks. Samas ilmnes, et CPSL oli üsna habras materjal, olles vähem kokkusurutav kui tselluloosid, mistõttu tema tableteerimisomadusi tuleb modifitseerida teiste abiainetega (kaas-abiainetega) enne tema kasutamist farmatseutilise abiainena otsepressimisel.

3. PRX-i amorfset vormi oli võimalik valmistada peenestades PRXAH I toatemperatuuril 180 minuti jooksul koos PVP25 ja PVP90-ga vahekorras 1:1 ja 1:2. Saadud PRX-i amorfne vorm oli füüsikaliselt stabiilne kuni kuus kuud.

Samamoodi tekkis PRXAH I-st amorfne vorm ka krüopeenestamisel 180 minuti jooksul PVP25 ja CPSC-ga vahekorras 1:3. Saadud PRX-i amorfse vormi stabiilsus sõltus kasutatavast polümeerist. CPSC-i kasutamisel muutus amorfne PRX kristalliliseks juba 24 tundi pärast toatemperatuuril säilitamist. PRX-i amorfsed dispersioonid PVP25-ga olid aga füüsikaliselt stabiilsed kuni üks nädal. PRX-i struktuur sisaldab O-H ja N-H funktsionaalseid gruppe, mis arvatavasti moodustavad vesiniksidemeid PVP25 karbonüülgruppidega. CPSC-i kasutamisel aga moodustub krüopeenestamise protsessi ajal hoopis füüsikaline maatriks, kus PRX asub tselluloosi mikrofiibrite vahel. Tulemused näitasid, et antud maatriks ei suuda PRX-i amorfset vormi piisavalt kaua stabiliseerida.
Raviaine tahke vorm ja vormi muutused tehti kindlaks kasutades *in situ* Raman spektroskoopiat simuleeritud maomahla keskkonnas. Amorfse PRX-i muutumist piroksikaammonohüdraadiks (PRXMH) jälgiti kõikide amorfsete tahkete dispersioonide puhul. PRX-i ja polümeeri füüsikalise segu puhul aga mingit PRXAH I muutumist monohüdraadiks ei toimunud. Amorfse tahke dispresiooni ja füüsilise segu käitumise erinevuse põhjuseks oli amorfse PRX-i madal füüsikaline stabiilsus.

Tahke raviaine (amorfne PRX) vormi muutuse ja abiainete mõju raviaine käitumisele hinnati *in vitro* dissolutsioonitestiga. Tulemused kinnitasid, et polümeeride lisamine ravimvormi tõstis lahustunud PRX-i kogust. Lisaks, polümeeri molekulmass mõjutas oluliselt amorfse PRX-i lahustumist mõlemast amorfsest tahkest dispersioonist ja füüsikalisest segust. Kui polümeerina kasutati PVP90-t, siis PRX-i vabanemine amorfsest tahkest dispersioonist toimus kõige kiiremini. Füüsikalise segu puhul aga, vastupidi, ravimvorm, mis sisaldas polümeerina PVP25-t, vabastas PRX-i kiiremini. Seda nähtust saab seletada arvatavasti erineva paksusega difusioonikihi moodustumisega osakeste ümber. Ilmselt, PVP90 moodustab füüsikalises segus paksema kihi võrreldes PVP25-ga, pärssides sellega raviaine osakeste vabanemist ja lahustumist. Amorfse tahke dispersiooni puhul antud paksemal difusioonikihil oli vastupidine toime. Selle tulemusena, lahustumiskeskkonnas toimuv raviaine penetratsioon läbi difusioonikihi oli madalam, mis pärssis amorfse PRX-i muutumist vees halvasti lahustavaks PRX MH- ks.

Lisaks, uuriti PRX-i lahustumiskiirust amorfsest tahkest dispersioonist, mis oli valmistatud krüopeenestamisel koos polümeeridega PVP25 ja CPSC vahekorras 1:3. Füüsikalise segu puhul polümeeride esinemine suurendas PRX-i lahustamiskiirust võrreldes puhta kristallilise veevaba PRXAH I-ga. See omakorda oli tingitud polümeeri osakeste omadusest katta raviaine osakeste pinda. Teatavasti kui polümeeriosakesed katavad raviaine osakeste pinda, siis nad suurendavad lahusega kontaktis olevat eripinda. Amorfsed tahked dispersioonid valmistatud PVP25-ga kiirendasid PRX-i lahustumist. Kui aga kasutati CPSC-d polümeerina, siis PRX-i vabanemine ja lahustumine olid aeglustunud. See võiks olla tingitud sellest, et CPSC on nõrk PRX-i amorfse vormi stabilisaator, ja PRX-i rekristalliseerimine toimus kohe pärast amorfse tahke dispersiooni valmistamist, millele järgnes PRXMH-i teke lahustumise käigus.

4. CPSL-i lisamine HPMC katetele tõstis nende katete tugevust, võrreldes puhas HPMC katetega. Katete iseloomustamiseks kasutati Youngs'i moodulit, mis näitab materjalide jäikust või kõvadust. CPSL-i kontsentratsiooni tõstmisega katete jäikus kasvas.

Katete stabiilsus niiskes keskkonnas on otseselt seotud tablettide/raviaine stabiilsusega, kuna paljud raviained on väga hügroskoopsed ning võivad vett imades säilitamisel laguneda. CPSL-i lisamine katete koostisele katete vastupidavust veele eriti ei mõjutanud.

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Tartu 2015

Anna Klugman

10. PUBLICATIONS

11. CURRICULUM VITAE

Name:	Anna Klugman (neé Penkina)
Date of birth:	January 18, 1988, Tallinn, Estonia
Address:	Vene 11–16, 10123, Tallinn, Estonia
Phone:	+37258077808
E-mail:	anna.penkina@hotmail.com

Education:

1994–2006	Maardu Gymnasium
2006–2011	University of Tartu, Faculty of Medicine, Institute of Pharmacy (MSc)
2011–2015	University of Tartu, Faculty of Medicine, Institute of Pharmacy (PhD)

Professional employment:

2012–	University of Tartu, Institute of Pharmacy, 0.5 specialist

Scientific work:

A total of 5 publications and 22 presentations at international scientific conferences.

My research activities and interests have been focused on the functionality-related characteristics of excipients for pharmaceutical dosage form formulations and solid state properties of pharmaceuticals.

12. ELULOOKIRJELDUS

Nimi:	Anna Klugman (neé Penkina)
Sünniaeg:	18.jaanuar 1988
Kodakondsus:	eesti
Aadress:	Vene 11–16, 10123, Tallinn, Estonia
Telefon:	+37258077808
E-post:	anna.penkina@ut.ee

Haridus:

1994–2006	Maardu Gümnaasium
2006–2011	Tartu Ülikool, Arstiteaduskond, Farmaatsia Instituut, proviisori õppekava
2011–2015	Tartu Ülikool, Arstiteaduskond, Farmaatsia Instituut, doktorant farmatseutilise tehnoloogia ja biofarmaatsia erialal (PhD)

Erialane täienduskäik:

2012–	Tartu Ülikool, Arstiteaduskond, Farmaatsia Instituut, 0.5 spetsialist

Teadustegevus:

Kokku on ilmunud 5 publikatsiooni ja 22 ettekannet rahvusvahelistel konverentsidel.

Teadustöö on seni olnud seotud järgmiste valdkondadega: abiainete funktsionaalsusega seotud omaduste karakteriseerimine farmatseutilise ravimvormide valmistamiseks; tahkete raviainete füsikokeemilised omadused ja nende modifitseerimise võimalused.

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