Nanofibrillar cellulose in drug delivery

by

Ružica Kolaković

ACADEMIC DISSERTATION

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Helsinki 2013
Abstract


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The choice of proper excipients is one of the key factors for successful formulation of pharmaceutical dosage forms. Increasing number of new therapeutic compounds suffers from poor solubility and/or bioavailability, creating a challenge from the drug formulation point of view. Problems have also been encountered in attempts to formulate biological drugs such as peptides and proteins, considering their sensitivity towards certain production processes and routes of administration. In both cases the choice of the right excipient(s) is essential to provide particular processability and development of systems with desirable drug delivery kinetics.

The aim of this work was to evaluate pharmaceutical applications of nanofibrillar cellulose (NFC), a renewable, biodegradable and widely available plant based material, as a potential excipient in the production of pharmaceutical dosage forms.

Initially, tablets with immediate drug release were manufactured by methods of direct compression and compression after wet granulation using spray dried NFC as a filler material. Addition of NFC improved the flow properties of commercially available and widely used microcrystalline cellulose.

The main focus of the thesis was to evaluate NFC material for long-term sustained drug release purposes. This goal was successfully achieved by two approaches: 1) by setting up a spray drying method for the production of drug loaded NFC microparticles, and 2) by developing a simple three-step method for the production of drug loaded NFC films with matrix structures. Both systems were able to sustain the drug release over long periods of time ranging from two months for the spray dried microparticles up to over three months for the films. The drug release kinetics were system and drug dependent, reaching, in several cases, zero order drug release kinetics.

The final part of the thesis work focused on studying the interactions between small molecular weight drugs, peptides and proteins with the NFC fibers. The purpose of this study was to further clarify and fully understand the mechanisms behind the successful performance of NFC as drug release controlling material. Binding of drugs to NFC due to the electrostatic interactions was observed. This kind of knowledge is beneficial when choosing the proper drug/excipient combination for the formulation process.

In conclusion, NFC was shown to be a versatile excipient for the production of pharmaceutical dosage forms, while the comprehensive evaluation of the full potential of NFC in pharmaceutical applications warrants further experiments in the future.
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Helsinki, January 2013

Ružica Kolaković
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### Abbreviations and symbols

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGU</td>
<td>Anhydrose glucose unit</td>
</tr>
<tr>
<td>API</td>
<td>Active pharmaceutical ingredient</td>
</tr>
<tr>
<td>CBD</td>
<td>Cellulose binding domain</td>
</tr>
<tr>
<td>DDS</td>
<td>Drug delivery system</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of polymerization</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>HFBI</td>
<td>Hydrophobin I</td>
</tr>
<tr>
<td>HFBI-DCBD</td>
<td>Hydrophobin I coupled with two cellulose binding domains</td>
</tr>
<tr>
<td>HPBC</td>
<td>Hydroxypropyl Beta Cyclodextrin</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>ITC</td>
<td>Isothermal titration calorimetry</td>
</tr>
<tr>
<td>MCC</td>
<td>Microcrystalline cellulose</td>
</tr>
<tr>
<td>NFC</td>
<td>Nanofibrillar cellulose</td>
</tr>
<tr>
<td>PVDF</td>
<td>Polyvinylidene fluoride</td>
</tr>
<tr>
<td>$P_y$</td>
<td>Yield pressure</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-tetramethylpiperidine-1-oxyl radical</td>
</tr>
<tr>
<td>XRPD</td>
<td>X-ray powder diffraction</td>
</tr>
<tr>
<td>$M_t$</td>
<td>Cumulative amount of a drug released from a drug delivery system (sphere, cylinder) at time $t$</td>
</tr>
<tr>
<td>$M_\infty$</td>
<td>Cumulative amount of a drug released from a drug delivery system (sphere, cylinder) at an infinitive time</td>
</tr>
<tr>
<td>$A$</td>
<td>Total surface area of a film-shaped drug delivery system</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusion coefficient of a drug within a drug delivery system</td>
</tr>
<tr>
<td>$C_s$</td>
<td>Saturated drug solubility of a drug inside a drug delivery system</td>
</tr>
<tr>
<td>$C_{ini}$</td>
<td>Initial total concentration of a drug in a drug delivery system</td>
</tr>
<tr>
<td>$R$</td>
<td>Radius of a drug delivery system (sphere or cylinder)</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Porosity of a film-shaped drug delivery system</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Tortuosity of a film-shaped drug delivery system</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density of a drug material loaded in a film-shaped drug delivery system</td>
</tr>
<tr>
<td>$\rho_{IND}$</td>
<td>Density of solid indomethacin</td>
</tr>
<tr>
<td>$V$</td>
<td>Volume of a film-shaped drug delivery system</td>
</tr>
<tr>
<td>$h$</td>
<td>Half-thickness of a film shaped drug delivery system</td>
</tr>
<tr>
<td>$K_b$</td>
<td>Binding constant drug-NFC/xylan interaction</td>
</tr>
<tr>
<td>$\Delta H$</td>
<td>Enthalpy of drug-NFC/xylan interaction</td>
</tr>
<tr>
<td>$\Delta S$</td>
<td>Entropy of drug-NFC/xylan interaction</td>
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1 Introduction

European Pharmacopoeia defines an excipient (auxiliary substance) as any constituent of a medicinal product that is not an active substance. Pharmaceutical excipients have vital roles in drug formulations and dosage forms.

Recent trends in drug discovery are leading towards new chemical entities with high molecular weight and increased lipophilicity [1]. Thus, an increasing number of drug candidates suffers from low aqueous solubility and, therefore, requires specialized formulations in order to fulfill their potential. On the other hand biological drugs, such as peptides, proteins and monoclonal antibodies, often possess poor membrane permeability, enzymatic instability, large molecular size and hydrophilic properties, which represent significant challenges for the choice of right excipients for successful formulations. Besides facing the challenges seen in new drug candidates, pharmaceutical industry strives to improve manufacturing processes, reduce costs and improve the performance of existing products by employing proper excipients.

In the area of production of immediate release tablets, direct compression is preferred manufacturing process, in order to improve the manufacturing costs and productivity [2, 3]. Direct compression requires excipients with certain physical characteristics in terms of flowability and compressibility. Thus, the ideal excipient would provide excellent compactibility at low pressures, have a high dilution potential, improve the flowability of powder blend and, at the same time have low price.

Simultaneously, drug manufacturers are aiming towards excipients to improve the performance of controlled release formulations, which enable faster development and easy manufacturing. Here the specific focus exist in the development of long-lasting sustained release systems, where difficulties rise when they are administered by implantation considering high requirements in terms of sterility, biocompatibility, physical and mechanical properties and also desirable drug delivery profile.

All mentioned challenges explain the constant demand in pharmaceutical industry for development of novel excipients.

Cellulose and its corresponding neutral, acidic and basic derivatives have a long history as pharmaceutical excipients in various types of formulations [4]. Recently, microscopic cellulose fibers of plant origin have been disintegrated to the level of nanoscale fibers and the material has been termed nanofibrillar cellulose (NFC). The material has found application in various research areas due to its excellent mechanical properties. When it comes to pharmaceutical application, NFC has been recognized as material for modification of rheological properties [5], stabilization of nanosuspensions [6] as well as formation of nanoparticles embedded aerogels [7].

This thesis work focused on evaluation of NFC as a novel versatile excipient for the formulation of pharmaceutical dosage forms. Our aim was to investigate applicability of NFC in formulation of different dosage forms ranging from immediate towards controlled drug release systems.

Initial hypothesis was that spray dried NFC could be used as filler in production of tablets with immediate release. Fillers for tablet production have to fulfill certain criteria
in terms of powder characteristics. Therefore our aim was to characterize spray dried NFC in terms of powder properties (e.g. bulk, tapped and true density, flow properties) and compare it to two grades of commercially available and widely used microcrystalline cellulose. Further we aimed at manufacturing the tablets with immediate drug release by methods of direct compression and compression after wet granulation using spray dried NFC as a filler material.

The main goal of the thesis was to test the applicability of NFC as excipient for the production of controlled drug release systems, which would provide release of a drug over time period of up to three months or longer. In order to achieve this goal two different types of drug delivery systems were to be produced and tested. First, the spray dried microparticles which would provide prolonged drug release of drugs soluble in aqueous solvents and second, drug loaded thin films produced by filtration method, which would be suitable for sustained release of water insoluble compounds. In both cases, microparticles and films, systems would have matrix structure with NFC fibers being matrix former. Thus, the concept that NFC can be successfully used as an excipient for controlled drug delivery systems could be proven.
2 Review of the literature

2.1 Cellulose

Biopolymers (such as cellulose and its derivatives, starch, chitosan, etc.) are often used and studied as pharmaceutical excipients due to their availability, low toxicity, biodegradability and renewable nature. Among all biopolymers used in formulation of drug delivery systems, cellulose and its derivatives are the most widely used ones. Main sources for cellulose isolation are plant-based materials such as wood, cotton, and straw. In plants cellulose is constituent of the cell wall and it has a reinforcing role [8]. Besides plants, it is also synthesized by some bacterial species (Gluconacetobacter xylinus) [9], algae (Oocystis apiculata) [10], and tunicates (Microcosmus fulcatus) [11].

Understanding of the cellulose organization within plants is necessary for successful understanding of the properties and production processes of NFC. The same phenomena will affect their self-assembly and organization in manufactured products.

2.1.1 Cellulose supermolecular structure

Anselme Payen discovered and isolated cellulose from green plants in 1838 and suggested that cell walls of almost any plant are constructed of the same substance [12]. However, it was only in the 20th century that the structure of cellulose was resolved [13, 14]. Cellulose is composed of linear chains formed of β (1→4) linked D-glucopyranosyl units. Every second anhydrose glucose unit (AGU) is rotated 180° in the plane to facilitate preferred angle for creation of acetal bond between two neighboring glucopyranosyl rings. Thus, the two neighboring residues form the structural unit called cellobiose (Figure 1) [8].

![Figure 1](image)

Figure 1 The structure of cellulose. Two cellulose monomers (anhydroglucose units) build dimer (cellobiose).
The length of the cellulose chains (degree of polymerization, DP) varies greatly depending on the material source and could be in the range of 300-10000 [15], although already 20-30 AGUs give the material properties of cellulose [16]. Each glucose unit possesses three hydroxylic groups, two primary groups at the positions C2 and C3, and one secondary at the position C6. These hydroxylic groups are able to establish intra and intermolecular hydrogen bonds, thereby forming crystalline structures. Based on the molecular orientation and hydrogen bonding pattern, four different polymorphs of cellulose (and their allomorphs) have been identified: I, II, III and IV [17, 18]. Forms I and II are the most studied ones and the difference in their structure is shown in the Figure 2. The dominant hydrogen bond for cellulose I is the O6-H---O3, whereas for cellulose II it is the O6-H---O2. Furthermore, the chains in cellulose I are organized in parallel direction, whereas cellulose II has an antiparallel packing. Native cellulose from plant sources occurs in form I with two allomorphs Iα and Iβ [19], and their ratio depends on the material source. Iα is a metastable form and it consists of triclinic unit cells, while Iβ allomorph (which is predominant in higher plants) exhibits a monoclinic type of unit cells. Cellulose II is rarely found in the nature, but it can be obtained from cellulose I by regeneration or mercerization processes. Regeneration includes solubilization of cellulose I in a solvent, while mercerization involves only swelling of cellulose in sodium hydroxide. Both processes end by a reprecipitation step, in which the more thermodynamically stable cellulose II form is obtained. Cellulose forms III and IV are formed from cellulose I and II via different chemical procedures [20-22].

![Figure 2](image)

**Figure 2**  *Major supramolecular difference between cellulose I and cellulose II.*

During the process of wood biosynthesis, the cellulose chains are synthetized in a parallel fashion, which leads to hydrogen bonding between the neighboring chains. In native cellulose 30-40 glucan chains are assembled and merged into a single microfibril, denoted as elementary fibril. These microfibrils are not completely crystalline, but they show less ordered, amorphous regions, creating an irregular pattern of amorphous and crystalline domains. These elementary fibrils have been shown to be 2.5-4 nm in diameter and up to a few micrometers in length. The elementary fibrils are then aggregated to form
bundles with diameters in a range of 10-20 nm and further microscopic cellulose fibers. The scheme of cellulose hierarchical organization is shown in Figure 3. In the wood cellulose the microfibrils are surrounded by an amorphous matrix of hemicelluloses and lignin.

![Figure 3](image)

**Figure 3** Schematic drawing of cellulose organization in cell walls of plants showing its hierarchical structure (This picture was provided by Dr. Antti Laukkanen from the UPM company). The existence of amorphous and crystalline regions is shown in lateral fiber structure.

### 2.1.2 Hemicelluloses

In the plant cell wall of wood fibers the cellulose microfibrils are surrounded by an amorphous matrix of lignin and hemicellulose. Lignins are water insoluble amorphous polymers of phenylpropane units, which are removed during the pulp preparation. The lignin matrix is crosslinked with the cellulose fibrils by hemicellulose. In contrast to cellulose, the hemicelluloses are heteropolysaccharides, with their monomeric components consisting of anhydrohexoses. In hardwood, the major hemicellulose component is O-acetyl-4-O-methylglucurono-β-D-xylan (Figure 4), sometimes called glucuronoxylan. Depending on the hardwood species, the xylan content varies within 15-30% w/w of the dry wood. The backbone consists of β (1→4) linked D-xylopyranose units. About seven of ten xylose units contain an O-acetyl group at the C2 or C3. In addition, per ten xylose units there is on average one (1→2) linked 4-Omethyl-α-D-glucuronic acid residue.
2.2 Nanofibrillar cellulose (NFC)

The structure of native cellulosic fibers results in two families of nanocellulose materials. Since the cellulose microfibrils consist of both amorphous and crystalline regions, treatment of them in strongly acidic conditions leads to an extensive hydrolysis of the amorphous fractions and formation of short rod-like cellulose nanowhiskers with high crystallinity and low aspect ratio (Figure 5). The diameter of these whiskers is typically around 2-20 nm, while they have wide length distribution of 100-600 nm. Several terms are used in the literature to denote the whiskers, nanowhiskers [23-25], nanorods [26, 27], and rod-like cellulose crystals [28]. When the macroscopic cellulose fibers are mechanically disintegrated avoiding the strongly acidic conditions, long nanoscale partly amorphous fibrils are produced (Figure 5). In the literature terms such as cellulose nanofibers [29-33], cellulose nanofibrils [32, 33], microfibrillated cellulose [34, 35], nanocellulose fibers/fibrils [36, 37], or nanofibrillated cellulose [38-40] have been used to describe these fibers. This thesis defines nanofibrillar cellulose as elementary fibrils and fibril bundles with a very high aspect ratio, which have diameters in nanometer scale and lengths of up to several micrometers.

In the experimental part of the thesis short- and long-term drug delivery applications of the NFC were explored. Thus, the contents of the following literature review chapters focus more on the production, properties and selected application fields of NFC, excluding the nanofibrillar whiskers.
2.2.1 Production of NFC

Wood is the most commonly used source for NFC production. As explained above, cellulose possesses high level of organization within plant based materials, where nanoscale fibers are grouped and bound by hydrogen bonding to form macroscopic structures. To isolate NFC from wood, one has to break down its highly organized hierarchical structure. The first successful attempt to isolate NFC was reported in 1983 [41, 42]. Turbak and coworkers used multiple passing of softwood pulp through a laboratory homogenizer to produce the material, which they named microfibrillated cellulose. This method produced fibers with 10-100 nm in diameter [42]. Even though the authors were aware of the material’s potential at the time, immediate applications were not found due to the high energy consumption of the production method. NFC started getting full attention at the beginning of the 21st century when a series of cost-effective methods for its production from various plant sources was reported [43]. These methods utilize mechanical fibrillation using various devices, which is often combined with chemical or enzymathical pretreatment. The most commonly used techniques for mechanical treatment involve refining and high-pressure homogenization [5, 44-49], cryocrushing [50-53], and grinding [28, 29, 48, 54]. The main drawback of the mechanical disintegration techniques is high energy consumption, which can be as high as 70000 kWh/tonne [55]. To decrease the energy consumption, mechanical disintegration is preceded by certain pre-treatments, which can be alkaline [52, 53, 56, 57], oxidation (most commonly 2,2,6,6-tetramethylpiperidine-1-oxyl radical - mediated oxidation or so called TEMPO oxidation) [58-62] or enzymatic [5, 63-65] pre-treatment. NFC is nowadays available product from
various companies and research institutes (UPM, Finland; Rettenmaier & Söhne, Germany; Daicel, Japan; Innventia, Sweden).

Wood is undoubtedly the biggest source of cellulose for NFC production. However, non-wood plants such as corn, wheat, rice, sorghum, barley, sugar cane, pineapple, bananas and potato, and especially their by-products, can be valuable sources of natural fibers. Non-wood plants generally contain less lignin than wood and, therefore, the bleaching processes are less demanding [43]. Also cellulose microfibrils are less tightly bound in the primary cell wall in these plants than in the secondary wall in wood, thus fibrillation to produce NFC should be less energy demanding [66]. Further by-products of these plants are normally burned or used for production of low-value animal food. Thus, their usage as a source for NFC production is desirable from an environmental point of view. So far the production of NFC from soy hulls and wheat straw [50], sugar beet pulp [56, 61, 66], potato pulp [49], swede root [67], bagasse [68], sisal [69], cladodes of cacti [70], pear fruit [71], banana rachis [72], carrot [73], and bamboo [74] has been reported.

Production processes of NFC include steps, which purpose is to remove the accompanying materials (e.g. lignin). However, the final material contains certain amounts of hemicellulose residues and their contents can vary highly depending on the material origin and production method [75]. The presence of hemicelluloses, which carry carboxylic groups, causes a slight negative charge for the NFC. The negative charge causes repulsion between fibers preventing in this way their aggregation in wet state [76]. Thus, the presence of hemicellulose promotes dispersion of otherwise non-dispersible NFC fibers in an aqueous medium.

NFC used in the experimental work of this thesis was produced using bleached birch pulp as a starting material and a controlled homogenization process using an industrial fluidizer. A more detailed description of the material production is given later in the materials section.

2.2.2 Properties of NFC

2.2.2.1 Morphology

Depending on the source of the raw material, the initial cellulose fibers used for NFC production can have different properties - lengths, microfibril angles, and amounts of residual lignin and hemicelluloses, or even other characteristics, when they come from the sources originally denoted as waste. These different qualities followed by different production methods result in NFC materials with similar morphologies, but different characteristics in terms of dimensions (diameter and length) and hemicellulose content. Table 1 lists diameters of the NFC fibers depending on the type of the mechanical treatment and pre-treatment used for production using the wood as starting material. However, it is difficult to accurately compare fiber dimensions since the raw materials are different. In general, it seems that the application of pre-treatment step results in more
narrow size distribution and can also produce fibers with diameters as low as 3-5 nm [58, 77]. These very low values are also due to the centrifugation step used after the fibrillation process, which eliminates the larger NFC fibers.

Table 1. Diameter of the NFC fibers produced from wood sources applying different mechanical treatments and pre-treatments.

<table>
<thead>
<tr>
<th>Mechanical treatment</th>
<th>Pre-treatment</th>
<th>Diameter (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenizer</td>
<td></td>
<td>20-40</td>
<td>[78, 79]</td>
</tr>
<tr>
<td>Grinder</td>
<td></td>
<td>15-50</td>
<td>[28, 29]</td>
</tr>
<tr>
<td>Blender</td>
<td>TEMPO oxidation</td>
<td>3-5</td>
<td>[58, 77]</td>
</tr>
<tr>
<td>Microfluidizer</td>
<td>carboxymethylation</td>
<td>10-15</td>
<td>[81]</td>
</tr>
<tr>
<td></td>
<td>enzymatic</td>
<td>20-30</td>
<td>[5]</td>
</tr>
</tbody>
</table>

NFC fibers extracted from plant sources other than wood can have higher or lower dimensions. Thus, diameters in range of 10-80 nm for wheat straw and 20-120 nm for soy hulls as raw materials have been reported [50]. On the other hand, NFC fibers isolated from sisal [82, 83], carrots [73], beet pulp [46, 56], and Luffa cylindrical [84] had diameters of 20-65 nm, 3-36 nm, 30-100 nm, and 40-70 nm, respectively.

Hemicellulose and lignin residues also influence the fiber morphology. The raw material used for production of NFC normally contains lignin only in low quantities. However, studies of the effect of lignin content on the fiber dimensions have shown that the diameter of NFC fibers produced from lignin-containing pulp was larger regardless of the origin of the used pulp [85, 86]. On the other hand, the hemicellulose content correlates with smaller nanofiber dimensions, which suggests that hemicellulose limits the association between the cellulose nanofibers [66, 75, 87, 88].

It is generally very difficult to create a clear picture on the effect of all factors on fiber dimensions, since each material source is specific and production methods differ from one study to another.

NFC used in this thesis had a fibril width between 20 and 30 nm. A more detailed description of the material morphology and the methods used for its determination are given later in the materials section.

2.2.2.2 Degree of polymerization and tensile strength

Degree of polymerization (DP) for cellulose is reported to correlate strongly with the aspect ratios of the nanofibers; longer fibrils are associated with higher DP. Further mechanical treatment during NFC isolation affects the DP by decreasing its value with the increasing number of treatment cycles. Hence, mechanical isolation may result in about 30–50% decrease in DP [39, 63]. The DP is also correlated to the NFC tensile strength,
where high DPs lead to high values of tensile strengths [63]. The exact values of tensile strength have not yet been precisely determined. However, the strength of NFC fibrils have been estimated to be at least 2 GPa based on the results for kraft pulp, which contained 70-80% of NFC fibrils distributed in a parallel direction [89]. NFC also possesses high toughness due to the high values of elastic modulus of the cellulose. Elastic modulus of a perfect crystal of native cellulose is estimated at 130 GPa to 250 GPa [47, 90].

2.2.2.3 Rheological properties

Aqueous suspensions of NFC form a highly entangled network, which behaves as a pseudoplastic gel [5, 42] showing a large decrease in viscosity with an increasing shear rate, a phenomenon which is referred to as shear thinning [42]. With regards to the influence of fibrillation method, the viscosity of NFC increases with the number of passages through the homogenization equipment. The rheological properties enable the use of the NFC as thickener or stabilizer in suspensions or emulsions [34, 38] in many applications such as foods, paints, cosmetics and pharmaceuticals.

2.2.2.4 NFC in dry state

In this thesis, the production of NFC based drug delivery systems was based on the property of NFC to aggregate upon water removal creating highly porous, water insoluble structures. Thus, the facts necessary to fully understand the mechanism behind this behavior must be understood.

NFC is normally processed and stored as an aqueous suspension. This is done to avoid the agglomeration of the fibers during drying, caused by a phenomenon called hornification. The hornification defines irreversible changes in the natural structure of the fibers upon water removal [91]. It can be explained by an increased degree in cross-linking in the material microstructure due to the formation of a large number of hydrogen bonds between the hydroxyl groups of neighboring fibers [88, 92]. Since this process is irreversible once dried, the material cannot be resuspended in water. This property has been used for the production of different dried forms of NFC. Hence, NFC has been converted into dry state to form aerogels, films and powders.

When the water is removed from NFC gel by freeze-drying, highly porous (250-389 m²/g), flexible and deformable aerogels are formed [5, 93]. These kinds of gels have been studied for numerous applications (e.g. drug delivery, catalysis, filtrations, grafts, cushioning and liquid storage) [7, 93-96].

Diluted NFC suspensions have been converted to a dried powder form by spray drying [97, 98]. However, the powder form has still not found any applications. Thus, in this thesis, not only its usage as an excipient in pharmaceutical formulations, but its application in general, was tested for the first time.
The most studied dry forms of NFC are NFC films produced by filtration method [28, 48, 99, 100], molding [101, 102] or spin coating [81]. When water is removed by any of these methods, films with tight fiber networks of high strength are formed. These films are sometimes referred to as nanofiber paper or nanopaper [81, 99-101]. Depending on the diameter of the NFC fibers the films show different degree of transparency which can be as high as 90% [103, 104]. Their application is based on their high strength and good barrier properties [99, 100]. However, the high hydrophilicity of NFC limits wide utilization of films made of pure NFC in some areas (e.g. in packaging). Thus, composite-type films are often prepared by mixing NFC with other polymers [43, 44, 77].

2.2.3 Applications of NFC

The most studied application of NFC is its utilization as a reinforcement material in nanocomposite materials. Numerous studies have reported superior mechanical properties of materials after the incorporation of NFC [39, 43, 47, 53, 77, 105]. However, in this thesis work, the focus was on the utilization of NFC as a pharmaceutical excipient. Therefore, the following chapter will describe only selected biomedical applications of NFC.

2.2.3.1 Biomedical applications of NFC

In recent years, NFC of bacterial origin has been the most widely studied form of NFC in the biomedical applications. However, several studies have also explored possible applications of plant based NFC for these purposes. These applications are often based on the mechanical properties of NFC fibers. Mathew et al. [106] used NFC as material for the development of artificial ligaments and tendons. Partially dissolving NFC films in ionic liquids, they prepared cylindrical composites with mechanical properties similar or better than the natural ligaments and tendons. The composites also had excellent cytocompatibility required for biomedical applications. The same authors have also reported production of collagen based implantable scaffolds reinforced with the NFC [107]. They exploited the potential of NFC in providing mechanical reinforcement and dimensional stability to the newly created material without affecting the biocompatibility and non-toxicity of collagen. Eyholzer et al. [108] have created biocomposite hydrogels reinforced with carboxymethylated NFC for the replacement of nucleus pulposus in intervertebral disks. The hydrogels were prepared by UV polymerization of a mixture containing N-vinyl-2-pyrrolidone with Tween 20 trimethacrylate as a cross-linking agent and carboxymethylated NFC powder. They created hydrogels that mimicked the swelling and mechanical behavior of human nucleus pulposus. Cherian et al. [109] have produced polyurethane matrices that were reinforced with NFC for implantable purposes. They tested the application of the composites in design of prosthetic heart valves as well as vascular grafts.
Besides its utilization based on excellent mechanical properties, NFC has found applications in biomedical sciences due to its high porosity and rheological properties. Bhattacharya et al. reported utilization of native NFC hydrogel as a scaffold for 3D cell cultures [110]. They found the rheological properties of NFC gel to be suitable for mixing with the cells and its fluid-like behavior at high-shear stress facilitated injecting. The spontaneously formed gel after the injection provided the required mechanical support for cell growth and differentiation [110]. Further, they showed that NFC material was not cytotoxic.

Shimotoyodome et al. [111] studied the effect of intake of NFC (native and TEMPO-oxidized) on postprandial blood metabolic variables. NFC was orally administered with glucose and glyceryl trioleate to mice and blood levels of metabolic variables were followed over time. It was found out that the levels of blood glucose, insulin, glucose-dependent insulinotropic polypeptide, and triglycerides were significantly decreased, especially 10 min after the administration of TEMPO-oxidized NFC.

So far, the only applications of NFC in the field of drug delivery have been reported by Valo et al. [6, 7]. They showed that NFC can be successfully used for the stabilization of nanosuspensions [6]. This was achieved by nanoprecipitation of the drug in the presence of NFC and genetically engineered hydrophobin fusion protein (HFBI-DCBD), where the hydrophobin (HFBI) was coupled with two cellulose binding domains (CBD). The protein was utilized in order to assist binding of drug nanoparticles to NFC. The NFC nanostructure prevented the nanoparticles agglomeration significantly improving storage stability. Further, the nanoparticles embedded in NFC network preserved their morphology during the freeze-drying. In addition, the dissolution rate of itraconazole was significantly increased in vitro and in vivo due to the effect of nanosizing [6]. The concept of freeze-dried NFC networks as drug carriers was broadened and four different NFC aerogels with embedded drug nanoparticles were tested using NFC originated from different sources [7]. Drug release from the aerogels was immediate or sustained, depending on the structure and interactions formed by the nanoparticles and the cellulose matrix during freeze-drying. Thus, the NFC aerogel made from red pepper, and microcrystalline cellulose aerogel released the drug immediately, while bacterial cellulose, quince seed cellulose and TEMPO-oxidized birch cellulose aerogels showed sustained drug release. Both studies showed the potential of NFC to be used in many pharmaceutical nanoparticle applications and in controlled drug delivery.

The publications listed above represent a collection of all reported biomedical applications of plant NFC so far. Evidently, NFC still has not found wide utilization in this area, especially not pharmaceutical field. Thus, this thesis aimed at exploring the applicability of NFC as an excipient in the formulation of pharmaceutical dosage forms.
2.3 Cellulose derivatives as pharmaceutical excipients

Cellulose and its derivatives are widely used as pharmaceutical excipients. Microcrystalline cellulose (MCC) is used as tablet binder and diluent in wet granulation or in direct compression, and as a tablet disintegrant, anti-adherent or capsule diluent. Powdered cellulose is available in several grades and is used as binder and disintegrant. Ethylcellulose is hydrophobic, inert, stable, cellulose derivative, frequently used in preparing sustained release dosage forms either as a tablet matrix-former material or film forming material [112-114]. Pure cellulose is not soluble in water due to its crystalline structure but, by incorporating substituents along the cellulose chains, the crystalline structure breaks down and cellulose derivatives such as hydroxypropyl (HPC), hydroxypropylmethyl (HPMC), and carboxymethyl cellulose (CMC) become water soluble. HPMC has found applications, depending on the molecular weight, in tablet film coating as well as rate controlling polymers in extended release hydrophilic matrices [115, 116]. Carboxymethylcellulose is a typical acidic cellulose derivative and is used as emulsifier, gel-former, but also as binding agent or disintegrant in tableting [117, 118].

Among all cellulose derivatives MCC could be recognized as the most commonly used as excipient in pharmaceutical formulations. It has been widely used as filler for tablet production and according to the survey conducted within the pharmaceutical industry it is considered the most useful filler for direct compression [119]. In this thesis, application of NFC as a filler for tablet production was studied and compared to MCC due to their physico-chemical similarities. Thus, the following chapter more closely describes the application of MCC in tablet manufacturing.

2.3.1 Microcrystalline cellulose as filler for tablet production

Microcrystalline cellulose is manufactured by controlled hydrolysis of dilute mineral acid solution of cellulose, obtained as a pulp from fibrous plant materials. Following hydrolysis, the hydrocellulose is purified by filtration and the aqueous slurry is spray-dried to form dry, porous particles of a broad size distribution. Considering this, MCC and NFC are chemically identical and they also utilize the same method of conversion of an aqueous slurry to the powder form (spray drying). Thus, it could be expected that spray dried NFC could be utilized for the same purpose as MCC when formulating tablets as dosage form. MCC has been employed as a tableting excipient in pharmaceutical manufacturing for more than 30 years [119].

A material should fulfill certain criteria in terms of powder characteristics in order to be successfully used as tablet filler. It should: i. possess good flowability to ensure uniformity of tablet weight, ii. be readily mixed with other ingredients without showing signs of segregation to ensure homogeneous mixtures and obtain tablets with an acceptable uniformity, iii. have suitable compression pressure – tablet strength profile to produce the tablets with high physical strength when applying low compression forces, iv. possess high dilution potential so that a high amount of another ingredient can be mixed with it while still obtaining tablets of acceptable quality, v. be physically and chemically
stable and inert with regard to moisture, air, and heat as well as active ingredients or other excipients, and vi. possess a known mechanism of consolidation during compression so that it complements the properties of an active ingredient. This thesis evaluated spray dried NFC in terms of some of the mentioned properties in order to estimate its potential as tablet filler and also in order to compare those properties of NFC to MCC. The popularity of MCC as tablet filler can be ascribed to its fulfillment of the majority of the above mentioned criteria. MCC is well known to deform in a predominantly plastic manner during the compaction. In general, early stages of compaction are characterized by particle rearrangement, break down of the particles agglomerates, and deformation or breaking of the granules for granulated powders. The density of the die content is increased and when the particles are close enough interparticulate bonds are created. Further increase in the pressure causes the change in particle shape and some type of deformations including plastic deformation or breakage of particles, consolidation of particle segments and bond formation between them. When the load is removed in decompression step, some particles are able to regain their original shape (elastic deformation), while other ones have experienced permanent deformation (plastic deformation) (Figure 6 A) [120]. Thus, the plastic deformation has been defined as a permanent change in the shape of a specimen due to the applied stress. This kind of deformation aids interparticulate bonding, because it increases the contact area between particles. In this way, strong tablets are prepared. The force required for initiation of plastic deformation is denoted as yield stress. On the other hand brittle particles undergo fragmentation when fracture occurs by the rapid crack propagation throughout the specimen crushing original particle in smaller units (Figure 6 B) [120, 121].

![Figure 6](image)

**Figure 6** Plasticity, elasticity and fragmentation in powder system during compression (A) and stress strain curve showing the behavior of a material under the stress (B).
A single particle may undergo several of these deformation stages during the compression process. Some materials mainly consolidate by plastic deformation (microcrystalline cellulose, mannitol, starch, sodium chloride) and some mainly by fragmentation (lactose, calcium hydrogen phosphate, sucrose), but all materials possess both elastic and plastic behavior depending on the applied force [121]. In the case of MCC, hydrogen bonding plays an important role in compact hardness [122, 123]. Hydrogen bonding is important because significant plastic deformation during compression brings an extremely large surface area into close contact and facilitates hydrogen bond formation between the plastically deformed, adjacent cellulose particles [124]. Considering that NFC also possesses hydroxylic groups capable of building hydrogen bonds it could be expected that similar phenomena takes place when NFC is used as tablet filler. Apart from the chemical similarity of MCC and NFC, certain degree of difference in terms of particles size and morphology, moisture content as well as crystallinity could be expected. It has been shown that these factors can influence the properties of the produced tablets [122, 123, 125-128]. Hence, certain differences in compression behavior of MCC and NFC were expected.

One of the aims of the thesis was to show if NFC could be used as tablet filler, on the other hand the main goal was to further explore its potential as pharmaceutical excipient in creation of controlled drug delivery systems. Thus, the following chapter focuses on discussion of the most important types of controlled drug delivery systems and mechanisms of drug release from those systems.

### 2.4 Controlled drug delivery

Periodic administration of a drug by conventional means, such as taking a tablet every four to eight hours or injecting a drug intravenously or subcutaneously, results in constantly changing systemic drug concentrations in the blood stream. This often produces a sharp initial increase in drug concentration to the level above the therapeutic range and it is followed by fast decrease in drug concentration below the therapeutic range [129] (Figure 7). Drug delivery systems (DDS) with controlled release attempt to maintain drug concentrations in the therapeutic level over a certain period of time. Thus, they offer several advantages over immediate release systems, including precise control of dose, decreased number of dosages, reduction in harmful side effects, and improvement in patient compliance and convenience.
A number of advancements have been made in the past 30 years in the development of new techniques for controlled drug delivery. These techniques are capable of regulating the rate of drug delivery, sustaining the duration of therapeutic action, and/or targeting the delivery of drug to a specific tissue [130, 131]. Based on the technical complexity, the DDSs with a controlled drug release that have been marketed so far, or that are under active development, can be classified as follows [132]:

- **Rate-preprogrammed drug delivery systems** - release of drug molecules from the delivery systems has been preprogrammed at a specific rate profile. This is accomplished by system design where the drug release is controlled by several physical and chemical phenomena and their combinations, e.g., wetting of the system’s surface with water, water diffusion in the system, drug dissolution, drug diffusion, polymer swelling, polymer dissolution, and/or polymer degradation, physical drug-excipient interactions, etc. [133, 134]. If several processes take place simultaneously, the one which is much slower than all the others is considered as dominant and needs to be taken into account for the quantitative description of the overall drug release rate from the device [133]. Thus, depending on the predominant/limiting process, the rate-preprogrammed DDSs can roughly be classified as:
  - Diffusion controlled DDS (reservoir and monolithic systems),
  - Water penetration controlled DDS (osmotic and swelling systems)
  - Chemicaly controlled DDS (biodegradable systems and ion exchange resins).
• **Activation-modulated drug delivery systems** - release of a drug takes place when triggered by specific internal or external stimuli, such as changes in pH [135-137] or temperature [138, 139], exposure to ultrasound [140], enzymes [141, 142] or light [143], or changes in electric [144, 145] or magnetic fields [146].

• **Feedback-regulated drug delivery systems** - ability to monitor the chemical environment and rapidly detect and appropriately respond to a biological event by changing the rate of release of a therapeutic agent [147].

• **Site-targeting drug delivery systems** – ideally have ability to deliver a drug exclusively to the targeted cells or cellular components thus offering the best controlled mechanism [148].

### 2.4.1 Diffusion controlled drug release

Diffusion is the most important mechanism used to control the drug release from DDSs [149]. Depending on the inner structure, the diffusion controlled DDSs are usually classified as reservoir type DDSs, which comprise “core-shell structure”, in which the drug is physically separated from the rate-controlling membrane, and monolithic DDSs, where the drug is homogeneously distributed within the matrix (Figure 8). Both the reservoir and monolithic DDSs can be further classified depending on the initial drug loading compared to the drug’s solubility.

#### 2.4.1.1 Reservoir systems

When talking about reservoir DDSs, it is possible to distinguish between “non-constant activity source” and “constant activity source” systems (Figure 8) [133, 150]. In non-constant activity source systems, the concentration of a drug within the system is below its solubility (e.g. coated pellets or tablets with a low drug loading). Thus, the drug concentration in the reservoir is decreasing over time. Drug release from these types of systems follows first order kinetics regardless of the geometry of the device given that the membrane does not dissolve or swell, the diffusion coefficient remains constant and sink conditions are provided [134]. In constant activity source systems, the drug concentration within the device exceeds the drug solubility. This results in the drug dissolution from the “depots” of amorphous or crystalline solid substance keeping the drug concentration constant over the time. As long as the excess of drug is present, zero order release kinetics is achieved regardless the system geometry, given the same conditions as mentioned above. Once the excess drug is dissolved and the concentration within the reservoir falls below drug solubility, the system becomes a non-constant activity source [134].
Figure 8  Classification system for primarily diffusion controlled drug delivery systems. Stars represent individual drug molecules, black circles drug crystals and/or amorphous aggregates. Only spherical dosage forms are illustrated, but the classification system is applicable to any type of geometry [134].

2.4.1.2 Monolithic systems

Similarly to drug delivery reservoirs, the monolithic systems can be classified based on the initial drug loading/drug solubility ratio. Here we can make distinction between monolithic solutions and monolithic dispersions (Figure 8) [133].

**Monolithic solutions**

The system is considered a monolithic solution if the initial drug concentration is below drug solubility, thus, the drug is molecularly dispersed in the matrix former or if the drug is rapidly completely dissolved upon water penetration into the system [150]. Drug release kinetics from monolithic solutions is significantly dependent on the system geometry. In this thesis work we produced systems which could be described as monolithic dispersions. Hence, the equations for the calculation of drug release from monolithic solutions are not presented here but later in the experimental part of the thesis.

**Monolithic dispersions**

The system can be classified a monolithic dispersion if the drug is homogeneously distributed within the matrix former in the form of crystalline and/or amorphous particles.
meaning that the initial concentration is above drug solubility (in the wetted state) [133]. When this type of a system is exposed to an aqueous medium, the drug in the outermost layer starts to dissolve and becomes available for diffusion. Once the drug from that layer has completely dissolved, the same process starts in the adjacent layer. Thus, the dissolved and non-dissolved drug fractions co-exist within the matrix. The drug release kinetics from these systems is also highly dependent on the geometry (Figure 9). Often the exact mathematical description of drug release can be rather complex. However, in case of thin films with the release from both faces and negligible edge effects (slabs), a quite simple equation has been derived 50 years ago by Takeru Higuchi [151, 152]:

\[
M_t = A \sqrt{D C_s (2C_{ini} - C_s) t}
\]

where \(M_t\) is the cumulative absolute amount of drug released at time \(t\); \(A\) is total surface area of the film exposed to the release medium; \(D\) is the diffusion coefficient of the drug within the system; \(C_s\) is the solubility of the drug in the wetted matrix (not in the release medium), and \(C_{ini}\) is the initial total concentration of the drug in the system. When applying the Higuchi equation, one should keep in mind that the equation is valid only in a case when: i. the drug transport through the matrix is the rate limiting step (while drug transport in release medium and water penetration into the system are rapid), ii. the dissolution of the drug particles within the system is rapid compared to the diffusion of the dissolved fraction, iii. perfect sink conditions are provided, iv. the initial drug concentration in the system is much higher than the drug solubility in the wetted system, v. the drug is homogeneously distributed and finely dispersed (the size of the particles is much smaller than thickness of the film), vi. the diffusion coefficient of the drug within the system is constant, and vii. the system does not swell or dissolve during the release.

![Figure 9](image-url)

**Figure 9** Overview on the mathematical equations, which can be used to quantify drug release from monolithic dispersions (initial drug concentration > drug solubility). The variables are explained in the text [150].
The best solutions to quantify drug release from monolithic dispersions with spherical and cylindrical shapes so far were offered by Baker and Lonsdale [153]:

\[
\frac{M_t}{M_\infty} - \frac{3}{2} \left[ 1 - \left( \frac{M_t}{M_\infty} \right)^{2/3} \right] = \frac{3D}{R^2 C_{\text{ini}}} t \quad \text{Spheres}
\]

where \( M_t \) and \( M_\infty \) are the cumulative amounts of drug released at time \( t \) and at infinite time, respectively; \( D \) is the diffusion coefficient of the drug within the system; \( C_s \) is the drug solubility in the wetted matrix (not in the release medium); \( C_{\text{ini}} \) is the initial drug concentration in the system, and \( R \) is the radius of the sphere.

\[
\frac{M_t}{M_\infty} + \left( \frac{M_t}{M_\infty} \right) \ln \left[ 1 - \frac{M_t}{M_\infty} \right] = \frac{4D}{R^2 C_{\text{ini}}} t \quad \text{Cylinders}
\]

where \( R \) is the radius of the cylinder.

This chapter gave an overview on main types of controlled drug delivery systems and mechanism of drug release from those systems. Considering that in the most cases different types of polymers are used for formulation of those systems the following chapter will focus on polymers and their application in formulation of DDSs with long-lasting drug delivery.

### 2.4.2 Materials for long-lasting parenteral drug delivery systems

The main aim of this thesis was to produce systems based on NFC as matrix former which would sustain release of a drug for long time period, preferably up to three months or longer. The idea originated from the existence of a continuous demand for new sustained release drug delivery techniques, which could be utilized for production of systems such as transdermal patches, intraocular devices or gynaecological devices such as intravaginal rings, intrauterine devices or implants for subcutaneous delivery. The duration of drug release from these systems can range from 24 h to 1 week for transdermal patches, 1 month to 28 months for intravaginal rings or 6 months to 1 year for implants.

The number of currently available polymers which could provide the needed duration of therapy is limited. It should also be taken into account that polymers whose mechanism of controlling drug release is based on swelling are not suitable for these purposes. Further biodegradable polymers (e.g. poly(lactide-co-glycolide)) are known to have disadvantages such as inter-patient variance, inability of system removal in the case of need for therapy discontinuation. Besides this, the design of some of the mentioned systems (intravaginal rings or intrauterine devices) would be rather complicated when biodegradable polymers are used due to the necessity of the systems to keep their shape and mechanical properties.
during the whole duration of therapy. Thus, only a small number of non-biodegradable, non-swelling polymers are available for the design of such long-lasting drug delivery systems. Nowadays, the most popular materials for this purpose are poly(ethylene-co-vinylacetate) (EVA) and silicone elastomers such as poly(dimethylsiloxane) (PDMS). Good example of the products present on the market which utilize EVA is the intravaginal contraceptive ring Nuvaring® (developed by Organon), which provides sustained release of etonogestrel and ethinylestradiol for a period of three weeks. The same material has been used for the production of the contraceptive implantable rod Implanon® (developed by Organon), which delivers progestin etonogestrel for a period of 3 years. PDMS has also been successfully employed in the products widely available on the market. The subcutaneous implant Jadelle® (developed by the Population Council and manufactured by Leiras Inc., nowadays Bayer Inc.) is used for the sustained release of levonorgestrel for a time period of 5 years. Another good example of successful application of PDMS is the intrauterine device Mirena® (developed by the Population Council in the late 1980s and later with Schering Inc., nowadays Bayer Inc.) which comprises of a T-shaped backbone of PDMS with a drug delivery cylinder wrapped around its stem. The cylinder is a PDMS/levonorgestrel mixture that allows a steady release of levonorgestrel through regulating surface membrane for period of 5 years.

From the examples above it is clear that intravaginal rings and implants have, to date, been primarily developed for the systemic delivery of contraceptive steroids and the localized and systemic delivery of steroids for hormone replacement therapy. However, it is likely that they will, in the future, be exploited for a much wider range of applications within women's health care in general. This brings to discussion the limitations of materials currently used for the production of the devices. The delivery of the drugs from the mentioned materials is based on the diffusivity of steroid compounds due to their high solubility in the hydrophobic material and the relatively low molecular weight/volume. Therefore, the currently used materials are not suitable for the delivery of hydrophilic and/or high molecular weight compounds. Furthermore, all the currently used materials are thermoplastic and the devices are often produced by extrusion or injection molding. Both the processes require relatively high temperatures which makes them unsuitable for incorporation of thermosensitive drugs. Furthermore, silicone elastomers require a curing step to achieve the desirable mechanical properties and the curing process is performed at an elevated temperature. All the mentioned limitations lead to conclusion that new, more versatile materials for long lasting drug delivery are needed. Thus, the aim of this thesis was to test application of NFC for these purposes.
3 Aims of the study

The aim of this thesis was to evaluate the potential of NFC to be used as an excipient in the production of pharmaceutical dosage forms, especially 1. as an excipient for immediate release tablets and compressions, and 2. for providing sustained drug release profiles from microparticle and film-type controlled release systems.

The specific objectives of this study were:

1. To study the applicability of NFC as a novel excipient for the production of tablets with immediate drug release properties. The specific goal was to investigate the use of spray dried NFC as a filler in tablet production in direct compression and wet granulated formulations (I)

2. To investigate the applicability of NFC in production of spray dried drug loaded microparticles which will act as long-lasting (several months) drug release systems (II)

3. To investigate preparation, drug-loading capability and sustained release properties of drug/NFC film-like matrix systems aiming at the drug loadings in the range of 20% – 40% and long-lasting drug release (up to three months) (III)

4. To study physical and chemical interactions between the NFC fibers and model drugs, peptides and proteins with different molecular properties (structure, molecular weight/size, charge) (IV)
4 Experimental

Complete details of the suppliers of the materials, production and analytical methods and equipment used in this work can be found in the original publications (I-IV).

4.1 Materials

4.1.1 Nanofibrillar cellulose (I-IV)

The nanofibrillar cellulose was produced and kindly donated by UPM-Kymmene Corporation, Finland. Bleached birch pulp was used as the NFC source. Purified pulp fibers were diluted with sterilized, ultra high quality water before the fibrillation. Fibers isolation was conducted via controlled homogenization process using an industrial fluidizer. The applied process resulted in the production of nanofibrillar cellulose hydrogel with the fiber content typically 1.7 wt%. Analysis of the NFC product with an electron microscope revealed that the most common fibril width is between 20 and 30 nm (Figure 10). The exact length scale cannot be estimated due to the entangled and bundled nature of the material. Detailed description of the fibers size distribution has been presented elsewhere [154]. The product also contains some soluble hemicelluloses, i.e. 23 wt% of xylan, due to the birch based raw material.

Figure 10  SEM image of NFC hydrogel (A), the scale bar 100 nm, fibril width distribution measured manually from FE-SEM image (B) (This picture was provided by Dr. Antti Laukkanen from the UPM company)
4.1.2 Model compounds (I-IV)

The list of the model drug compounds used in the studies and their most important physicochemical properties are given in Table 2.

**Table 2. List of the model drug compounds, their aqueous solubilities, \(pK_a\) values, and molecular weights.**

<table>
<thead>
<tr>
<th>API</th>
<th>Solubility in aqueous media</th>
<th>(pK_a/pI)</th>
<th>Molecular weight (g/mol)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>17.39 mg/ml</td>
<td>9.5</td>
<td>151.2</td>
<td>[155, 156]</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>&lt; 1 mg/ml</td>
<td>6 mg/ml pH=7.4</td>
<td>5.2</td>
<td>206.3</td>
</tr>
<tr>
<td>Nadolol</td>
<td>6.77 mg/ml</td>
<td>9.4</td>
<td>309.4</td>
<td>[159]</td>
</tr>
<tr>
<td>Atenolol</td>
<td>26 mg/ml</td>
<td>9.6</td>
<td>266.3</td>
<td>[160, 161]</td>
</tr>
<tr>
<td>Metoprolol tartrate</td>
<td>freely</td>
<td>9.5</td>
<td>684.8</td>
<td>[162, 163]</td>
</tr>
<tr>
<td>Verapamil hydrochloride</td>
<td>70 mg/ml</td>
<td>8.9</td>
<td>491.1</td>
<td>[164-167]</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>6 (\mu g/ml) MQ</td>
<td>13 (\mu g/ml) pH=5</td>
<td>4.4</td>
<td>357.8</td>
</tr>
<tr>
<td></td>
<td>302 (\mu g/ml) pH=7.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>&lt;1 (\mu g/ml)</td>
<td>3.7</td>
<td>705.6</td>
<td>[171]</td>
</tr>
<tr>
<td>Beclomethason dipropionate</td>
<td>&lt;1 (\mu g/ml)</td>
<td>15.6</td>
<td>521.1</td>
<td></td>
</tr>
<tr>
<td>Nafarelin acetate</td>
<td>&gt;10 mg/ml</td>
<td>5.9/9.9/12.5</td>
<td>1322.5</td>
<td>[172]</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>&gt;10 mg/ml</td>
<td>11.1</td>
<td>14300.0</td>
<td>[173, 174]</td>
</tr>
</tbody>
</table>

4.2 Production techniques

4.2.1 Tableting (I)

Spray dried NFC powder was compared to two commercially available grades of microcrystalline cellulose (MCC); Avicel PH 101 and Avicel PH 102. MCC was chosen for the comparisons due to its similarity to NFC in chemical structure, and Avicel PH 101 and Avicel PH 102 are the two most commonly used grades of MCC. Determination of the powders’ main physical characteristics was done as well as examination of their compression behavior. Tablets were compressed from both dry powder mixtures and granules. For direct compression studies, dry powder mixtures were prepared by mixing the ingredients using a Turbula blender, (System Schatz, Willy A. Bachofen AG, Switzerland) for 2 minutes. For compression after granulation, granules were produced manually (using mortar and pestle) by a wet granulation process. Tablet compression was performed using an instrumented single punch tableting machine (Korsch EK-0, Erweka...
Experimental

Apparatebau, Germany) equipped with flat-faced 9 mm punches. The adjustments of the tablet machine were kept constant in all compressions in order to ensure the same speed (37 rpm) of the upper punch and the even thickness of the tablets in all cases. The powder die was filled manually with gradually increasing amounts of powder/granules to provide desirable increase in compression force.

4.2.2 Spray drying (II)

Suspensions for the spray drying process were prepared by dissolving a drug in a suitable solvent (MQ water or 50 mM aqueous NH₄OH). The solutions were then mixed with NFC suspension so that the total concentration of the both, dissolved and suspended material, was 0.5%. The prepared suspensions were sonicated for 15 min using a high intensity ultrasound processor (Sonics, Newtown, USA) equipped with a 13 mm probe, and then mixed with a mechanical stirrer for 15 min at a speed of 1800 rpm. The spray drying was performed using a Mini spray dryer Büchi B-191 (Büchi, Switzerland). The operating parameters were set as following: inlet temperature 220 °C, outlet temperature in a range from 120 – 127 °C, spray flow 700 l/h, air pressure 7 bar, aspirator setting 95% and pump setting 18% (corresponds to approx. 7 ml/min). The spray dried microparticles were collected from the dryers’ collection vessel and stored in closed vials at room temperature. The spray dryer was equipped with a two-fluid nozzle and operated in a co-current mode (the feeding suspension and the drying air flow in the same direction).

4.2.3 Drug-loaded NFC films production (III)

Drug loaded NFC matrices were produced using water insoluble model drug compounds. The chosen drugs were mixed with a highly viscous NFC suspension and the mixtures were sonicated for 2 min using a high intensity ultrasound processor (Sonics, USA) equipped with a 2 mm stepped microprobe adjusted at power of 750 W and 20 kHz frequency. The prepared mixture was diluted with water at 1:1 ratio to make pipetting of the highly viscous mixture easier, and to have an even distribution during the filtration process. The suspension was then filtered through a PVDF membrane filter with 0.2 µm pore size. During the filtration process, the water insoluble drug particles remained on the filter and were entrapped within a network of fine cellulose fibers. After the filtration, the wet matrices were dried in an oven for 4 h at a 65 °C.
4.3 Characterization techniques

4.3.1 Drug interactions with NFC fibers (IV)

Interactions between the model drug compounds and NFC fibers, as well as xylan, were studied by following thermodynamic changes during the binding events. This was done using an isothermal titration calorimetry technique (ITC). Calorimetric experiments were performed using a Microcal Isothermal Titration Calorimeter (MicroCal, MA). A sample cell (with a volume of 1.8 ml) was filled with either aqueous NFC suspension or aqueous xylan solution. The xylan concentrations were chosen to match the concentration of xylan present in NFC suspension used for titrations. The experiments were performed at 25 °C by injecting 10 µl of aqueous drug solutions to the sample cell which was stirred at 300 rpm. The observed released heat included the heat of the drug binding to the NFC fibers and the heat of dilution of the drugs to the water. To determine the heat of dilution, blank titrations were made by injecting the drug solutions into the sample cell filled with MQ water. The differential enthalpy curves of heat of dilution were then subtracted from the curves of the binding of drugs to the NFC fibers. Data analysis was performed using Microcal ORIGIN software. Titrations were also performed using a genetically engineered fusion protein (HFBI-DCBD) as a positive control, since its affinity and capacity of binding for NFC has already been reported [175].

4.3.2 Characterization of produced drug release systems (I-III)

4.3.2.1 Tablet characterization (I)

Produced tablets were characterized in terms of their thickness, mass and mechanical properties. The thickness of the tablets was measured 24h after the compression and the obtained values were used to calculate the tablet porosity. The Heckel plots were then constructed by plotting the natural logarithm of the inverse of the tablet porosities as a function of the compression pressures. Regression analysis was performed on the linear portion of the curve. The slope values obtained were converted to yield pressures ($P_y$) using the relationship: $P_y = 1/\text{slope}$.

4.3.2.2 Electron microscopy (I-IV)

Morphology of the starting material for compression (I), spray dried drug-loaded microparticles (II), drug loaded NFC films (III) and plain NFC films (IV) were studied by scanning electron microscope (SEM) Zeiss DSM 962 (Carl Zeiss, Germany) and FEI Quanta™ FEG (FEI, Oregon, USA). For the powder imaging (I-II), the dried powder
samples were fixed onto a two-sided carbon tape. For the imaging of drug-loaded NFC film (III) the samples were fractured manually or cut with a scalpel to study the morphology of films cross-section. For the imaging of plain NFC films (IV), the samples were cryo-fractured using liquid nitrogen for studying the inner structure. All the samples were sputtered with platinum for 25 seconds with an Agar sputter device (Agar Scientific Ltd., UK).

4.3.2.3 Solid state analyses (II-III)

Thermal analyses of the spray dried drug-loaded NFC microparticles (II) and drug-loaded NFC films (III) were performed by differential scanning calorimeter (DSC) Mettler Toledo DSC 823e (Mettler Toledo, Switzerland). The samples were placed in sealed aluminum pans with pierced lids and held at 25°C for 5 min before heating. The experiments were conducted at a heating rate of 10 °C/min in the temperature range between 25°C and 200°C or 250°C depending on the sample. The data was analyzed with STARe software (Mettler Toledo, Switzerland).

Physical state of the drugs within the drug-loaded NFC films (III) was estimated by X-ray powder diffraction (XRPD) using a theta-theta X-ray diffractometer (D8 Advance, Bruker AXS GmbH, Germany). The measurements were performed in symmetrical reflection mode with Cu Kα radiation (λ=1.54Å) using a Göbel mirror. The angular range was 5° to 40°. The measurements were made with the steps of 0.02°, and the measuring time was 2 s/step.

4.3.2.4 Dissolution studies (I-III)

Dissolution tests for the tablets (I) were performed using a paddle type dissolution apparatus (Erweka DT-D6, Germany) with a rotation speed of 50 rpm and 900 ml of purified water at 37±0.5°C as a medium. After each sample (500 µl), the volume was replaced by the same amount of medium. The samples were filtered through 0.45 µm filters and analyzed using UV spectrophotometer (Pharmacia LKB Ultrospec III, Sweden).

Dissolution tests for the drug-loaded spray dried particles were performed after the surface unbound fraction of the drug had been removed. The surface fraction was washed and quantified by placing 40 mg of the microparticles on a hydrophilic polypropylene membrane with a pore size of 0.2 µm and washing with 400 ml of the medium using a vacuum filtration system. After this step the samples were transferred to 50 ml glass bottles with 10 ml of medium. The bottles were placed into a shaking water bath (Julabo SW 23, Germany) equipped with a tray for the Erlenmeyer flasks. Shaking frequency was set at 100 min-1. At various time points the total volume of dissolution medium was replaced by fresh medium. This was done by filtering the particles from the dissolution medium and resuspending them in the same volume of fresh medium. The amount of the released drug was quantified by a suitable HPLC method (Agilent 1100 series, CA, USA).
Dissolution tests for the drug-loaded NFC films were also performed after the fraction of the drug located on the film surface was removed. For the determination of surface fraction, pieces of the films (4.5 mg) were cut and placed into vessels of the standard dissolution equipment (Sotax, Switzerland, paddle method, Ph. Eur. 7th) that contained 400 ml of a medium. The samples were left in the medium overnight with a paddle rotation speed of 60 rpm and then transferred to 50 ml glass bottles with 25 ml of medium and placed into a shaking water bath (Julabo SW 23, Germany) equipped with a tray for the Erlenmeyer flasks. Shaking frequency was set to 100 min⁻¹. Samples of the medium were taken at various time points and analyzed by a suitable HPLC method. The total amount of medium (25 ml) was exchanged with a fresh medium after every sampling.
5 Results and discussion

5.1 NFC as a tableting excipient (I)

5.1.1 Physical characterization of the spray dried NFC

Spray dried NFC particles were characterized in terms of their main physical properties relevant for the potential application as a novel filler in tablet production. Its main characteristics were then compared to those of two commercially available grades of microcrystalline cellulose (MCC): Avicel PH 101 and Avicel PH 102. The commercially available MCCs show broad particle size distributions with main diameters of 50 µm for Avicel PH 101 and 100 µm for Avicel PH 102. Spray dried NFC had more uniform, smaller particles which were 5 - 10 µm in diameter (Figure 11). A difference in surface morphology was also apparent with irregularly shaped MCC particles versus more spherical, porous NFC particles formed by the web of fibrils.

Figure 11  SEM images of Avicel PH 101 with ×1,000 magnification; scale bar 20 µm (a), spray-dried NFC with magnification ×2,000; scale bar 20 µm (b), and ×10,000; scale bar 2 µm (c)

The particle size and spherical shape play important roles in powder flow, thus resulting in better flowability of the NFC than Avicel PH 101 and also improved flow properties of Avicel PH 102 with the addition of NFC (Figure 12).

Figure 12 Flow properties of Avicel PH102 (closed circle) and mixtures containing Avicel PH102 and 10% (open square), 20% (closed triangle), and 30% (open circle) of NFC
5.1.2 Direct compression studies

For the direct compression studies, tablets were prepared of pure NFC powder as well as of the mixtures of NFC and MCC. The Heckel plots were constructed and values of yield pressure calculated (Table 3) in order to gain more knowledge on the material deformation properties during the compression process. Ductile materials which undergo plastic deformation during the compression process are characterized by a dominant linear region in the Heckel plots. The slope of the Heckel plots and, consequently, the values of yield pressure are a measure of the degree of material plasticity, with steeper curves and lower values of yield pressure indicating a more pronounced ductile component.

Table 3. Regression analysis of mechanical properties of Avicel PH 102, spray-dried NFC, and their mixtures (A2 and C denote Avicel PH 102 and NFC, respectively, and A2C1, A2C2, and A2C3 mixtures containing Avicel PH 102 and 10%, 20%, and 30% of NFC, respectively).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Compression pressure range (MPa)</th>
<th>R²</th>
<th>Slope</th>
<th>Yield pressure P_y (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>25-76</td>
<td>0.9935</td>
<td>0.0133</td>
<td>75</td>
</tr>
<tr>
<td>C</td>
<td>30-97</td>
<td>0.9984</td>
<td>0.0090</td>
<td>111</td>
</tr>
<tr>
<td>A2C1</td>
<td>25-72</td>
<td>0.9905</td>
<td>0.0128</td>
<td>76</td>
</tr>
<tr>
<td>A2C2</td>
<td>25-72</td>
<td>0.9920</td>
<td>0.0131</td>
<td>76</td>
</tr>
<tr>
<td>A2C3</td>
<td>25-72</td>
<td>0.9973</td>
<td>0.0131</td>
<td>76</td>
</tr>
</tbody>
</table>

It has been shown that MCC experiences a plastic deformation during the compression process [122-124, 176]. Values of the slope of the Heckel plots lead to a conclusion that NFC has less pronounced ductile characteristics. These findings were further supported by the values of yield pressure. The values of yield pressure lower than 80 MPa indicates a plastic behavior of the material, while higher values imply brittle characteristics [177]. Thus, compared to MCC, NFC has more pronounced brittle characteristics and its deformation is characterized by particle fragmentation.

Figure 13 Effect of compression pressure on the tensile strength of tablets made of Avicel PH102 (closed diamond), spray dried NFC (open circle), and mixtures containing Avicel PH102 and 10% (open diamond), 20% (closed triangle), and 30% (asterisk) of spray-dried NFC by direct compression method.
The mechanical properties of tablets were evaluated and the relationship between tablet strength and compression pressure analyzed. Linear relation was found for all the tested batches under the studied conditions. Spray dried NFC formed noticeably weaker tablets than Avicel PH 102 under the same applied pressure (Figure 13). The same behavior was noted when a model active compound (paracetamol) was added to the tablet formulation.

5.1.3 Tableting after the granulation

Tablets were compressed from two batches of granules containing 40% and 60% of paracetamol as a model active compound. The mechanical properties of the tablets were evaluated in the same way as after the direct compressions. Plots of compression force against tensile strength were constructed as well and showed the same trend as in the cases of direct compression as a tablet production method. The tablets made of the granules containing NFC were weaker than those containing Avicel PH 101 and Avicel PH 102, when the same compression pressure was applied (Figure 14). However, this difference in tablet strength was less pronounced than in the case where the direct compression method was used. This is explained by the fact that the plasticity of MCC is significantly reduced after the wet granulation step is applied [122].

![Figure 14: Effect of compression pressure on the tensile strength of tablets made from granules containing 40% (A) and 60% (B) of paracetamol and Avicel PH101 (closed squares), Avicel PH102 (open triangles), spray dried NFC (open diamonds), and mixture of Avicel PH101 and spray dried NFC (closed circles).](image)

The study showed that spray dried NFC can be successfully employed as an excipient for tablet production. However, this thesis further aimed at exploring the applicability of NFC as pharmaceutical excipient, while specifically focusing on its application as material for sustained drug delivery.
5.2 Spray dried drug loaded NFC microparticles for sustained drug release (II)

5.2.1 Microparticles production by spray drying

Spray drying has been utilized since the early 1940s [178, 179] as a tool for processing pharmaceuticals. It offers a possibility of adjusting the product properties (such as particle size, shape) and production in a continuous mode. We produced matrix structured microparticles with NFC as a carrier material and drug incorporated within a porous fiber network (II). The microparticles were produced by spray drying the suspension prepared by mixing drug solution in a suitable aqueous solvent and dispersion of NFC fibers. Since NFC creates highly viscous gel-like dispersions in concentrations over 1.5% [5, 42], the concentration of feeding suspension for the spray drying had to be kept low (total solid content of 0.5%). This factor combined with a high affinity of NFC to water and short retention time of drying material in the drying chamber required a high temperature for successful drying. Thus, drugs with suitable thermal stability and melting point were chosen as model active pharmaceutical ingredients (APIs). During the spraying process, a problem of adherence of non-completely dried particles was encountered, which resulted in low yields of the production process (yields were in a range of 19.2% - 35.2%). Further, due to the different drying kinetics of the drugs and NFC, a certain portion of drug was dried separately causing a decrease in the targeted drug loading (final loading in the range of 4.5% - 15.1%).

5.2.2 Microparticle characterization

As a consequence of agglomeration of the NFC fibers in the drying process, the produced microparticles had diameters of around 5 µm, roughly spherical shapes and fibrous surface structures (Figure 15). The particle morphology was similar as in the study (I), which meant that the incorporation of an API does not affect the particle formation during the spray drying process.

Spray drying, as many other processing steps, is known to cause changes in the solid state of the processed drugs [180]. For that reason, a physical state of the drugs within the spray dried microparticles was evaluated by thermal analysis. It was found that the particles production process mainly caused a transformation of the crystalline drug form to an amorphous state, which is in accordance with previous findings that spray drying results in the production of amorphous materials [180-182]. However, small portion of the drug crystallized after the spraying. In indomethacin loaded microparticles, the existence of two polymorphic forms, α and γ, was detected.
Results and discussion

Figure 15  SEM images of the spray dried NFC microparticles: (A) particles containing 20% of metoprolol imaged with lower magnification (scale bar 5 µm) and (B) higher magnification (scale bar 3 µm), and (C) particles containing 20% of verapamil imaged with lower magnification (scale bar 10 µm) and (D) higher magnification (scale bar 4 µm).

5.2.3 Drug release studies

The drug release from the microparticles was characterized by a burst phase during the first 10-14 days, which was followed by slow release up to 60 days (Figure 16). This burst release is the consequence of the release of the loosely bound drug fraction and the portion of the drug located close to the particle surface.

Figure 16  Drug release curves of indomethacin, metoprolol tartrate and verapamil hydrochloride loaded in the NFC microparticles.
Results and discussion

The drug release curves were fitted to a mathematical model describing the drug releasing kinetics from a spherical matrix (equation (2)) developed by Baker and Lonsdale [153]. The equation (2) can be modified as following:

\[
\frac{3}{2} \left[ 1 - \left( 1 - \frac{M_t}{M_\infty} \right)^{2/3} \right] \frac{M_t}{M_\infty} = kt
\]

where \(M_t\) is the released drug amount at time \(t\) and \(M_\infty\) is the amount of drug released at an infinitive time, the release constant \(k\) corresponds to the slope of the curves obtained when the left side of the equation is plotted against time. Linear fittings were obtained for all the analyzed batches indicating diffusion through the matrix system.

Although this study confirmed the fact that NFC can be used as a material to sustain drug release, a more efficient production method was needed to overcome the yield and other manufacture problems of spray drying.

5.3 Drug loaded NFC films for controlled drug release (III)

5.3.1 Production of the films

Method for the production of drug loaded NFC films was considered due to the existence of several drawbacks in the spray drying method used in the previous study (II). The proposed method is based on the property of NFC fibers to aggregate upon water removal from the material creating porous structures. This hornification phenomenon was discussed in the literature part of this thesis. In study (II) water removal was achieved by spray drying, while in study (III) this step was accomplished by a filtration method. During the filtration the NFC fibers collapse forming tight networks around the solid, water insoluble drug particles. Small amount of water that remained in the matrices after the filtration was removed during the following oven-drying step. In this way drug/NFC films with matrix structures were formed. The applied production method, besides simplicity, had an advantage of possible adjustments based on the desired product properties. Thus, by changing the concentration of drug/NFC mixture before filtration, matrices with different thicknesses and loadings could be fabricated.

5.3.2 Characterization of the films

The thickness of the produced films was in the range of 150-200 µm, which affected their mechanical properties. The NFC films produced from pure NFC with a thickness of 60 µm can be easily folded like conventional paper [99]. A greater thickness and presence of
incorporated solid materials lead to increased rigidity of the film, but their mechanical properties still allow an easy bending and cutting with scissors. When it comes to the morphology of the inner part, SEM imaging of the films cross-section revealed layered structures of the NFC fibers organized in lamellar phases entrapping and surrounding the solid drug (Figure 17).

Solid state of the incorporated drugs was evaluated by thermal analysis (DSC), as well as by XRPD. It was concluded that the production method did not change the solid state of the drugs, and that their crystalline structure remained intact, which was expected considering the simplicity of the production method.

Figure 17  Shematic drawing of the matrix structure of the films (middle), appearance of the film and dissolution profiles of beclomethasone dipropionate loaded films (left), SEM image of indomethacin loaded film before (up) and after (bottom) the dissolution studies

5.3.2.1 Dissolution studies

The specific target of the NFC films was to achieve sustained drug release profiles for long time periods, preferably for at least 3 months. The dissolution behavior was expected to be dependent on the penetration of the medium into the matrix structure and the diffusion of the drug through the tight fiber network. The long-lasting release was achieved from all the tested batches with differences in duration and rate of drug dissolution. The drug release did not affect the morphology of the films and their structure remained unchanged after the drug had been released (Figure 17). Beclomethasone dipropionate and itraconazole loaded matrices gave constant drug release profiles over the entire period of three months, with the release kinetics closest described with zero order kinetics ($R^2 > 0.986$ for itraconazole and $R^2 > 0.982$ for beclomethasone dipropionate films). However, the drug release curves for indomethacin had a different shape caused most likely by a different release mechanism (Figure 18).
Results and discussion

**Figure 18** Drug release curves of the indomethacin loaded NFC matrices.

The films were flat and had a large aspect ratio. Hence, the release curves could be fitted to the model derived from the simple Higuchi equation [151]:

\[
M_i = A \sqrt{\frac{D \varepsilon}{\tau} (2 \rho - \varepsilon C_s) C_s t}
\]

where \(M_i\) is the amount of drug released, \(A\) is the surface area of the film, \(D\) is the diffusion coefficient of the drug inside the film, \(\varepsilon\) is the porosity of the film, \(\tau\) is the tortuosity of the film, \(\rho\) is the density of the drug material in the film, \(C_s\) is the saturated solubility of the drug inside the film and \(t\) is time. The density of the drug in the above equation can be assumed to be:

\[
\rho = f \rho_{\text{IND}}
\]

where \(f\) is the volume fraction of the drug in the film and \(\rho_{\text{IND}}\) is the density of solid indomethacin. Assuming that the drug has a low solubility in water, i.e. \(\rho \gg C_s\), the equation (5) reduces to:

\[
\frac{M_i}{M_s} = \frac{A}{V} \sqrt{\frac{2 D \varepsilon \rho C_s}{\tau}} = \frac{1}{h} \sqrt{\frac{2 D \varepsilon}{\tau} \frac{1}{f \rho_{\text{IND}}} C_s t}
\]

where \(V\) is the volume and \(h\) is the half-thickness of the film. When the equation (7) was used to plot released fraction of drug against the square root of time, good correlation was obtained (Table 4).
Results and discussion

<table>
<thead>
<tr>
<th>Loading (%)</th>
<th>Slope (%/day)</th>
<th>( R^2 )</th>
<th>( \varepsilon/\tau : (\varepsilon/\tau)_{\text{IND20}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.7</td>
<td>19.688</td>
<td>0.997</td>
<td>1.000</td>
</tr>
<tr>
<td>27.7</td>
<td>16.725</td>
<td>0.996</td>
<td>1.231</td>
</tr>
<tr>
<td>37.0</td>
<td>14.698</td>
<td>0.995</td>
<td>1.165</td>
</tr>
</tbody>
</table>

It is obvious that there is difference in mechanism of drug release from matrices loaded with indomethacin, beclomethasone dipropionate, and itraconazole. The possible reasoning for the differences in the releasing mechanisms between the films with different incorporated drugs was found in the particle sizes of the incorporated drugs, where the dissolution of small evenly distributed particles causes an increased tortuosity of itraconazole matrices compared to the indomethacin films. Also pH of the medium used in the test could possibly cause slight changes in the NFC structure (due to the presence of xylan). Further reasons could be seen in the possible binding of the drug to the NFC fibers in a molecular form after it has dissolved from the drug particles. This could lead to desorption limited kinetics in the case of slow diffusion, which could cause the release to follow zero-order kinetic. However, at this point it was not possible to create a clear picture on the presence and influence of the binding. Hence, further investigations of the drug-NFC interactions were needed (see below).

5.4 Drug interactions with the nanofibrillar cellulose (IV)

5.4.1 Permeation studies

There was an obvious need for the studies which would clarify the influence of factors affecting the drug release from the matrices. This knowledge could then also be used as a tool for drug screening when choosing suitable drug candidates for the NFC-containing drug formulations. Thus, we performed drug permeation tests through plain NFC films as a quick technique to estimate the rate of drug diffusion through a porous NFC network. Series of permeation tests at different pH-values were conducted using model compounds with different sizes. It was shown that the ability of NFC to slow down the drug diffusion was not changing significantly with small increases in the molecular size of the permeating compounds (Table 5). It was also concluded that the pH change of the used medium did not greatly change the membrane structure and, thus, did not influence the drug diffusion rate.
Results and discussion

<table>
<thead>
<tr>
<th>Drug</th>
<th>Medium</th>
<th>Diffusion coefficient (cm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beclomethasone dipropionate</td>
<td>1% HPBC</td>
<td>0.602x10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>1% HPBC/pH 1.2</td>
<td>0.601x10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>1% HPBC/pH 7.4</td>
<td>0.738x10⁻⁷</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>MQ</td>
<td>2.128x10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>Phosphate buffer pH 5</td>
<td>1.710x10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>Phosphate buffer pH 7.4</td>
<td>2.540x10⁻⁷</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.03M NaCl/pH 1.2</td>
<td>1.548x10⁻⁷</td>
</tr>
<tr>
<td>Nafarelin acetate</td>
<td>MQ</td>
<td>1.541x10⁻⁷</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>MQ</td>
<td>0.930x10⁻⁸</td>
</tr>
</tbody>
</table>

5.4.2 Quantification of drug binding to NFC

5.4.2.1 Incubation studies

The affinity of five different model compounds to the NFC fibers was evaluated by determination of the binding capacity. The bound amount was quantified by incubation of fiber suspension with a drug, followed by determination of the unbound fraction. It was found that the charge of the drugs and NFC (charge originating from the xylan residues in NFC) at the tested pH values (pH 3, pH 5.5, pH 7) plays an important role in the binding process. pKa of xylan is 3.7 [183], which means that it is mainly negatively charged at both pH-values of 5.5 and 7, thus, favoring the binding of positively charged molecules. The binding capacity is decreased at lower pH values (pH 3). Although the previous results showed that certain drugs show affinity and binding to the NFC, indicating electrostatic binding as the main mechanism, these studies did not provide information on the binding affinities. Hence, an ITC method was introduced to gain more quantitative information on the binding and the binding mechanisms.

5.4.2.2 ITC studies

The incubation studies above pointed out the electrostatic interactions between the xylan fraction in NFC and charged drugs as the main mechanism in the binding process. To evaluate the potential influence of charge to drug/NFC and drug/xylan interactions, and the corresponding enthalpic responses, the ITC titrations were performed at different pH values. Figure 19 shows a typical titration data of HFBI-DCBD binding to the NFC.
Results and discussion

Figure 19  Typical titration data of HFBI-DCBD to NFC. Raw data (upper curve) - each heat flow peak corresponds to one injection of a HFBI-DCBD. Titration isotherm (bottom curve) obtained by the integration of upper curve and subtraction of the dilution enthalpy.

The results confirmed that the drugs bind to the NFC material and, again, indicated the pH dependence of the binding and electrostatic forces as the main mechanism (Table 6).

Table 6.  Thermodynamic parameters (enthalpy \( \Delta H \), entropy \( \Delta S \), binding constant \( K_b \), number of binding sites \( N \)) of the drug binding to NFC and xylan. Titrations for which the pH value is not noted were performed using aqueous solutions/dispersions without adjusting the pH. The abbreviations LYS, NAF and XYL state for lysozyme, nafarelin acetate and xylan respectively.

<table>
<thead>
<tr>
<th>Titration</th>
<th>Binding capacity (µmol/g)</th>
<th>( K_b (M^{-1}) )</th>
<th>( \Delta H (Jmol^{-1}) )</th>
<th>( \Delta S (Jmol^{-1}deg^{-1}) )</th>
<th>( ^a N ) (sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYS to NFC</td>
<td>1.48</td>
<td>3.99×10^6</td>
<td>-8.59×10^4</td>
<td>-162</td>
<td>3.85×10^3</td>
</tr>
<tr>
<td>LYS to NFC pH3</td>
<td>0.46</td>
<td>9.16×10^6</td>
<td>-4.65×10^4</td>
<td>-22.6</td>
<td>2.17×10^4</td>
</tr>
<tr>
<td>LYS to XYL</td>
<td>16.91</td>
<td>1.41×10^7</td>
<td>-4.38×10^4</td>
<td>-10.1</td>
<td>3.12×10^2</td>
</tr>
<tr>
<td>LYS to XYL pH3</td>
<td>4.82</td>
<td>3.27×10^7</td>
<td>-6.78×10^4</td>
<td>-83.1</td>
<td>1.30×10^3</td>
</tr>
<tr>
<td>HFBI-DCBD to NFC</td>
<td>3.48</td>
<td>1.63×10^6</td>
<td>-1.84×10^5</td>
<td>-499</td>
<td>2.44×10^3</td>
</tr>
<tr>
<td>HFB to NFC</td>
<td>2.62</td>
<td>1.92×10^6</td>
<td>-5.97×10^4</td>
<td>-79.9</td>
<td>3.03×10^3</td>
</tr>
<tr>
<td>NAF to XYL</td>
<td>69.44</td>
<td>1.10×10^8</td>
<td>-1.58×10^5</td>
<td>-413.0</td>
<td>3.57×10^3</td>
</tr>
</tbody>
</table>

\( ^a \) Number of binding sites calculated as number of glucose or xylose units needed for binding of one drug molecule

However it is not completely clear, whether electrostatic binding is the only mechanism involved. It has been well known that aromatic residues play an important role in the binding of proteins to the crystalline part of cellulose via an interaction of three tyrosine residues organized in a planar surface and glucopyranose units of cellulose chains supported by hydrogen bonds [184, 185]. Lysozyme protein molecules are rich in
aromatic amino acids (3 tyrosine, 6 tryptophan, and 3 phenylalanine residues out of 129 residues in total) [186], and nafarelin acetate also possesses the aromatic rings [187] which could potentially interact with cellulose. Therefore, the possibility that lysozyme binds not only to xylan part via electrostatic interactions, but also to cellulose chains via π stacking and hydrogen bonding, should not be excluded.

As a conclusion, the study provided deeper knowledge on the processes and mechanisms occurring when NFC is used as drug release controlling material.
Conclusions

6 Conclusions

Nanofibrillar cellulose (NFC) of wood (birch) origin was tested as a novel pharmaceutical excipient. Standard methods for tablet production were used to evaluate the applicability of spray dried NFC as filler for tablet manufacturing. The spray dried NFC powder was characterized in terms of particle size and morphology, density, and flowability. The addition of NFC to commercially used microcrystalline cellulose (MCC) improved its flow properties. Tablets made of NFC were successfully prepared either by direct compression method or compression after wet granulation. Compared to MCC, NFC was found to deform less plastically and have more pronounced brittle characteristics. NFC did not affect the drug release from the tablets manufactured with active model compounds.

Spray drying process was developed to produce drug loaded NFC microparticles for longer-lasting controlled drug release. NFC fibers formed network structures during the drying process entrapping the drug within the microparticles. The formed network was tight enough to sustain the release of the drug for up to two months. The results of this study indicated the capability of NFC fibers to sustain the release of a drug. However, the spray drying process suffered from drawbacks, such as limitation to water soluble compounds, low loadings due to the different drying kinetics of drug and NFC, thus warranting the need for better manufacturing process to overcome the mentioned problems.

An easy three step method for the production of drug loaded NFC films was introduced for this purpose. The method resulted in highly loaded (20% – 40%) NFC films with excellent mechanical properties, which provided easy handling and shape and size adjustment. The films had matrix structures with NFC fibers as a matrix former, and uniformly dispersed drugs in crystalline form. The systems gave controlled drug release profiles for a period of over three months, which for some drugs was very close to zero order drug release kinetics. The systems completely retained their shapes after the drug had been released. This work clearly confirmed the applicability of NFC as drug delivery reate controlling material.

More in depth interactions between the NFC fibers and a range of model compounds were also studied. It was found out that the main mechanism of interaction lies in the binding of positively charged drugs to the negatively charged xylan fraction of the NFC material via electrostatic interactions.

As a summary, this thesis represents the very first steps on a way to explore the potential multiple applications of NFC in the manufacture and formulation of pharmaceutical dosage forms and further work is needed to advance its utilization in drug delivery systems.
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