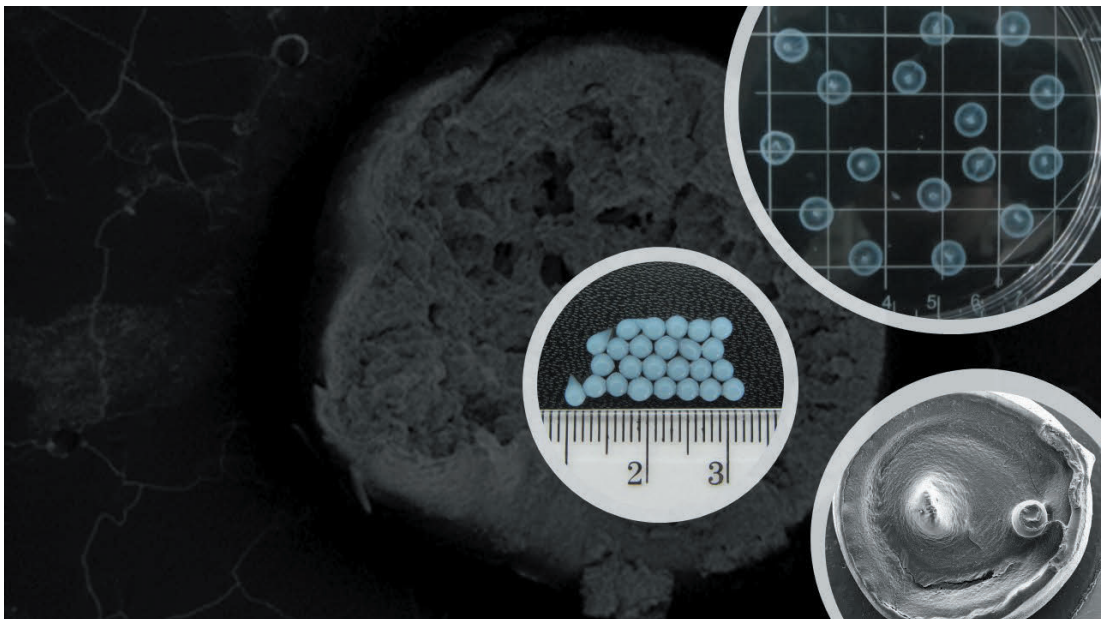


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Physically and Chemically Modified Cellulose for Drug Delivery

A study in Pharmaceutical Sciences





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Cover image; Various images of cellulose beads and discs.



Physically and Chemically Modified Cellulose for Drug Delivery

A study in Pharmaceutical Sciences

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List of Original Publications

The thesis is based on the following original publications. The publications were published in peer-reviewed scientific journals and are referred by their Roman numerals (I-IV) throughout the thesis.

Article I. Yildir, Emrah; Kolakovic, Ruzica; Genina, Natalja; Trygg, Jani; Gericke, Martin; Hanski, Leena; Ehlers, Henrik; Rantanen, Jukka; Tenho, Mikko; Vuorela, Pia; Fardim, Pedro; Sandler, Niklas. **Tailored beads made of dissolved cellulose-investigation of their drug release properties.** *International Journal of Pharmaceutics* **2013**, 456, 417–423

Article II. Trygg, Jani; Yildir, Emrah; Kolakovic, Ruzica; Sandler, Niklas; Fardim, Pedro. **Anionic cellulose beads for drug encapsulation and release.** *Cellulose* **2014**, 21, 1945-1955

Article III. Trygg, Jani; Yildir, Emrah; Kolakovic, Ruzica; Sandler, Niklas; Fardim, Pedro. **Solid-state properties and controlled release of ranitidine hydrochloride from tailored oxidised cellulose bead.** *Macromolecular Materials and Engineering* **2015**, 300, 210-217

Article IV. Yildir, Emrah; Sjöholm, Erica; Preis, Maren; Trivedi, Poonam; Trygg, Jani; Fardim, Pedro; Sandler, Niklas. **Investigation of dissolved cellulose in development of buccal discs for oromucosal drug delivery.** *Pharmaceutical Development and Technology* **2017**, published online (in press)
DOI: 10.1080/10837450.2017.1397163

Contribution of the Author

The author of the thesis contributed to the publications as described;

Article I. Performing most of the experiments (except FE-SEM and cell viability studies). Writing the manuscript.

Article II. Performing drug loading and release experiments. Contributing the manuscripts by interpreting the results.

Article III. Performing drug loading and release experiments. Contributing the manuscripts by interpreting the results.

Article IV. Performing most of the experiments (except FE-SEM). Writing the manuscript.

For article I-III, cellulose beads were received ready from the Fibre and Cellulose department, Åbo Akademi University.

Abstract

Emrah Yildir

Physically and chemically modified cellulose for drug delivery

Doctor of Philosophy in Pharmaceutical Sciences Thesis

Pharmaceutical Sciences Laboratory, Åbo Akademi University

Modern advances in pharmaceutical industry have raised the interest in designing, modifying and regenerating polymers which can improve the quality of final dosage forms. Cellulose, as the most abundant biopolymer on the world, has been extensively studied by scientists over decades. Characteristics of the natural form of cellulose can be altered by physical and chemical modification methods depending on the attributes needed for the end product. These methods aim to break the hydrogen bonds between cellulose chains or replace a hydroxyl group with a functional group, in order to obtain the desired properties.

The aim of the present work was to investigate the pharmaceutical applications of cellulose beads and discs which were produced by a novel, environmentally friendly, physical and chemical modification technique of natural cellulose. First, we investigated drug entrapping and release abilities of novel porous cellulose beads which were formed by dissolving cellulose in sodium hydroxide-urea-water solvent system and then dropping the solution into an acidic medium. Next, the cellulose beads were chemically modified by oxidation and these oxidized, negatively charged beads were loaded with cationic drugs to study the effect of opposite charges on drug entrapment and release. As final step, dissolved cellulose was shaped into another geometric form; namely discs, by changing the properties of the precipitation medium to explore their possible usage in oromucosal drug delivery.

In conclusion, the results showed that non-oxidized beads, oxidized beads and discs can be used as a versatile excipient for the production of different pharmaceutical dosage forms. However, comprehensive evaluation of the full potential of cellulose beads and discs need further investigation.

Abbreviations

CB	Cellulose beads
CD	Cellulose discs
DDS	Drug delivery systems
API	Active pharmaceutical ingredient
AGU	Anhydroglucose unit
PC	Powdered cellulose
MCC	Microcrystalline cellulose
LCPC	Low crystallinity powdered cellulose
MC	Methyl cellulose
EC	Ethyl cellulose
HEC	Hydroxyethyl cellulose
HPC	Hydroxypropyl cellulose
HPMC	Hydroxypropylmethyl cellulose
CMC	Carboxymethyl cellulose
NaCMC	Sodium carboxymethyl cellulose
DMSO	Dimethyl sulfoxide
CA	Cellulose acetate
CAP	Cellulose acetate phthalate
CAB	Cellulose acetate butyrate
CAT	Cellulose acetate trimelitate
HPMCP	Hydroxypropylmethyl cellulose phthalate

RSP	Riboflavin 5`-phosphate sodium
LiHCl	Lidocaine hydrochloride
Thp	Theophylline
RaHCl	Ranitidine hydrochloride
TAA	Triamcinolone acetonide
PEG	Polyethylene glycol
TEA	Triethanolamine
PG	Propylene glycol
NaOH	Sodium hydroxide
NaClO ₂	Sodium chlorite
NaClO	Sodium hypochlorite
UV/Vis	Ultraviolet–visible
USP	United States Pharmacopeia
HCl	Hydrochloric acid
FE-SEM	Field emission scanning electron microscopy
ATR/FTIR	Attenuated total reflectance/Fourier-transform infrared
DSC	Differential scanning calorimeter
NIR	Near-infrared
min	Minutes
rpm	Revolutions per minute
e.g	exempli gratia (for example)
etc.	et cetera (and the rest)
i.e.	id est (that is)

1. Introduction

Polymeric materials have been extensively studied and utilized by the pharmaceutical industry. The initial use of polymers was limited to polystyrene vials, rubber closures, polyvinyl chloride bags and simple gelatine capsule shells. However, advancements in polymer and pharmaceutical sciences led to the amalgamation of these two scientific fields. Nowadays, different types of novel or functionalised polymers are used in the design and development of novel drug-delivery systems (DDSs) [1,2].

Cellulose, the most abundant, naturally occurring biopolymer, is a pharmaceutically acceptable and compendial polymer for tableting and capsule filling purposes. Moreover, the polysaccharide can be physically modified to form different shapes such as fibres of different geometry, films, discs, or spherical particles. In addition, cellulose derivatives with specific properties, ranging from hydrophilic to hydrophobic, from non-charged to an-, cat- or poly-ionic can be obtained by chemical modification of the cellulose. In the pharmaceutical industry, modified cellulose is used as an excipient to control and/or sustain the drug release, to coat tablets (osmotic and enteric coating), to increase the mucoadhesiveness of the formulation and to enhance the compressibility of the powder mixture. Even though there are many excipients derived from cellulose used in drug formulations, the pharmaceutical industry tries to improve manufacturing processes, decrease the costs and improve the performance of existing products by investigating and developing different modification methods to create desired cellulose-based excipients [3,4].

In the literature it has been described that cellulose microspheres, films or discs can be created by first dissolving the cellulose with a non-derivatizing or derivatizing solvent system and later dispersing or dropping the dissolved cellulose into a coagulation bath to form desired final shape (physical and chemical modification) [5,6]. Most of these procedures use derivatizing solvents and include various disadvantages such as using an excess of chemical because of the regeneration step. Recently, the utilization of

environmentally friendly and non-derivatizing solvent systems to dissolve cellulose and then shape and/or modify the desired cellulose product have raised a lot of interest [7,8].

The aim of this thesis was to investigate the potential of physically and chemically modified cellulose for drug delivery purposes. Physical modification was performed by first dissolving the cellulose in an environmentally friendly solvent system and dropping the dissolved cellulose into an acidic medium to coagulate desired shapes such as beads and discs. Later, the chemistry of cellulose beads was changed by altering the functional group with an oxidation procedure. All the generated beads and discs were studied as potential drug carriers due to their tailorable specific surface area and high porosity, uniform volume, weight, shape, and ionic charge.

In order to achieve the goal of this thesis, first, cellulose was physically modified by dissolving it in a water-based solvent system and then dropping the dissolved cellulose into an acidic medium to obtain bead like shapes. Freely and poorly water soluble active pharmaceutical ingredients (APIs) were loaded into physically modified beads and drug entrapment, release and distribution were studied. Then, some of these cellulose beads were oxidized to obtain functionalised, anionic beads by chemical modification. A cationic model drug was incorporated into oxidized and unoxidized beads and subsequently the effect of the amount of anionic groups in the cellulose beads on drug incorporation and release were investigated. As a final step, disc shaped cellulose precipitates were shaped by changing the properties of the acidic precipitation media and their potential for oromucosal drug delivery was investigated. Hardness, drug content and in vitro release profiles were studied and compared with a marketed product.

2. Literature review

A polymer is a macromolecule formed by the repetition of small structural chemical units called monomers. Polymeric materials are divided into two groups; biopolymers (natural) and synthetic polymers (man-made). Biopolymers are extracted from living organisms and can be protein based such as collagen and albumin or polysaccharides such as cellulose, chitosan and starch. On the other hand, synthetic polymers are derived from petroleum oil and can be biodegradable such as polyesters, polyanhydrides and polyamides or non-biodegradable such as some cellulose derivatives (e.g. ethyl cellulose), silicones and acrylic polymers [9–11].

Polymeric materials have found vast numbers of applications in different biomedical fields. For instance, they have been utilized to create novel drug formulations, to develop scaffolds in tissue engineering, to aid bone repair processes due to their surface and bulk properties [12,13]. In the last decades, novel drug delivery systems have been developed with the recent advancements in polymer science. Different polymers used to provide controlled release of active pharmaceutical ingredients (API) in constant doses over long periods, increase the stability of the API by entrapping it within the matrix of the polymer, tune release of both hydrophilic and hydrophobic drugs by the swelling, eroding nature of the polymers [14–16]. As most abundant natural polymer, cellulose, has been investigated and used in pharmaceutical sciences for decades.

2.1. Cellulose



Cellulose was used in the form of wood, cotton, and other plant fibres for building materials, clothing and making paper for hundreds of years prior to its discovery by the French chemist Anselme Payen in 1838 (Figure 1) [17]. He isolated cellulose as solid fibrous material after treating plant tissues with acids and ammonia and applying extraction with water, alcohol and ether.

Figure 1. Anselme Payen.

He determined the molecular formula as $C_6H_{10}O_5$ with elemental analysis and the word “cellulose” was first used in 1839 in a report of the French academy on the work of Payen [18].

Cellulose is the most abundantly occurring biopolymer on earth with 1.5 trillion tons/year biomass production [19]. Cellulose, as renewable natural polymer, is found within the plant cell walls and synthesized by some bacterial species, algae, fungi and tunicates [20]. Among these sources plants remain the main source of cellulose.

Even though cellulose was discovered and isolated from green plants by Anselme Payen in 1838, resolving its structure and discovering its polymeric nature had to wait until the 20th century [21,22].

2.2. Cellulose structure

Plant cell walls consist of primary and secondary cell walls which both contain cellulose and hemicellulose within their structure. In addition, primary cell walls furthermore contain pectin and secondary cell walls include lignin (Figure 2) [23,24].

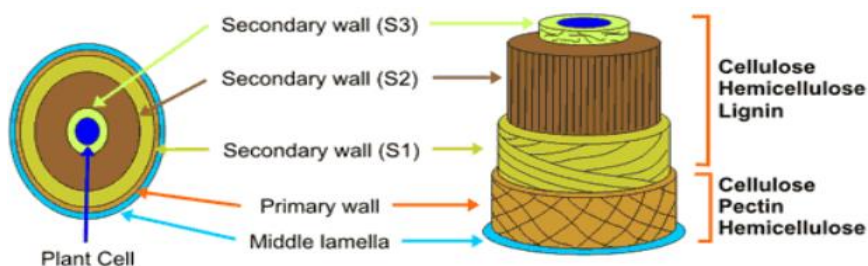


Figure 2. Illustration of structural design of plant cell walls.
(source: <http://bioenergy.crc.uga.edu/Background/background.htm>).

Cellulose is a carbohydrate polymer constructed from repeating β -D-glucopyranose molecules which are linked by 1,4- β glucoside bonds. In a cellulose structure, every second β -D-glucopyranose unit (anhydroglucose unit-AGU) rotates in a 180 degrees angle to create covalent bonds between hydroxyl groups of the C4 and the C1 atoms (Figure 3) [25,26].

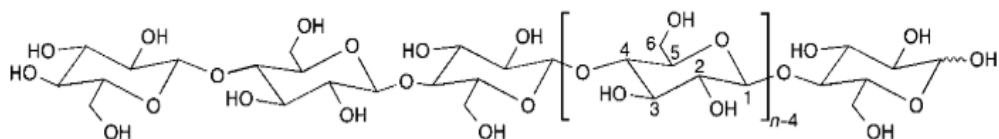


Figure 3. Structure of cellulose (n =Degree of polymerization (DP)).

In addition, inter- and intramolecular hydrogen bonds occur due to the affinity of hydroxyl groups to each other. These hydrogen-bonding interactions contribute greatly to the mechanical strength and chemical stability of cellulose by creating different cellulose polymorphs [27].

2.3. Cellulose polymorphs

In the cellulose chain, each AGU unit has three hydroxyl groups which are positioned at the C2, C3 and C6 carbons (Figure 3). These OH groups can form hydrogen bonds within the same chain via interaction of C2-OH and C6-OH groups (intramolecular bond) or with neighbouring chains via interaction of C3-OH and C6-OH groups (intermolecular bond). The orientation of these hydroxyl groups within single units and the alignment of chains within neighbouring chains create different polymorphs of cellulose (and their allomorphs), these polymorphs (I_{α} , I_{β} , II, III_I, III_{II}, IV_I and IV_{II}) are well documented in the literature [28,29]. For example, when OH groups at C2 or C3 carbons bond to C6-OH of the parallel chain, different crystalline forms of cellulose occur, cellulose I and II (Figure 4).

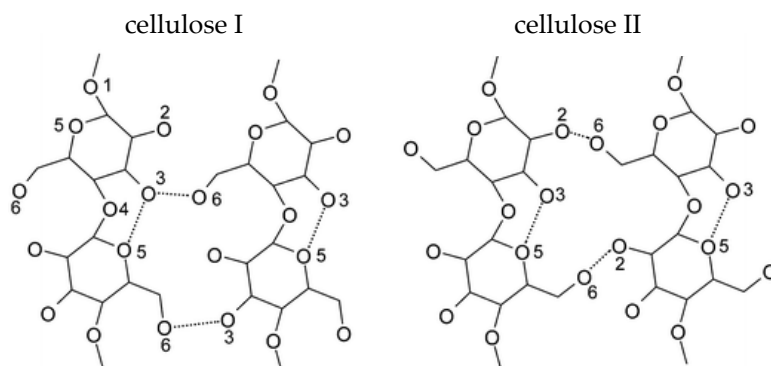


Figure 4. Positions of intermolecular hydrogen bonds for cellulose I and II.

Cellulose I, sometimes referred to as native cellulose, is produced in nature by different organisms such as trees, plants, tunicates, algae, and bacteria. The crystalline structure of cellulose can have triclinic (cellulose I_α) and monoclinic (cellulose I_β) unit cells. The ratio of cellulose I_α and I_β in the native cellulose depends on the source of cellulose. Native cellulose is thermodynamically metastable and can be converted into the most stable form cellulose II or into the cellulose III. Cellulose II is formed from cellulose I, either by swelling plant fibres in concentrated sodium hydroxide (NaOH) solution (mercerization) or by solubilizing cellulose I in liquid ammonia and re-precipitating in water (regeneration). Cellulose III_I and III_{II} are formed when cellulose I and II are treated in liquid ammonia or some amines. Cellulose IV_I and IV_{II} can be formed by heating cellulose III_I and III_{II} in glycerol at 250 °C, respectively (Figure 5) [30–32].

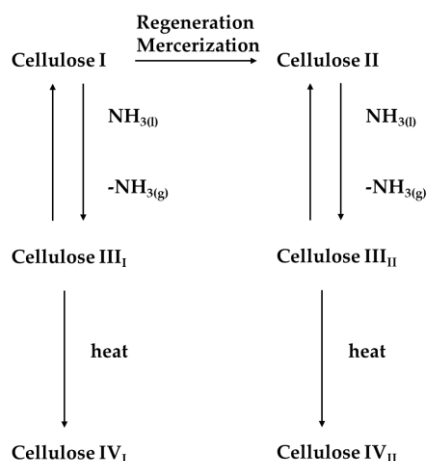


Figure 5. Transformation of polymorphic forms of cellulose.

Cellulose has several characteristic properties such as high moisture absorbance, good biocompatibility, high thermal stability, relatively low cost, high strength and durability and low density yet good mechanical properties. However, there are several drawbacks of this most abundant polymer, such as its insoluble nature in water and organic solvents, low thermoplasticity, high hydrophilicity (can be undesired in specific applications) and lack of antimicrobial properties. In order to overcome these drawbacks, cellulose can be regenerated or derivatised by different physical and chemical modification methods. It can be physically modified by dissolving it in non-derivatizing

solvent systems or chemically modified by introducing functional groups (derivatization). Thus, new desired attributes can be engineered without eliminating its desirable properties [33].

2.4. Cellulose dissolution, shaping, regeneration, derivatization

Cellulose is insoluble in water and most common solvents. The reason behind the poor solubility are the strong hydrogen bonds within a cellulose chain and also with neighbouring chains. To improve some of the characteristics of cellulose including the solubility in water, hydrogen bonds can be weakened by dissolving the cellulose in non-derivatizing solvents and derivatizing solvent systems and then it can be coagulated, regenerated or modified (Figure 6). There are variety of methods to dissolve cellulose and each has its own benefits and drawbacks [34–36].

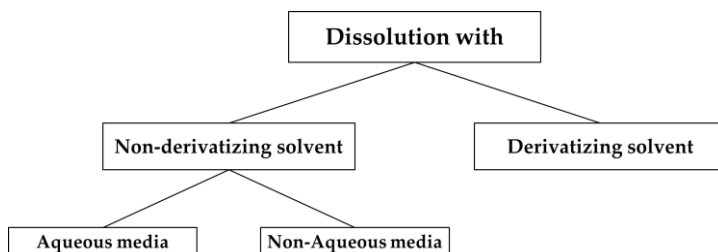


Figure 6. Classification of cellulose dissolving solvents.

Different types of solvent compositions can be used as non-derivatizing solvent system such as ionic liquids, amine oxides and polar-aprotic-organic liquids to dissolve the cellulose. Dissolved cellulose can be coagulated into different geometries and properties via dropping or dispersion techniques [37–39].

Derivatizing solvents are used to chemically modify the cellulose by functionalizing it with different molecule groups. Thus, the properties of cellulose can be tailored for specific industrial applications. Chemical modification such as esterification and etherification at the hydroxyl groups of cellulose are typical modification process. Fundamental property changes can be achieved with these substitution reactions. Oxidation, acetylation, ionic and radical grafting and deoxyhalogenation can be given as examples for

other chemical modification methods to create cellulose derivatives. In addition, a derivatised cellulose solution can be regenerated as cellulose to be used as functional materials in various forms, such as fibres, films, membranes, beads, microspheres, hydrogels, aerogels, bioplastics via polymer spinning or casting [40,41].

2.5. Cellulose based materials for drug delivery

Cellulose, regenerated cellulose and cellulose derivatives are widely used as pharmaceutical excipients. Different forms of pure cellulose can be obtained by various manufacturing processes. Powdered cellulose (PC), low crystallinity powdered cellulose (LCPC) and microcrystalline cellulose (MCC) are examples of different forms of pure cellulose. α -cellulose purification and mechanical size reduction is used to manufacture powdered cellulose. Microcrystalline cellulose is partially depolymerized cellulose prepared by controlled treating of α -cellulose. The shape, size, degree of crystallinity and polymerization create the differences between the forms. Powdered cellulose and microcrystalline cellulose are used in the formulation of oral solid dosage forms as bulking agent in direct compression, or as adsorbent and thickening agent in topical formulations. Moreover, MCC is used as tablet binder, diluent in wet granulation or in direct compression as tablet disintegrant, anti-adherent or as capsule diluent. Some of the grades of powdered cellulose is available also to be used as binder and disintegrant [3,4,29].

Regenerated cellulose materials are manufactured by first dissolving the cellulose to soluble cellulose derivative and then subsequently regeneration by polymer spinning or casting. For example, cellulose can be dissolved in alkali and carbon disulphide to make the soluble cellulose derivative called viscose. Then, viscose can be reconverted to cellulose by passing it through diluted sulfuric acid and sodium sulphate solution. The reconverted cellulose undergoes several other processes to remove the sulphur, bleach and add plasticizer to form transparent films called cellophanes. The cellophanes are used in pharmaceutical packaging due to the good compatibility, elasticity, durability [4,37].

The most commonly used cellulose derivatives in the pharmaceutical industry are cellulose ether and ester derivatives. Cellulose ethers are manufactured by replacing the hydroxyl groups in AGUs of cellulose with alkyl or substituted alkali groups. This class of cellulose derivatives are mostly water soluble and can be used as thickener, binders, film former or lubricant. Cellulose ethers can be employed to design matrix tablet where they swell and form a hydrogel barrier in contact with water, thus sustained release can be achieved. Methyl cellulose (MC), ethyl cellulose (EC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), hydroxypropylmethyl cellulose (HPMC), carboxymethyl cellulose (CMC) and sodium carboxymethyl cellulose (NaCMC) are examples for mostly used cellulose ethers [4,42–44]. Cellulose ester derivatives can be generated by using different types of derivatizing solvents such as N-alkylpyridinium halides, especially N-ethylpyridinium chloride and N-benzylpyridinium chloride DMSO/paraformaldehyde. Thus, acetates, butyrates, benzoates, phthalates and anthranilic acid esters of cellulose can be generated. Cellulose esters are mostly water insoluble and mainly used for osmotic and enteric coating of drug formulations. Cellulose acetate (CA), cellulose acetate phthalate (CAP), cellulose acetate butyrate (CAB), cellulose acetate trimelitate (CAT), hydroxypropylmethyl cellulose phthalate (HPMCP) are commonly used cellulose esters in the pharmaceutical industry [4,45–47].

2.5.1. Cellulose beads as drug carrier

As it is described above, cellulose based materials with different characteristics can be manufactured and used to formulate different types of drug formulations depending on the needs of the final formulation. Cellulose pellets and granules are produced from cellulose or cellulosic materials, mainly by extrusion, spheronization and subsequent exposure to mechanical stress to obtain a spherical shape [48]. However, there are physical and chemical differences between the spherical bead like particles produced from cellulose suspension and from cellulose solution. For example, the microscopic morphology, pore size, distribution, surface area and functional groups may differ at the end product by using these different production techniques [5,40]. In addition, producing cellulose beads from cellulose

solution eliminates the energy consuming procedures such as extrusion, spherization, thus they can be cost effective, too. Therefore, we have investigated drug carrier potential of novel cellulose beads which were manufactured by dissolving cellulose in environmentally friendly dissolution and shaping them by coagulation in acidic medium.

2.5.2. Cellulose disc for oromucosal drug delivery

Different types of drug formulations have been investigated and used in oromucosal drug delivery such as solutions, pastes, ointments, films, tablets. However, solutions, sprays or pastes possess some limitations, such as unintentional swallowing of the active ingredient, continuous dilution with salivary flow, and short duration at treatment site causing extended treatment periods [49,50]. However, unidirectional release from mucoadhesive films, patches or tablets with various properties (swelling, non-swelling, bilayered) can overcome the drawbacks mentioned above, and thus these formulations have been investigated and reported in the literature, extensively [51–53].

Cellulose derivatives such as HPC, HPMC, HEC, CMC are employed to manufacture formulations which are suitable for buccal administration due to their mucoadhesivity, hydrophilicity and swelling properties [49]. However, to the best of our knowledge, no study exists, which uses the non-dispersing buccal patches, made out of dissolved cellulose which were produced via a novel environmentally friendly method. Therefore, the potential of cellulose discs as patch like drug carrier for oromucosal drug delivery needed to be explored and investigated.

3. Aims of the study

The aim of this thesis was to investigate the potential of cellulose beads and discs as drug carriers and introduce these novel excipients which have a tailorable specific surface area and high porosity, uniform volume, weight, shape, and ionic charge for pharmaceutical applications.

The specific objectives of the thesis were:

1. To study loading freely soluble APIs into physically modified cellulose beads and investigate the potential of controlling the drug release (**Article I**).
2. To study entrapment of cationic drugs into chemically modified cellulose beads (anionic cellulose beads) and drug release properties from these beads which had different oxidation time, thus different amount of anionic groups (**Article II&III**).
3. To investigate the potential of cellulose discs as drug delivery systems for buccal treatment (**Article IV**).

4. Materials and methods

More detailed description of materials and methods used in this work can be found in original publications (I-IV).

4.1 Materials

4.1.1. Drug carriers; cellulose beads and discs

Non-ionic and anionic CBs were received ready from Fibre and Cellulose Technology laboratory, Åbo Akademi University, Finland.

Cellulose discs were created from 5% cellulose solution in the Pharmaceutical Science laboratory, Åbo Akademi University, Finland.

4.1.2. Active pharmaceutical ingredients

Active pharmaceutical ingredients (API) with different aqueous solubilities were riboflavin 5'-phosphate sodium (RSP) (Fluka Analytical, Sigma Aldrich, France), lidocaine hydrochloride monohydrate (LiHCl) (Sigma Aldrich, India) and anhydrous theophylline (Thp) (Orion Pharma, Finland). They were used for the loading of non-ionic CBs (Article I). Ranitidine HCl (RaHCl) (Sigma Aldrich, Germany) was used as cationic API in Article II-III to incorporate into anionic CBs. In the case of cellulose discs (CDs), lidocaine hydrochloride monohydrate and triamcinolone acetonide (TAA) (Fagron, Italy) were used as model APIs (IV).

4.1.3. Shrinkage preventing co-polymers and other substances

The other components used in the Article IV to avoid shrinkage were polyethylene glycol 400 (PEG 400) (Fluka Analytical, Sigma Aldrich, Germany), polyethylene glycol 6000 (PEG 6000) (Aldrich Chemistry, Sigma Aldrich, Germany), triethanolamine (TEA) (Fluka, Biochemika, Sigma-Aldrich, Switzerland), propylene glycol (PG) and ethyl cellulose (EC) (Sigma Aldrich, Germany). In addition, PEG 6000 and PEG 400 were also used in solubility enhancement studies.

4.2. Methods

4.2.1. Preparation of non-ionic cellulose beads (I)

The detailed preparation method of non-ionic cellulose beads used in this study can be found in the literature [40]. It can be described briefly as follows; first pretreated dissolving pulp was dissolved in a 7% NaOH-12% urea-water solution so that the dry content of cellulose was 5% in the solution. Later, the 5% cellulose solution was extruded through a 50 mm long needle with diameter of 0.8 mm and dropped into various nitric acid solutions with different molarity and temperature from 1-2 cm above to form spherical cellulose beads by coagulation (Table 1). Three different types of CBs were prepared by controlling the temperature and concentration of the coagulation media. 5% cellulose solution were dropped into 25 °C 0.5 molar, 25 °C 2 molar and 50 °C 2 molar HNO₃ solution to form type 1, 2 and 3 CBs, respectively. The beads were left in the acidic solution for 2 hours to ensure complete coagulation. In addition, the physical properties of these CBs are presented in Table 1 [54].

Table 1. Physical properties of three different types of beads [54,55].

Type of CBs	c _{HNO₃} and temperature of HNO ₃ solution	Specific Surface area m ² .g ⁻¹	Water swollen Volume mm ³ /bead	Water swollen Weight mg/bead	Porosity %/bead
T1	0.5 M, 25°C	470	11.2	14.1	93.6
T2	2 M, 25°C	447	14.9	17.0	93.8
T3	2 M, 50°C	381	17.2	17.8	94.7

The coagulated CBs were washed for at least 4 hours under running tap water. This procedure was followed by several washing cycles with distilled water.

4.2.2. Preparation of anionic cellulose beads (II-III)

Anionic CBs were prepared from never dried water swollen (type2, T2) non-ionic cellulose beads. The detailed explanation of the preparation method of these negatively charged CBs can be found in Article II [56]. It can be described briefly as follows; intensively washed and water swollen cellulose beads were immersed into a TEMPO/NaClO₂/NaClO solution for 2, 5, 24 and 48 hours at 20, 40, 60 and 80 °C for oxidation. Only cellulose beads which were oxidized for 48 hours at 20, 40 and 60 °C were washed thoroughly and were used for drug entrapment and release studies [56,57].

Some properties of anionic CBs such as porosity, amount of carboxylic groups and re-swelling abilities in sodium phosphate buffers with different pH values were investigated and the results are presented in Table 2. In addition, the porosity of the all samples was found to be between 94% and 95%. [56,57].

Table 2. Properties of non-oxidized and oxidized beads [56,57].

Type	Oxidation temperature for 48 hours	Amount of carboxylic acid groups mmol.g ⁻¹	Re-swelling in pH 1.2 %	Re-swelling in pH 3.6 %	Re-swelling in pH 7.4 %
CB (T2)	NA	0.1	54.6	52.7	49.5
OCB20	20 °C	0.92	57.1	63.0	77.8
OCB40	40 °C	1.23	56.5	64.3	85.3
OCB60	60 °C	1.85	54.7	64.9	87.8

4.2.3. Preparation of cellulose discs (IV)

A 5% cellulose solution, which was prepared by dissolving pretreated pulp in 7 % NaOH-12 % urea-water at -10 °C, was received ready from Fibre and Cellulose Technology laboratory, Åbo Akademi University, Finland and used for the manufacturing of cellulose discs.

There are several parameters affecting the formation of CDs in nitric acid, such as the dropping height, temperature and the molarity of the coagulation

medium [54,55]. Therefore, two of those formation determining parameters were investigated to obtain CDs; the molarity of the coagulation medium and the drop height of the cellulose solution. Four different molarities (0.3, 0.5, 0.7 and 1 molar) for the coagulation medium and five different drop distances (1, 3, 7, 10 and 15 cm) as dropping height were investigated by changing one factor at the time. Instead of manual dropping of the solution by a pipette, gravitational dropping using a Mohr pipette was chosen to have minimum weight and volume variation among the created discs. Coagulated CDs were left in the nitric acid solution for 1–2 minutes for successful formation of cellulose discs. The nitric acid, urea and NaOH were removed by washing under tap water flow for approximately 2 hours and several washing cycles with ethanol were performed to change the water in the CDs with ethanol.

4.2.4 Drug incorporation into cellulose beads (I-III)

Undried, empty, water swollen CBs were loaded with model drugs by immersion technique, i.e. model drugs were dissolved in distilled water and empty CBs were immersed into drug loading solutions so that there would be 2 beads per ml of the loading solution. The concentration of the drug loading solutions and the types of CDs used are presented in Table 3. APIs; Thp, RSP and LiHCl. After the immersion, the loading solutions were gently agitated with a magnetic stirrer for 24 hours. After the loading was completed, loaded CBs were dried on a glass plate at constant temperature and humidity (22.5 °C, 50 %) for at least 48 hours until there was no change in the mass of the loaded CBs.

Table 3. Summary of the model drugs, drug loading concentrations and cellulose bead types [55–57]

Article No	Model drugs	Drug loading concentrations mg/ml	CB types used for loading
Article I	Thp	4	3
			2
			1
	RSP	20	3
			2
			1
		4	3
			2
			1
	LiHCl	20	3
			2
			1
		4	3
			2
			1
Article II & III	RaHCl	20	OCB0 (T2)
			OCB20
			OCB40
			OCB60

4.2.5 Drug incorporation into cellulose discs (IV)

Drug loading solutions were prepared by dissolving the APIs in polymer-ethanol solutions. Three different API concentrations (5, 20 and 80 mg/ml for LiHCl and 0.5, 1 and 2 mg/ml for TAA) and six different PEG 6000:PEG 400:ethanol weight ratios were used to prepare the drug loading solutions. Table 4 presents the overview of drug loading solution compositions and concentrations.

Table 4. Summary of the model drugs, drug loading concentrations and compositions [58].

Model drug	Drug concentration mg/ml	Polymer-ethanol ratios %-weight			Coding (abbr. for the formulations)
		PEG 6000	PEG 400	Ethanol	
LiHCl	80	50	0	50	L80-50:0:50
		35	15	50	L80-35:15:50
		25	25	50	L80-25:25:50
		15	35	50	L80-15:35:50
		0	50	50	L80-0:50:50
		0	0	100	L80-0:0:100
	20	50	0	50	L20-50:0:50
		35	15	50	L20-35:15:50
		25	25	50	L20-25:25:50
		15	35	50	L20-15:35:50
		0	50	50	L20-0:50:50
		0	0	100	L20-0:0:100
	5	50	0	50	L5-50:0:50
		35	15	50	L5-35:15:50
		25	25	50	L5-25:25:50
		15	35	50	L5-15:35:50
		0	50	50	L5-0:50:50
		0	0	100	L5-0:0:100
TAA	2	50	0	50	T2-50:0:50
		35	15	50	T2-35:15:50
		25	25	50	T2-25:25:50
		15	35	50	T2-15:35:50
		0	50	50	T2-0:50:50
		0	0	100	T2-0:0:100
	1	50	0	50	T1-50:0:50
		35	15	50	T1-35:15:50
		25	25	50	T1-25:25:50
		15	35	50	T1-15:35:50
		0	50	50	T1-0:50:50
		0	0	100	T1-0:0:100
	0.5	50	0	50	T0.5-50:0:50
		35	15	50	T0.5-35:15:50
		25	25	50	T0.5-25:25:50
		15	35	50	T0.5-15:35:50
		0	50	50	T0.5-0:50:50
		0	0	100	T0.5-0:0:100

Undried, ethanol swollen CDs were immersed into these solution (one disc per ml) and gently agitated with a magnetic stirrer for 24 hours. In the case where PEG 6000 was used, the drug loading process was performed at 55 °C in a water bath due to the melting point of PEG 6000 ($T_m=55-60$ °C) [59]. After the loading, loaded CDs were dried on a glass plate at constant temperature and humidity (22.5 °C, 50%) for at least 48 hours until there was no change in the mass of the loaded CDs.

4.2.6 Drug content analysis (I-IV)

To be able to extract the all the incorporated drug content, first, the loaded never dried CBs were mechanically crushed into small pieces and immersed into 100 ml of distilled water. In the case of the loaded CDs, the crushed pieces were immersed into 10 ml ethanol solution due to the low solubility of TAA in water. Then, the solutions which had the crushed CDs & CBs were stirred with magnetic stirrer for 24 hours at room temperature until CBs & CDs were fibrillated. Fibres were separated from the supernatant by centrifugation (4500 rpm, 5 minutes). After the centrifugation, the amount of drug incorporated into the CBs was examined spectrophotometrically by measuring the UV light absorption of the solutions with a UV/Vis spectrophotometer (PerkinElmer, Lambda 25, Germany) at fixed wavelength; 267 nm for RSP, 218 nm for LiHCl, 267 nm for Thp, 228 nm for RaHCl, 218 nm for LiHCl and 239 nm for TAA (see Table 5). Each sample was analysed in triplicate for all cases. The table below presents the model drugs, used wavelengths and solvents in the respective articles.

Table 5. Model drugs, wavelengths and solvents used to determine the drug content in CBs and CDs [55–58].

Article No	Model drugs	Wavelength nm	Solvent
Article I	RSP	267	water
	LiHCl	218	
	Thp	267	
Article II & III	RaHCl	228	ethanol
Article IV	LiHCl	218	
	TAA	239	

Furthermore, to measure any possible effects of the remaining cellulose fibres in the solutions on the absorbance, empty CBs and CDs were immersed and crushed in water and ethanol respectively and then centrifuged under the same conditions as mentioned above. Also, two times more than the maximum PEG 6000 and PEG 400 amount which were entrapped in the CDs were dissolved in ethanol and the absorbance measured at 218 and 239 nm. There was no absorbance interference of the remaining fibres or polymers at the wavelengths used for the detection of the drug substances.

4.2.7 In-vitro drug release studies (I-IV)

The in vitro drug release for the loaded CBs was performed according to the USP paddle method (United States Pharmacopeia, 35th Ed.). The number of the beads used for the dissolution experiments for each study was chosen carefully in order to stay within sink conditions in the dissolution media even if there would be a 100% drug release from all the beads. The 500 ml dissolution media were 0.1 N HCl buffer solution (pH 1.0) and sodium phosphate buffer (pH 1.2, 3.6 and 7.4). The in-vitro drug release experiments were performed with the Sotax AT7 smart dissolution tester (SOTAX, Switzerland), thus sampling was done automatically.

In the case of the cellulose discs, drug and polymer loaded and dried CDs, which were covered with ethyl cellulose film from one side, were immersed into glass vessels filled with 50 ml phosphate buffer (pH 6) at 37 °C. A thermostatic water bath (Julabo SW22, Germany) was used to preserve 37 °C during the analyses. 100 rpm was chosen as shaking speed. The sample collection was performed manually at specific time intervals for 4 hours. The collected sample volume was replaced by an equal volume of fresh buffer solution.

The drug release amounts at the different time intervals were measured by using a UV/Vis spectrophotometer (PerkinElmer, Lambda 25, Germany). The absorption was measured at fixed wavelengths: 267 nm for RSP, 218 nm for LiHCl and 272 nm for Thp (I), 228 nm for RaHCl (II-III), 218 nm for LiHCl and 242 nm for TAA (IV). All the experiments were done in triplicate (n = 3).

Table 6 summarizes the model drugs, wavelengths, dissolution media and volumes used for in-vitro drug release studies for each article.

Table 6. Model drugs, wavelengths , dissolution medium and volume used to investigate the drug release from CBs and CDs [55–58].

Article No	Model drugs	Wavelength nm	Dissolution medias	Dissolution volume
Article I	RSP	267	0.1 N HCl buffer solution (pH 1)	500 ml
	LiHCl	218		
	Thp	267		
Article II	RaHCl	228	sodium phosphate buffer (pH 7.4)	
Article III	RaHCl	228	sodium phosphate buffer (pH 1.2 & 3.6)	
Article IV	LiHCl	218	potassium phosphate buffer (pH 6)	
	TAA	242		

4.2.8 Morphology studies with field emission scanning electron microscopy (FE-SEM) (I & IV)

The surface and interior morphology of empty and loaded dried cellulose beads and discs were investigated by using the Leo Gemini 1530 field emission scanning electron microscope (FE-SEM) with an In-Lens detector. The samples were carbon coated with the Tamcarb TB500 sputter coater (Emscope Laboratories, Ashfold, U.K) prior to FE-SEM observation. Then, the optimum accelerating voltage of 8 kV (I) & 10 kV (IV) was used to obtain FE- SEM images and the magnifications used were between 30x (I-IV), 500x (IV) and 10kx (I).

4.2.9 Solid state characterization

4.2.9.1 *Fourier transform infrared (FTIR) & Raman spectrometer (II-III)*

ATR/FTIR and Raman spectra of empty non-ionic and anionic cellulose beads were collected by using a Nicolet iS 50 FT-IR spectrometer which has a Raman module (Thermo Fisher Scientific, USA). Tungsten-Halogen source and DLaTGS-KBr detector-splitter with 4.000 cm^{-1} resolution and 64 scans were used to collect spectra during FTIR analyses. During the Raman analyses, a diode laser was used as source and a InGaAs-CaF₂ detector-splitter with 8.00 cm^{-1} and 1,024 scans was used for spectra collection. Gold plates were used during the Raman measurements.

4.2.9.2 *Differential scanning calorimeter (DSC) (III-IV)*

DSC Q2000 (TA Instruments, USA) was used to understand the solid state of the model drugs in the cellulose beads and discs. In article III, empty and loaded anionic and non-ionic CBs, pure RaHCl; in article IV, pure model drugs, polymers, physical mixtures and empty-loaded cellulose discs were subjected to DSC measurements. The samples were placed in aluminium T-zero pans (for solid samples) and in hermetic pans for (liquid samples) over a temperature range between 20-300 °C with a heating rate of 10 °C/min. Nitrogen as inert gas was used during the measurements at the flow rate of 50 ml/min.

Moreover, DSC analyses were performed on washed and unwashed CDs to prove the removal of residual nitric acid after precipitation of the CDs (IV).

4.2.10 Other analyses performed on CBs and CDs

4.2.10.1 *Hyperspectral NIR imaging on CBs-drug distribution (I)*

The empty and loaded cellulose beads (I) were dissected with a doctor blade for the analysis of the internal structure. Drug distribution within the loaded dried cellulose beads was studied by collecting hyperspectral NIR images with the hyperspectral, a chemical imaging workstation SisuCHEMA (SPECIM, Spectral Imaging Ltd, Oulu, Finland), which utilizes a SPECIM

MCT based Spectral Camera. Multivariate visualizations were conducted with the Evince (Umbio AB, Sweden) software.

4.2.10.2 Mechanical tests on CDs, strength and mucoadhesion (IV)

A Texture analyser (TA.XTplus, Stable Micro Systems, UK) was used to determine the hardness and mucoadhesion properties of the bilayered CDs. Hardness studies were performed by applying force on the bilayered CDs with a 20 mm diameter cylindrical aluminium probe at 1 mm/sec speed. All measurements were performed at ambient conditions in triplicate. The results were compared with the marketed buccal tablet, Aftab (MedaPharma, Germany). For mucoadhesion studies, loaded CDs were attached to aluminium cylindrical probe and then brought in contact with the porcine tongue tissue. The tongue was wetted with filtered human saliva and with a phosphate buffer (pH 6). In addition, the tongue was kept in phosphate buffer (pH 6) at 37 °C thus the tissue could be kept at a constant temperature to mimic the oral conditions. The probe with the sample were moved toward the tongue with 0.5 mm/s pre-test speed and when the probe reached the tissue, it was held in place for 3 seconds for Aftab and 60 s for the CDs with 2 kg load power. The reason for time difference was due to the low mucoadhesion of the CDs, since the 3 seconds contact time for the CDs did not result in any significant value. After pressing the samples which were on top of the probe, to the tissue, the probe was withdrawn at a speed of 0.5 mm/s to a distance of 15 mm. The Texture Exponent 32 software were used to calculate the detachment force with respect to time (adhesion impulse, unit N.s). The collected data was used to compare the mucoadhesion of the formulation with the marketed product.

5. Results and discussions

5.1 Properties of non-ionic and anionic CBs (I-III)

Porosity, volume, specific surface area of non-ionic CBs (I) and ionic charge, re-swelling abilities, porosity of the anionic CBs (II-III) are significant physical properties since they can have a direct influence on the drug entrapment capacity of CBs, the drug release from CBs and the solid state of drugs within CBs (Table 1, 2). These properties have been discussed below.

In the process of creating non-ionic cellulose beads, coagulation happened faster when the molarity of the coagulation media was kept constant and the temperature altered from 25 °C to 50 °C. This caused the skin layer to become thicker and firm faster, thus the dimensions of the droplet were preserved. Since bigger beads could hold more water inside, the weight and volume increased. In addition, the porosity increased due to a higher proportion of micro and small mesopores (2-50 nm) at 25 °C and macropores at 50 °C [54]. On the other hand, when the temperature was kept constant, but the molarity was altered, it was observed that coagulation in nitric acid solution with lower molarity resulted in denser internal structure and coarser surface morphology since aggregation continued for a longer time within the beads. Higher molarity of nitric acid resulted in faster coagulation and intercepted the aggregation of the cellulose fibrils. Thus, decrease in porosity, water swollen volume and weight was observed with the CBs coagulated in 0.5 molar compared to the beads which were coagulated in 2 molar nitric acid solution. On the contrary, the specific surface area of CBs decreased with the molarity and temperature rise. Slow diffusion and continuous agglomeration processes occurred when nitric acid with low temperature and molarity was used, thus cellulose molecules arranged themselves into specific internal structures which resulted in maximized specific surface area [54]. It could be expected that cellulose beads with higher water swollen volume and weight can be loaded with higher drug amounts since there would be more space within the empty beads for the drug loading solution to diffuse. However, a decrease in the specific surface areas while weight and volume increase can affect the drug entrapment and drug release negatively due to less surface to attach for the drug molecules.

The amount of anionic groups within the oxidized CBs were elevated when the temperature of the oxidation reaction was increased from 20 to 60 °C and the time of reaction elevated from 2 to 48 hours (see Table 2). The reason behind the increase in the amount of anionic groups is that specific oxidation of C6 hydroxyl groups by N-oxoammonium ions occurs more efficiently when higher temperatures and longer time were employed during the oxidation [56]. In addition, higher amount of anionic groups resulted in higher re-swelling abilities in the dissolution medium with high pH. However, there was no significant change in the porosities of anionic CBs when oxidization conditions were altered. 94-95% porosity was observed within all oxidized samples [56,57]. It was expected that cellulose beads which were oxidized for a longer time period can entrap higher amount of cationic drug molecules, since the amount of anionic groups within the beads was increased and the bonding of these negatively charged groups with a positively charged drug would be higher. In addition, higher re-swelling in a high pH medium could result in faster drug dissolution since the pores would enlarge more and the entrapped drug could diffuse easier into the dissolution medium.

5.2 Formation of cellulose discs and their shape (IV)

5 % cellulose solution, was dropped from five different heights into the nitric acid coagulation medium with five different molarities. These parameters were altered to find out the optimal dropping height and coagulation medium molarity to create the desired CDs. The experiments showed that higher molarities than 0.7 molar resulted in thicker and bead like structures regardless of the dropping height due to faster skin formation due to the high molarity of the coagulation media. However, when the molarity was 0.7 molar and lower, flatter and disc shape cellulose formations could be created because of slow coagulation and skin formation. After the molarity was chosen as 0.7 molar, the effect of the drop height was investigated. When the dropping distances were 1 and 3 cm hollow bead like shapes were formed and at the drop height of 10 and 15 cm, the discs got uneven edges due to increased surface impact of the nitric acid medium. The dropping height of 7 cm resulted in flatter disc-like shapes (Figure 7).

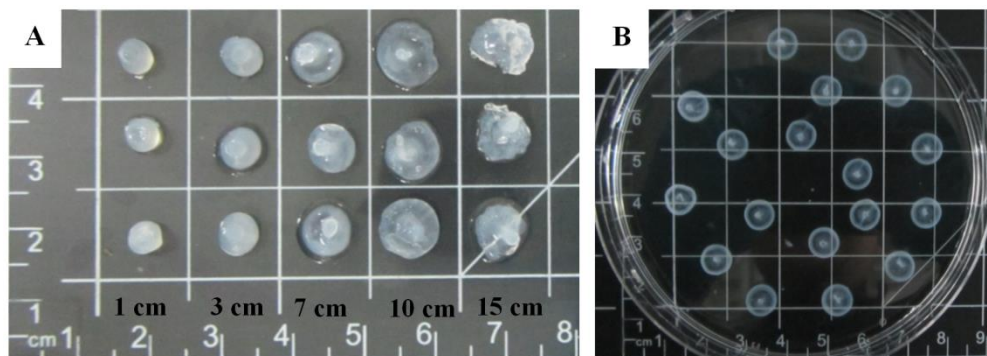


Figure 7. A. Dropping height effect of cellulose disc formation. B. Cellulose discs formation with 7 cm dropping height, coagulation media 0.7 molar nitric acid.

In addition to the dropping height and the molarity of coagulation medium, the dropping method was investigated to create uniformly shaped cellulose discs. Manually dropping the cellulose solution with an automatic pipette created greater a weight deviation while forming cellulose solution drops. However, dropping the solution with a Mohr pipette and using the gravitational force created cellulose discs with a uniform weight distribution. Therefore, cellulose discs used in this study were produced by employing the later mentioned dropping method [58].

5.3 Morphological studies (FE-SEM)

5.3.1 Cellulose beads (I-III)

Empty dried CBs and drug loaded dry CBs had a regular spherical shape with an average size of 1 mm. FE-SEM analyses proved that there is a significant difference between the internal structure of empty and loaded CDs (Figure 8 A-B).

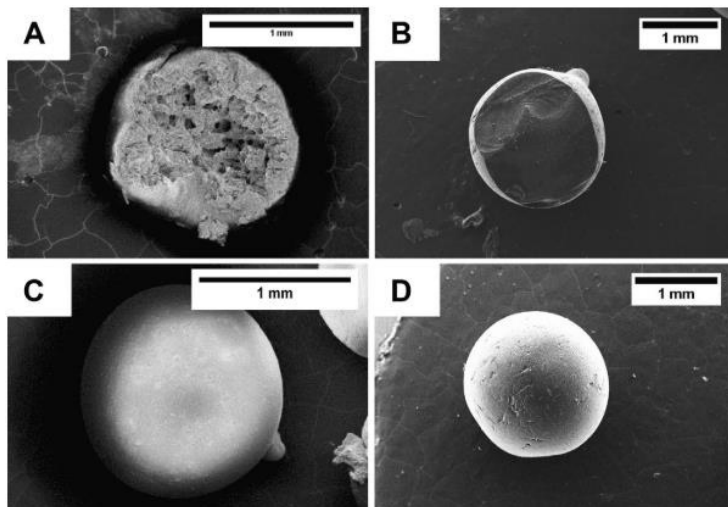


Figure 8. Internal morphology of an unloaded (A) and lidocaine loaded beads (B) magnification: 100 \times , 200 \times , respectively. External/surface morphology of an unloaded (C) a lidocaine loaded bead (D), magnification 100 \times , 200 \times , respectively [55].

The pores of empty beads (Figure 8 A) were filled with the model drug compounds and there was no observable crystallization within or on the surface of the beads (Figure 8 B-D). In addition, it can be stated that the concentration of the loading solution, type of the beads and the selected model drugs did not have a noticeable effect on the external surface morphology of the beads (Figure 8 C-D).

The oxidization process resulted in reduction of big agglomerates which were observed in non-ionic CBs [54,56]. In addition, opening of agglomerates was observed along the cross section of oxidized CBs regardless of the distance from the edges or the oxidation conditions. Thus, opening of agglomerates resulted in even distribution of anionic groups within the CBs and changed in pore size distribution compared to the non-ionic CBs [56]

5.3.2 Cellulose discs (IV)

FE-SEM studies on dried empty CDs, only polymer containing CDs and drug-polymer loaded CDs indicated that PEG 6000 and PEG 400 embedded in the voids within the CDs (see Figure 9 A-D). Similar findings were reported in the literature, when PEG 400 and PEG6000 were loaded into porous CBs [60].

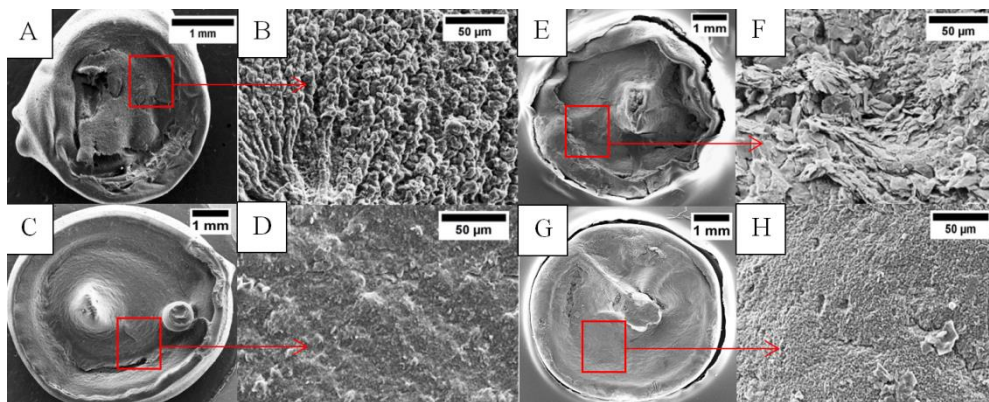


Figure 9. A,B. Empty dried CDs, 10x-500x. C,D CDs loaded in PEG 6000:PEG400:EtOH solution (15:35:50 w:w:w %), dried, contains no drug, 10x-500x. E,F. Partial crystallization of LiHCl on the surface of CDs loaded in PEG 6000:PEG400:EtOH solution (15:35:50 w:w:w %) with 80 mg/ml LiHCl concentration, dried, 10x-500x. G,H Surface of CDs loaded in PEG 6000:PEG400:EtOH solution (15:35:50 w:w:w %) with 1 mg/ml TAA concentration, dried, 10x-500x.

In addition, partial crystallization of LiHCl on the surface of the loaded CDs was observed due to the high loading concentration of this model drug (see Figure 9 E-F). No crystallization was observed when the drug loading concentration of LiHCl was lower than 80 mg/ml or when TAA was loaded with in the CDs with 2, 1 and 0.5 mg/ml concentrations (see Figure 9 G-H).

5.4 Assay of drug content

5.4.1 Cellulose beads (I-III)

The amount of entrapped drug within the beads correlated with the drug loading concentration and the water swollen volume and porosity of the beads (Table 7). The incorporated drug amount was increased from the bead type 1 to 3 where the water swollen volume-weight and porosity was highest. In addition, the loading efficiency was affected by the water solubility of the APIs. The amount of incorporated Thp was lower than for the other model drugs due to lower water solubility of Thp.

Table 7. Drug loading shown as the amount of drug incorporated per one bead and as mass percentage. Uniformity of drug loading is also presented as variation in the amount of drug loaded per one bead (n=5).

Model drug	Drug loading concentration mg/ml	Bead type ^a	Incorporated drug amount in one bead mg	Drug amount percentage per one dry bead %
Thp	4	T3	0.072 ± 0.002	5.0
	4	T2	0.052 ± 0.002	4.2
	4	T1	0.049 ± 0.004	3.7
LiHCl	20	T3	0.396 ± 0.032	27.3
	20	T2	0.333 ± 0.050	26.6
	20	T1	0.313 ± 0.052	23.2
	4	T3	0.118 ± 0.022	8.1
	4	T2	0.095 ± 0.036	7.6
	4	T1	0.065 ± 0.008	4.8
RSP	20	T3	0.207 ± 0.005	14.3
	20	T2	0.163 ± 0.015	13
	20	T1	0.172 ± 0.038	12.7
	4	T3	0.081 ± 0.005	5.6
	4	T2	0.063 ± 0.002	5.1
	4	T1	0.068 ± 0.002	5

^athe average weights of empty dried T1, T2 and T3 beads were 1.35, 1.25 and 1.45 mg, respectively, n=25.

The observed correlation between drug content in the beads and drug loading concentration, porosity, water swollen weight and volume was qualitatively predictable. Since the drug could diffuse into empty CDs more when the concentration gradient was higher and the porosity was higher, the volume had increased the entrapment ability of the CDs too.

In the case of anionic CBs (II & III), it was found that oxidation has widened the cavities (small meso and macropores) within the beads, opened some of the closed pores by opening agglomerates and increased the anionic groups within the beads [56,57].

Table 8. Drug loading shown as the amount of drug incorporated per one bead and as mass percentage. Uniformity of drug loading is also presented as variation in the amount of drug loaded per a one bead.

Model drug	Drug loading concentration mg/ml	Bead type ^a	Incorporated drug amount in one bead mg	Drug amount percentage per one bead %
RaHCl	20	OCB0 (2)	1.13 ± 0.01	20.7
	20	OCB20	1.18 ± 0.01	17.8
	20	OCB40	1.77± 0.01	25.8
	20	OCB60	1.49 ± 0.01	21.8

^a the average weights of empty water swollen OCB0 (2), OCB20, OCB40 and OCB60 beads were 4.33, 5.43, 5.09 and 5.34 mg, respectively, n=50.

The amount of ranitidine HCl incorporated into the matrix system of the CBs increased when the cavities within the bead were widened and the amount of carboxylic groups (anionic groups) in the beads was increased by higher degree of oxidation (Table 8). However, a decrease was observed in the total amount of the drug in the beads, which were oxidized at 60 °C (OCB60). A possible explanation for this might be the higher amount of bounded water within the mass of empty OCB60 [57]. Moreover, the difference in the weight of empty beads resulted in a variation of the drug amount percentage per bead between 17.8% and 25.8% (Table 8).

5.4.2 Cellulose Discs (IV)

Two model drugs, LiHCl as freely water-soluble API and TAA as poorly water-soluble API were incorporated into CDs by immersion technique as described in section 4.2.3 (Table 4). The aim of investigating different drug concentrations and solvent ratios was to understand the significance of these parameters on drug intake and drug release. The average content of LiHCl and TAA in the CDs is presented as bars in Figure 10.

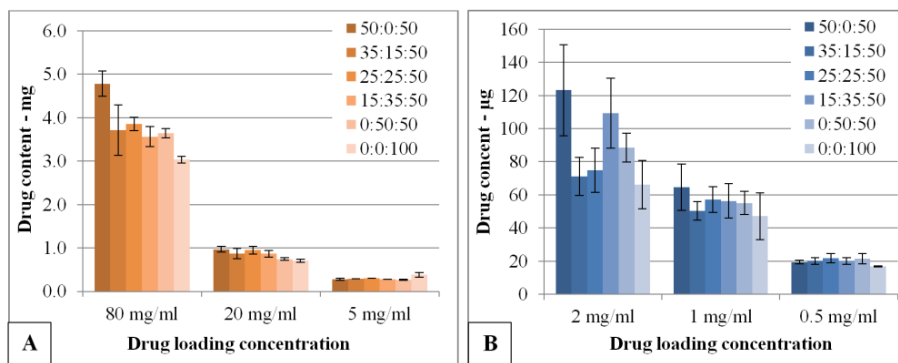


Figure 10. Amount of LiHCl and TAA in loaded cellulose discs (A, B, respectively) where CDs had been loaded in drug loading solutions with three specific drug concentrations and six different PEG 6000:PEG400:EtOH solvent composition ratios, presented in legends ($n = 3$).

The results presented in the graphs show that there was a significant positive correlation between the drug loading concentration and the drug content within the beads. In addition, it is important to state that the solubility of APIs is also a limiting factor on the loading efficiency within the beads since the drug loading concentration is limited by that factor, too. A similar finding was observed in the previous studies that have examined the effect of the drug loading concentration on the drug content in the drug carriers [55].

Moreover, Modde (Umetrics AB, Sweden) as a design of experiment software was used to mathematically identify the influence of drug loading solution concentration, polymer type and interaction factors (drug loading concentration*polymer type considered together) on the drug content (response) within the discs. The software performs the task by fitting a polynomial model to the data (factors and response values) and then determining the numerical values of the model parameters (regression coefficients). These coefficients used to understand how significantly the factors influence the response. Polynomial model can be summarised with the equation:

$$y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 + \epsilon, \quad (\text{Eq. I})$$

where y is the response, x are the factors, β_0 is the constant term, β are the model parameters, and ε the residual response. For the data we obtained, when the factors and response values were entered into the software and scaled-centred regression coefficients of the polynomial model for CDs were evaluated. All the other regression coefficient values were smaller than the uncertainty coefficients (confidence intervals) except the regression coefficient of the drug loading concentration, thus we can once more conclude that effect of the polymer type on the drug content in the CDs was not significant within these sets of variables and drug concentration ranges. The concentration of drug loading solution was the only major factor influencing the drug content and correlating with it.

The drug contents in the final formulations were chosen to be at the same level as in similar commercial products. For example, Kamistad N is an oral gel with 20 mg/g LiHCl concentration and the approximate amount of applied gel on aphtha would contain 2 mg LiHCl (PIL of Kamistad N describes three times a day on 0.5 cm surface). Moreover, the marketed mucoadhesive tablet, Aftab contains 25 μ g TAA. Since the final formulation of CDs was covered with EC film from one side to obtain a unidirectional release to the aphtha side and CDs were aimed to be kept at the site of action for four hours, the drug dose in the final formulation in the CDs was aimed to be approximately two times higher than the marketed products (4 mg for LiHCl and 50 μ g for TAA). Therefore, the CDs were loaded in the drug loading solutions with concentrations of 80 mg/ml and 1 mg/ml for LiHCl and TAA, respectively.

Once the drug loading concentration was chosen, the Modde software was used to study how the drug content varies as a function of PEG 6000, PEG400 and ethanol (Figure 11).

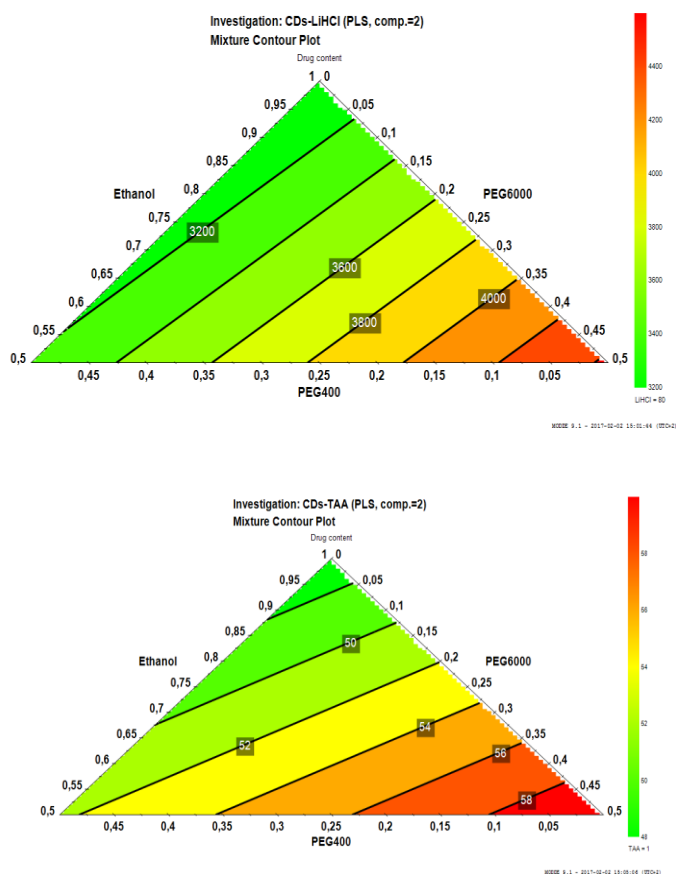


Figure 11. Mixture contour plots for CDs, which were loaded in 80 mg/ml LiHCl and 1 mg/ml TAA solutions. Both mixture contour plots illustrate the changes in the drug content when PEG 6000, PEG400 and EtOH ratios have been altered from 0 to 50% and sum up to 100%.

All the drug loading solutions with different PEG 6000: PEG 400: ethanol ratios can give the desired drug dose in CDs, 3.2-4.2 mg and 50-60 µg for LiHCl and TAA respectively (Figure 10). We can conclude that the polymer concentrations in the drug loading solutions do not have a significant influence on the drug diffusion into the cellulose discs since the size of all polymer molecules used was small enough to diffuse through macropores. Therefore, it was decided that CDs which were immersed in 80 mg/ml for LiHCl and 1 mg/ml for TAA solutions with different PEG 6000:PEG400:EtOH ratios must be analysed further. All the formulations created need to be analysed further to decide which formulation can give the desired release and mechanical properties.

5.5 In-vitro drug release kinetics

5.5.1 Non-ionic cellulose beads (I)

The release profile of Thp loaded CBs reveals an initial burst release followed by a steady release. However, RSP loaded CBs showed a prominent difference since the burst release was not observed here and only a steady release of the API could be seen (Figure 12). The reason behind this phenomenon can be attributed to the low solubility of RSP in the studied dissolution medium (2 mg/ml). In the case of LiHCl loaded beads, the pure substance was released totally after 30 minutes in 0.1 N HCl solution. Even though LiHCl is a freely water-soluble substance, the common ion affect decreased the solubility of the substance in the medium thus the release rate was slower. Moreover, LiHCl was not released totally from the matrix. The reason behind that phenomenon might be that the concentration of the substance in the media and matrix reached an equilibrium.

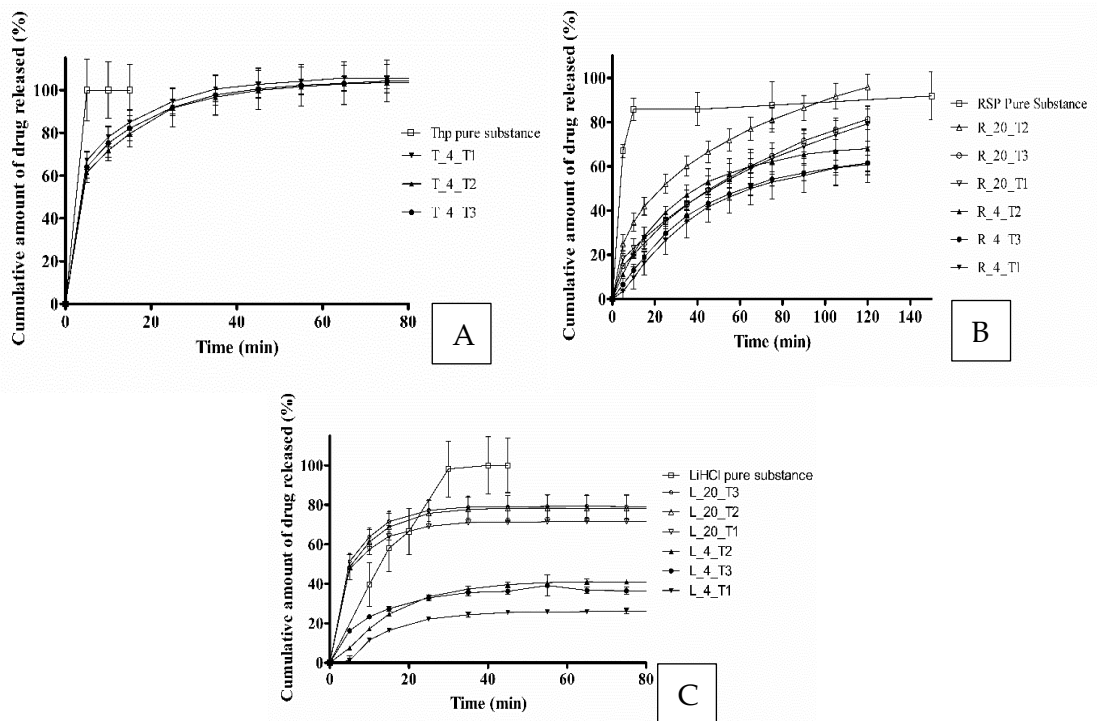


Figure 12. Cumulative drug release of from Thp (A), RSP (B) and LiHCl (C) loaded CBs and release profiles of pure substances.

Overall, the dissolution profiles prove that the drug release from the loaded CBs is related to the solubility of the APIs in the dissolution media and also the loading degree had an effect on the release profile as it can be seen from RSP and LiHCl loaded beads. However, the choice of different types of CBs did not result in a difference in the release profiles. It can be concluded that when the API is freely soluble in the medium, cellulose beads provide a controlled release of the substance. Thus, cellulose beads can be utilized for controlling the release.

5.5.2 Anionic cellulose beads (II-III)

As mentioned in section 5.4.1, drug incorporation into negatively charged CBs (anionic) was higher than with the reference beads which were not oxidized (see Table 8). The reason behind this finding is the higher of amount of cavities (small meso and micropores) where RaHCl could accumulate in the oxidized beads. It is apparent that the significant difference of the released amount of drug on the cumulative release profile comes from the drug incorporation differences (Figure 13 A). However, regardless of the oxidation state of the beads, the drug release levelled off after 2 hours and all types of beads released almost an equal percentage of the drug in any given time (Figure 13 B). Therefore, it can be concluded that there are no stronger interactions between the cationic drug and the anionic surface of the pores regarding the drug release rates.

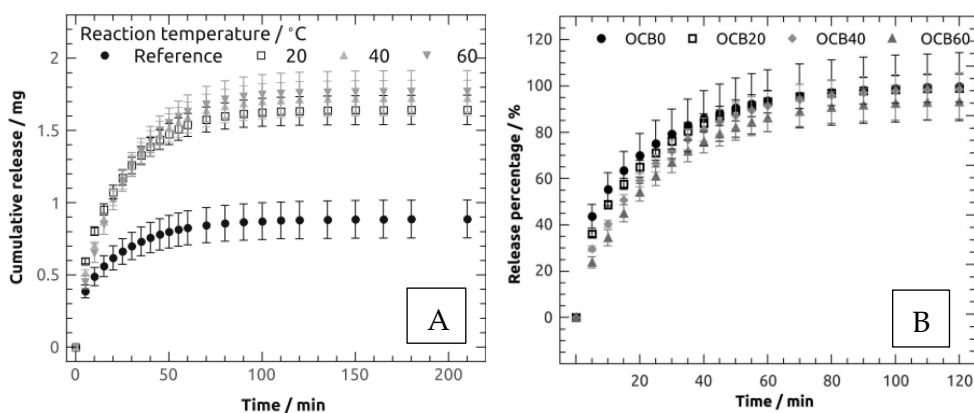


Figure 13. A. Cumulative release profiles B. Release rate (%) of Ranitidine HCl from non-oxidized and oxidized CBs at pH 7.4.

To investigate the effect of dissolution media with different pH on the drug release, dissolution studies were also performed in sodium phosphate buffer media with pH values of 1.2 and 3.6, in addition to 7.4. It was found that the swelling of the beads increased with higher oxidization time and higher pH of the dissolution media [57]. However, the swelling did not affect the release profiles significantly, the drug release profile exhibited a similar pattern regardless of the bead type and pH of the dissolution media. The similarity of the release profiles indicates that the pores were open and the drug could dissolve easily even without swelling and additional opening of the cellulose matrix which were reached by higher oxidation.

5.5.3 Cellulose discs (IV)

Content studies on CDs have shown that CDs which were loaded in drug loading solutions with two specific drug concentrations (80 mg/ml for LiHCl and 1 mg/ml for TAA) can provide sufficient content amount within the discs. Sink conditions were maintained during the drug release studies since the drug content was approx. 4 mg for LiHCl loaded CDs, and 50 μ g for TAA loaded CDs and dissolution studies were done in 50 ml sodium phosphate buffer solutions.

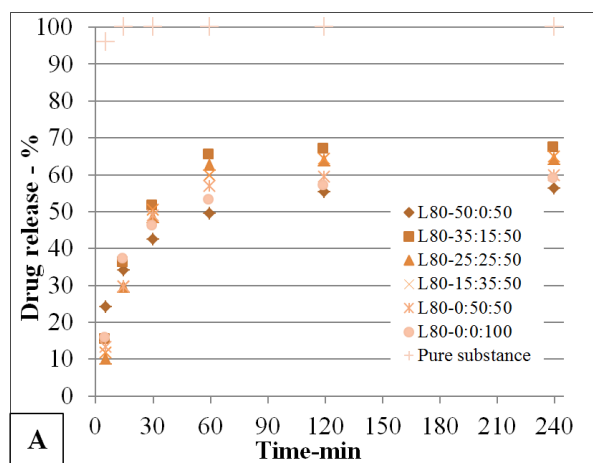


Figure 14. Cumulative drug release rates of LiHCl loaded bilayered buccal patches and pure LiHCl in pH 7.4 phosphate buffer (n=3, standard deviations <10% for all the cases).

Figure 14 shows that the LiHCl release from bilayered patches has a characteristic 10-20% initial release in the first 5 minutes and is followed by a steady release for the next 4 hours. However, pure LiHCl dissolves very fast in the dissolution medium (within 5 minutes). Thus, we can state that incorporating LiHCl into CDs with PEG 6000-PEG 400 and obtaining 4 hours of release can reduce the frequency of application of LiHCl at the ulcer site due to sustained release.

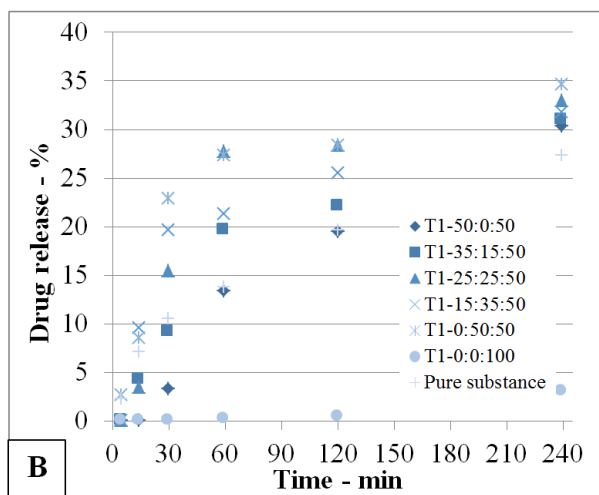


Figure 15. Cumulative drug release rates of TAA loaded bilayered buccal patches and pure TAA in pH 7.4 phosphate buffer (n=3, standard deviations <10% for all the cases).

In the case of bilayered patches which were formed with TAA loaded CDs, a sustained release of the API from the formulation was also observed, except the formulation where loaded CDs did not include any polymers (T1-0:0:100). The reason behind the very low and slow release in this specific formulation was the entrapment of the API within the matrix due to shrinkage and slow diffusion due to the low solubility of the API in the dissolution medium. However, Figure 15 shows that the TAA release rates from other formulations were similar to each other and approx. 20 μg of the TAA content from all formulations were released within 4 hours. A similar effect could be achieved with employing CDs compared to the marketed product since the TAA content in the marketed product is 25 μg .

5.6 Solid state characterization

5.6.1 FTIR and Raman spectroscopy on empty cellulose beads (II)

FTIR and Raman spectroscopy studies mainly were conducted by the first author of article II. However, it is important to present the findings regarding the polymorphic form of empty cellulose beads in the thesis. Therefore, the results obtained from spectrophotometric studies on non-ionic and anionic CBs which were performed by using FTIR and Raman spectrometers can briefly be explained here in this section [56]. The results show that in the Raman spectra, vibrations at 1.463 cm^{-1} and 1266 cm^{-1} are specific for the cellulose II polymorphic form [61]. Thus, it could be concluded that both non-ionic and anionic CBs were in that specific polymorphic form [61]. In addition, the vibrational difference between non-oxidized and oxidized cellulose beads are most profound in between the wavenumbers $1400\text{-}1600\text{ cm}^{-1}$ in FTIR, $1400\text{-}1650\text{ cm}^{-1}$ in the Raman spectrum. Thus, we can state that increasing the oxidation temperature resulted in a rise in the linear trend at the vibrations which were related to R-COO stretching in these regions. This finding implies that an anionic nature (total amount of AGs) can be predicted quantitatively by using FTIR/Raman.

5.6.2 DSC on empty and loaded cellulose beads and discs (III-IV)

5.6.1.1 Cellulose beads (III)

DSC analyses were performed after empty and loaded non-ionic and anionic CBs were dried at room temperature for at least two days. The results of the DSC analysis on empty CBs are presented in Figure 16. The rise on the endothermic dehydration peaks at $200\text{-}210\text{ }^{\circ}\text{C}$ proves that the hydrophilicity of the anionic CBs was increased with oxidation time. Moreover, endothermic dehydration for non-ionic (OCB0) was not observed in the region of $200\text{-}210\text{ }^{\circ}\text{C}$ but later at $225\text{ }^{\circ}\text{C}$. The reason behind this finding was the tighter and stronger water bound to the OCB0 internal structure compared to the oxidized CBs.

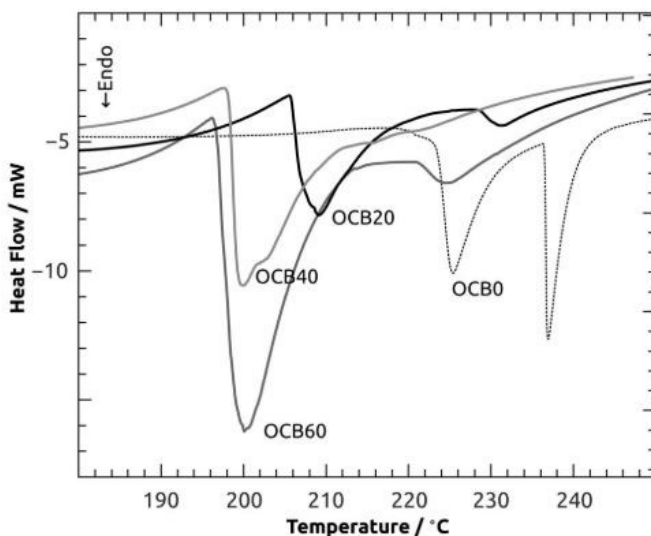


Figure 16. Results of DSC analyses on empty non-oxidized (OCB0) and oxidized (OCB20-40-60) CBs.

Figure 17 presents the results of DSC analyses of pure ranitidine HCl and loaded non-oxidized and oxidized CBs. In the literature, the melting point of RaHCl is described as 134-140 °C for the polymorphic form I, and 144 °C for the polymorphic form II [62]. From the graph below, we can see that pure RaHCl used in this study has a melting point around 144 °C, thus it can be stated that RaHCl was in the polymorphic form II. Also, it is observed that the melting is followed by an instant exothermic decomposition. However, an endothermic peak which signifies the melting point of RaHCl, could not be observed with the drug loaded beads, indicating the amorphous distribution of the drug in the cellulose matrix (Figure 17). In addition, the exothermic decomposition of RaHCl was delayed 20-50 °C. The reason behind this phenomenon could be the slower heat transfer from the cellulose beads to the API.

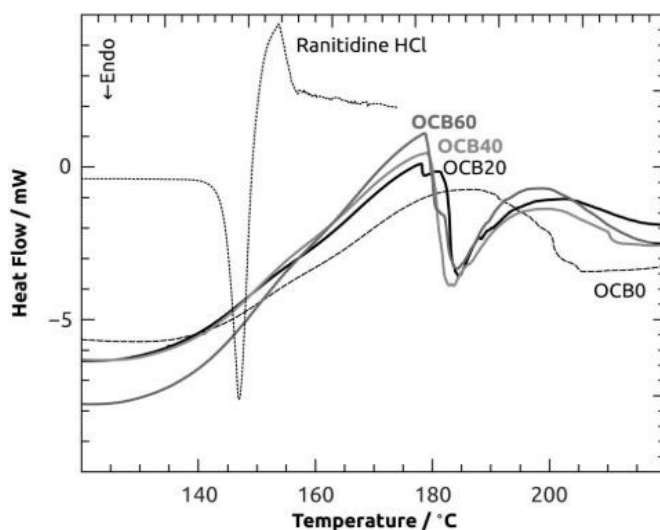


Figure 17. Results of DSC analyses of RaHCl, loaded non-oxidized (OCB0) and oxidized (OCB20-40-60) CBs.

In RaHCl loaded beads the endothermic dehydration peaks (around 180 °C) were very close to each other. However, these dehydration peaks had significant differences in unloaded CBs (Figure 17). This result indicates the partial replacement of crystal water embedded in the cellulose matrix with RaHCl. In addition, a higher drug loading as function of anionic charges with longer oxidation time can be confirmed with increasing exothermic heat flow from loaded OCB0 to OCB60.

5.6.1.2 Cellulose discs (IV)

As it was mentioned in section 4.2.1-3, the cellulose solutions were dropped into the nitric acid solution to form cellulose beads/discs. After the formation was completed, the beads/discs were removed from the nitric acid solution and washed under tap water for several hours. Washed cellulose beads/discs then were immersed into distilled water or ethanol depending on the drug loading solution they will be incubated in later. Therefore, DSC studies were performed to prove that the washing procedure was efficient enough for the removal of nitric acid. Figure 18 compares the DSC analyses of the washed and unwashed CDs.

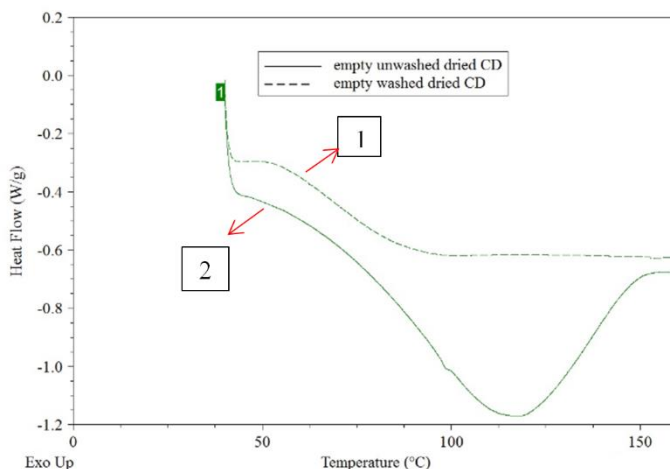


Figure 18. Results of DSC analyses of empty washed (1) and unwashed (2) cellulose discs.

In figure 18, the endothermic thermal event before 100 °C indicates the evaporation of trapped humidity in the internal structure of the empty washed CDs. The absence of any endothermic or exothermic peak proves the successful removal of nitric acid or any other residuals such as sodium hydroxide or urea, thus we could conclude that the washing technique was adequate. On the other hand, unwashed CDs had multiple endothermic peaks due to remaining nitric acid in the matrix of the cellulose discs.

It was essential to determine the solid-state properties of the APIs within the CDs. Therefore, pure substances (LiHCl, TAA, PEG 6000, PEG 400), physical mixtures, and empty-loaded cellulose discs were examined with DSC (Figure 19).

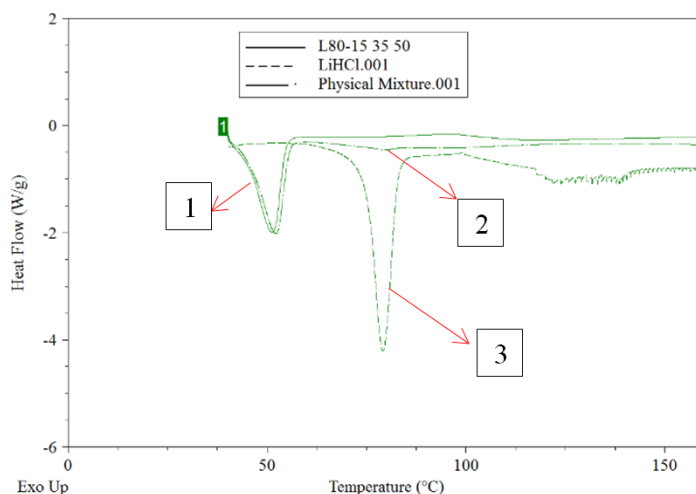


Figure 19. Results of DSC analyses of (1) LiHCl loaded CDs (L80–15:35:50), (2) physical mixture of empty CDs, LiHCl, polymers and (3) pure LiHCl.

PEG 6000 has a melting point around 55–60 °C [59] and PEG 400 has a melting point around 4 °C [63]. Therefore, in figure 19, an endothermic peak assigned to the melting of PEG 6000 can be observed in both loaded CDs and physical mixtures (1,2). However, due to the low melting point of PEG400, there was no observable endothermic change related to PEG 400, both loaded CDs and physical mixtures.

In the literature, the melting point of LiHCl is stated as 70 °C [64]. The enthalpy changes due to the melting of LiHCl can be seen in the DSC spectrum of pure LiHCl as a sharp endothermic peak (3) and in the physical mixture as a small endothermic peak (2). Moreover, no melting point for LiHCl was observed in loaded CDs (1). As conclusion we could state that LiHCl is in an amorphous state within the matrix of the CDs. However, as it was discussed in section 5.3.2, crystalline LiHCl on the surface of the loaded discs was observed with FE-SEM analyses. Therefore, it could also be stated that absence of an endothermic melting peak in the DSC spectrum of loaded CDs may also be a result of dilution rather than conversion to the amorphous form, as the endothermic peak assigned to the melting of pure crystalline LiHCl in the physical mixture was not very evident, too.

Due to the very low amount of TAA in the loaded CDs, it was not possible to confirm the solid state of TAA within the CDs by employing DSC. However, since the pure crystalline TAA release is similar to the release of TAA from loaded CDs (section 5.5.3), it can be assumed that the API is in a crystalline state in the CDs in the case of TAA loaded CDs.

5.6 Other analyses performed on CBs and CDs

5.6.1 Hyperspectral NIR imaging on empty & loaded CBs (I)

Dried empty and LiHCl loaded CBs were first cut in half with a razor blade and scanned by the hyperspectral NIR imaging equipment. The spectral data NIR was visualized by multivariate analysis (Fig. 19 A–D).

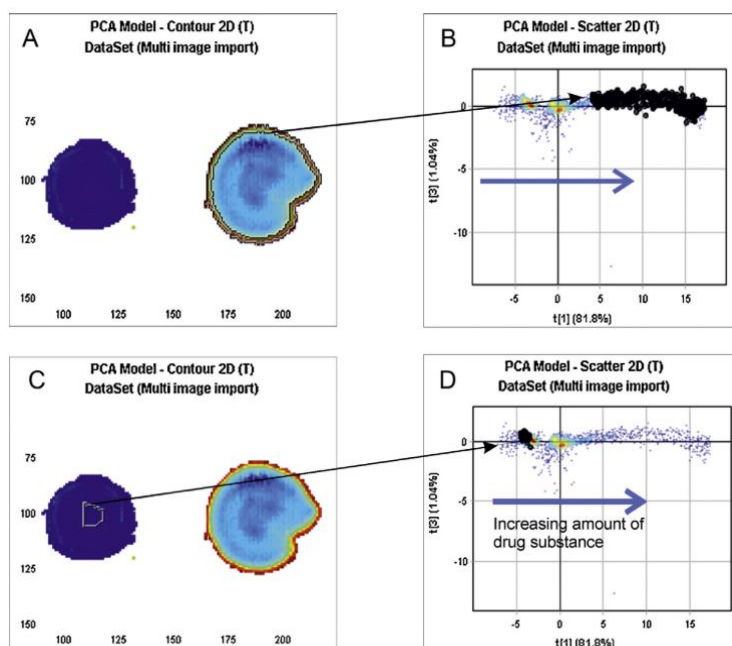


Figure 19. A contour plot (PCA model) of the original image with an empty cellulose bead (left) and a LiHCl loaded CB (right) with chosen pixels of interest from the surface of the loaded bead (A) and from the middle of the unloaded bead (C), APCA scatter 2D density plot indicating the chosen pixels of interest (coloured in black) (B–D). The scatter plots show the first and the third principal component (PC), which reveal 81.8 and 1.0% of the variation in the spectral data respectively.

From the NIR spectral data of the original samples, contour and scatter 2D plots can be created by employing principal component analysis (PCA) [65]. In Figure 19 A and C in the contour 2D plots, selected pixels of interest can be visualized in the 2D scatter density plots as in Figure 19 B and D. These plots reveal clusters of pixels with similar NIR spectral properties. In addition, selecting pixels in the scatter plot indicate the corresponding pixels in the contour 2D image plot. The scatter and contour plots of empty and loaded beads indicate that higher drug loading was detected on the outermost of the LiHCl loaded beads (Figure 19 A-B). Moreover, when the pixel was chosen from the middle of the unloaded beads and spectral data converted to scatter plot, there was no drug substance detected (Figure 19 C-D). In addition, it is important to state that when analysed further the distinct cluster in the middle of the scatter plots corresponds to values in the middle of the loaded bead. Similar information was gained for TAA and RSP (data not shown).

In section 5.5.1, the burst release of LiHCl shown in Figure 12, since NIR imaging of loaded LiHCl showed increased drug amount on the outmost layer, we could state that burst release can be explained by the location of the drug on the outer layer. Overall it can be concluded that hyperspectral imaging with NIR appears to be a very powerful analytical tool supporting the drug distribution behaviour within the beads.

5.6.2 Mechanical tests on CDs – strength and mucoadhesion (IV)

Strength and mucoadhesion studies were performed only on CD formulations which were used in in-vitro drug release studies. The analyses showed that CDs which were prepared by immersing the empty beads into the drug loading solutions with 15:35:50 and 0:50:50 (PEG 6000:PEG 400:ethanol, w:w:w %) solvent ratios were not breaking, but instead flattened under the maximum force of 130 N. All the other formulations were fragile and broke at approximately 30 N forces or below. As it was observed and stated in the literature, PEG 6000 solidifies within the CDs at room temperature [66], therefore these formulations which were loaded in solvents with high PEG 6000 amounts depicted a fragile morphology. However, it was decided to continue studying the formulation which were loaded in a 15:35:50 (PEG 6000:PEG 400:ethanol, w:w:w %) solvent ratio due to the stronger shrinkage preventing abilities of PEG 6000 [58].

For mucoadhesion tests, cellulose discs loaded in drug loading solution which were prepared by dissolving 80 mg/ml LiHCl and 1 mg/ml TAA in 15:35:50 (PEG 6000:PEG 400:ethanol) w/w/w solution, and the marketed product Aftab were used. The maximum detachment impulse (N.s) described in section 4.2.10.2 was determined for each sample and the data is shown in Table 9.

Table 9. Mucoadhesion test, comparison of positive area under the curve (detachment force vs. time, n=3).

Wetting 0.1 ml	Formulation- Codes	Positive area under peak N.s
With buffer	L80-15:35:50	0.37 ±0.09
	T1-15:35:50	0.28 ±0.13
With saliva	L80-15:35:50	0.41 ±0.13
	T1-15:35:50	0.26 ±0.10
No wetting	Aftab	0.88 ±0.05

As it can be seen in table 9, the detachment impulse needed to separate the CD formulations from the tissues were 30 to 60% of the marketed product Aftab. This result indicates that CD formulations are not as mucoadhesive as the marketed product. The marketed product, Aftab, includes carbomer and polyvinylpyrrolidone (PVP) as excipients which have better mucoadhesivity than PEG 400 and PEG 6000 [67,68]. Thus, different polymers with better oral mucoadhesion properties such as carbomer, PVP, poly(acrylic acid), cellulose ester derivatives, chitosan, etc. [69,70], should be studied and used in the formulation stage in the future. On the other hand, it can be stated that using the filtered saliva collected from volunteers or buffer for wetting did not create any significant difference on the mucoadhesion. Therefore, buffer solution can be used as a substitute for the saliva in future studies.

6. Summary and conclusion

The aim of this thesis was to investigate the potential of the physically and chemically modified cellulose for drug delivery purposes. Cellulose was dissolved in a water-based environmentally friendly solvent system and then the dissolved cellulose was dropped into an acidic medium to form cellulose beads and discs (physical modification). Coagulated beads were oxidized to obtain functionalised beads (chemical modification).

Freely and poorly soluble drugs were incorporated into porous non-ionic CBs by immersing technique. The results showed that the porosity of the beads and the concentration of the drug loading solution were the parameters controlling the drug entrapment efficacy. However, the drug release rates were only influenced by the solubility of the APIs in the dissolution medium since the release was diffusion controlled. When the APIs were freely soluble in media, controlled release was achieved by incorporating the drug substance into the cellulose bead matrix. Thus, cellulose beads could be employed for controlling the release of freely soluble APIs.

Non-ionic beads were functionalised by using oxidation as a chemical modification method. Oxidized (anionic) and unoxidized (non-ionic) beads were loaded with a cationic model drug to understand the effect of oxidation and the amount of anionic groups in cellulose beads on drug incorporation and release. Oxidized beads were able to entrap almost double the amount of the model drug compared to non-oxidized beads due to the opening of small pores and mesopores with oxidation. However, release profiles were not affected by the amount of anionic groups within the beads. Thermal and spectroscopic analyses showed that the drug is solidified in amorphous form in both bead types. These findings point out that chemically modified beads could be utilized in the delivery of poorly soluble drugs.

In the last stage of this research, a different geometry than beads, namely discs, were formed by altering the properties of the precipitation medium. Cellulose discs were investigated as drug carrier for oromucosal drug delivery. The final formulation consisted of discs, PEG 6000, PEG 400, and EC.

Sufficient hardness, desired drug content and in vitro release properties were achieved by choosing the correct amount of PEG combination in the formulation. A prolonged release of the API for 4 hours in the mucosal area could be achieved. However, the mucoadhesive properties were not sufficient enough compared to the already marketed product and further studies are needed to achieve proper mucoadhesivity.

In addition to the benefits mentioned above, cellulose beads or discs could carry equal amounts of drug content. Thus, acceptable drug content uniformity could be achieved due to even volume and weight of the produced beads and discs. It could be concluded that due to the easy loading procedure with the immersion technique and high content uniformity, cellulose beads and discs could be utilized when fast dose adjustment is needed for the final formulation.

As summary, the thesis represents the research performed on regenerated non-oxidised cellulose beads, discs (formed by physical modification) and oxidized cellulose beads (functionalised by chemical modification) as potential drug carriers for different drug delivery systems. This thesis created a new understanding for how the novel cellulose beads can be employed to control the release of freely soluble and cationic drugs and furthermore how cellulose discs can bring new opportunities to oromucosal drug delivery systems due to their non-eroding nature which provides a sustained release of the API. However, further research is needed to improve their properties in order to enrich their attributes as drug carriers.

7. Sammanfattning (Summary in Swedish)

Moderna framsteg inom läkemedelsindustrin har väckt intresse för att designa/utforma, modifiera och regenerera polymerer som kan förbättra kvaliteten på de slutliga doseringsformerna. Cellulosa, den mest rikliga biopolymeren i världen, har grundligt undersökts av forskare under årtionden. Egenskaper hos den naturliga cellulosan kan förändras genom fysikaliska och kemiska modifieringsmetoder enligt de egenskaper som behövs för slutprodukten. Dessa metoder syftar till att bryta vätebindningarna mellan cellulosakedjorna eller ersätta en hydroxigrupp med en funktionell grupp för att erhålla de önskade egenskaperna.

Syftet med föreliggande arbetet var att undersöka läkemedelsapplikationer av cellulosapärlor och -skivor som framställdes av naturlig cellulosa genom en ny/modern, miljövänlig, fysikalisk och kemisk modifieringsteknik.

Först undersöktes läkemedelsintagning och frisättnings förmågor av porösa cellulosapärlor, vilka formulerades genom att upplösa cellulosa i ett natriumhydroxid-urea-vatten-lösningssystem och varefter lösningen droppades in i ett surt medium.

Därefter modifierades cellulosapärorna kemiskt med oxidation. Dessa oxiderade, negativt laddade pärlorna laddades med katjoniska läkemedelssubstanser för att undersöka effekten av motsatta laddningar vid läkemedelsinfångning och frisättning. Till sist formades den upplösta cellulosan till en annan geometrisk form; nämligen skivor, genom att modifiera utfällningsmedlets egenskaper för att undersöka deras möjliga användning vid läkemedelstillförsel i munhålan.

Sammanfattningsvis visade resultaten att icke-oxiderade pärlor, oxiderade pärlor och skivor kan användas som ett mångsidigt hjälpämne för framställning av olika farmaceutiska doseringsformer. En omfattande utvärdering av den fulla potentialen hos cellulosapärlor och -skivor behöver emellertid ytterligare undersökning.

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Physically and Chemically Modified Cellulose for Drug Delivery

In the scope of this work, physically and chemically modified cellulose was investigated for the drug delivery purposes. The research was performed on regenerated non-oxidised cellulose beads, discs (formed by physical modification) and oxidized cellulose beads (functionalised by chemical modification) as potential drug carriers for different drug delivery systems. This thesis created a new understanding for how the novel cellulose beads can be employed to control the release of freely soluble and cationic drugs and furthermore how cellulose discs can bring new opportunities to oromucosal drug delivery systems due to their non-eroding nature which provides a sustained release of the active pharmaceutical ingredients.

