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KOPROCESIRANE DIREKTNO STISLJIVE POMOŽNE SNOVI PREDHODNO OBDELANE CELULOZE IN FUKOZE

CO-PROCESSED DIRECT-COMPRESSION EXCIPIENTS OF PRETREATED POWDERED CELLULOSE AND FUCOSE

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I, Teja Pucelj, student of pharmacy at the University of Ljubljana, Faculty of Pharmacy, performed this thesis research work within Erasmus student mobility exchange program, at the University of Tartu, Faculty of Medicine, at the Department of Pharmacy. Host supervisor was Jyrki Tapio Heinämäki, Ph.D., and home supervisor was prof. dr. Stanko Srčič. Preparation of co-processed excipients and DSC were made at the Department of Pharmacy in Tartu, XRPD and laser diffractometry were performed at the Institute of Chemistry in Tartu, SEMs were made at the Institute of Physics and powder flow, tablet compression and testings were carried out at the Faculty of Pharmacy, University of Helsinki.

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Statement

I declare that I made this thesis research work under the supervision of prof. dr. Stanko Srčič and Jyrki Tapio Heinämäki, Ph. D.

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ABSTRACT

Co-processed excipients (CPEs) are combinations of two or more excipients that can be used as new excipients for tablet direct compression. The great advantage of the preparation process of CPEs is special modification without altering the chemical mixture. CPEs have the synergistic effect of both co-processed materials in terms of better compressibility, improved flow properties, better dilution potential, fewer fill-weight variation problems, and reduced lubricant and moisture sensitivity.

The purpose of this research was to develop co-processed binary mixture of catalytic pretreated softwood cellulose (CPSC) and rare sugar, representing novel CPE for direct compression. The specific aims were: (a) To develop a sugar thin layering and thermally induced surface engineering technique for preparing CPE(s) and (b) To investigate physico-chemical properties, particle and powder properties, as well as compression properties of the present CPEs.

After the preliminary test L-Fucose was selected, and used for thin layering of CPSC (1, 2 and 3 L-Fucose layers) in the laboratory-scale and exposed to a short-term thermal treatment at elevated specified temperature (150˚C), slightly above the melting temperature of the sugar component. Tablets were compressed using an instrumented Korsch EK-0 eccentric tableting machine. Melting point of sugar was determinated by differential scanning calorimetry (DSC), particle size was measured by laser diffractometry, solid-state properties were investigated using X-ray powder diffractometry (XPRD) and for more detailed information about surface morphology, shape and size, scanning electron microscope (SEM) was used. The compactibility of CPEs was evaluated by determining the relationship between the upper punch compression force and tablet crushing strength.
In XRPD graphs of CPEs after 3 and 5 consecutive thermal treatments, the same 2θ diffraction angles were clearly noticed as in L-Fucose XRPD graphs. The shape of CPEs particles in SEMs was irregular and elongated. Regarding tablet compression, two pure materials, MCC and lactose, exhibited the highest and the lowest mechanical strength values for tablets. All CPEs had almost identical compression pressure - crushing strength profiles, also equivalent to the pure CPSC, used as a reference.

To some up, co-processed excipients of powder cellulose, prepared by sugar thin layering and thermally induced particle engineering are potencial excipients for pharmaceutical direct compression application.

Key words: direct compression, co-processed excipients, catalytic pretreated softwood cellulose, L-Fucose, thermally induced surface engineering.

RAZŠIRJEN POVZETEK

Uvod: V sodobni industriji tablete predstavljajo 80 % vseh uporabljenih farmacevtskih oblik. Uporabljene metode za izdelavo tablet so vlažno in suho granuliranje ter direktno stiskanje. Ravno slednja je sedaj v največjem razcvetu. Metoda direktnega tabletiranja ima veliko prednosti, vendar z glavno omejitvijo: manj kot 20 % materialov je primernih za izdelavo po tej metodi. To je zadosten razlog, da se veliko vlaga v iskanje primernih pomožnih snovi, ki bi izboljšale sam proces stiskanja. Ker pa je načrtovanje, izdelava in testiranje popolnoma novih pomožnih snovi stroškovno neučinkovito, se industrija raje odloča za že poznane materiale. S spremembo premreženja polimera (npr. želatiran škrob, natrijeva karboksimetil celuloza, krospovidon), z načrtovanjem površin delcev (α-laktoza monohidrat) ali s kombinacijo dveh ali več pomožnih snovi, lahko dobimo pomožne snovi z želenimi fizikalno-kemijskimi lastnostmi. Materiale s kombinacijo dveh ali več pomožnih snovi imenujemo koprocesirane pomožne snovi. Uporabimo jih lahko kot nove pomožne snovi za direktno tabletiranje. Največja prednost koprocesiranih pomožnih snovi je priprava, ki ne vpliva na osnovno kemijsko strukturo. Koprocesirane pomožne snovi imajo sinergistični učinek vseh izvornih pomožnih snovi v smislu izboljšanja stisljivosti, boljših pretočnih lastnosti, večje kapacitete polnila, izogibanja problemov z variacijo teže ob tabletiranju ter manjši občutljivosti na drsila in vlago. Poleg vsega lahko pripomorejo k zmanjšanju stroškov zaradi izboljšane funkcionalnosti znotraj ene formulacije ter manjšega števila potrebnih testov. Tako lahko v primeru, ko ima vezivo-polnilo slabše lastnosti razgrajevanja, pripravimo koprocesirano pomožno snov. Vezivo-polnilo združimo skupaj z razgrajevalom s sposobnostjo močenja in ustrezno poroznostjo, da dosežemo povečan prevzem vode v farmacevtsko obliko. Prav tako lahko z metodo koprocesiranja dveh razgrajeval dobimo učinek sinergije pri delovanju; tako krospovidon deluje na razgradnjo tablet z močenjem, Na-kroskarmeloza pa z nabrekanjem. Koprocesirane pomožne snovi lahko pripravimo s fizikalno spremembo, mletjem, kristalizacijo, metodo sušenja z razprševanjem, aglomeracijo ali dehidracijo. Stopnje, ki jih moramo izvesti pred izdelavo koprocesiranih pomožnih snovi so sledeče: najprej preučimo fizikalno-kemijske lastnosti materialov in zahteve glede funkcionalnosti, nato izberemo primerne količine posamezne pomožne snovi za dosego ustreznih mehanskih lastnosti med stiskanjem tablet, optimalno velikost delcev in najboljšo metodo za izdelavo koprocesirane pomožne snovi. Na koncu izboljšamo še sam proces, z namenom, da se izognemo odstopom med posameznimi

izdelanimi serijami. Pri celotnem procesu težimo k izdelavi koprocesirane pomožne snovi z minimalnimi stroški.

Namen: Namen naše raziskave je bil, da razvijemo koprocesirano binarno mešanico s kombinacijo katalitično predhodno obdelane celuloze iz mehkega lesa (CPSC) in redkih sladkorjev, ki naj bi predstavljala novo koprocesirano snov za direktno tabletiranje. Cilji naloge so bili: (a) Razviti tankoplastno oblaganje s sladkorjem in metodo za površinsko obdelavo delcev spodbujeno s toploto, za pripravo koprocesiranih pomožnih snovi in (b) Raziskati splošne fizikalno kemijske lastnosti snovi, lastnosti delcev in prahov ter stisljivosti koprocesiranih pomožnih snovi.

Materiali in metode: L-fukozo in D-manozo smo izbrali na osnovi predhodnih testiranj. Predhodno obdelana celuloza (CPSC) je bila izolirana na osnovi teorije nove katalitične predhodne obdelave za ločitev celuloze in lignina iz lignocelulozne biomase. Redka sladkorja in CPSC smo 30 minut mikronizirali v krogličnem mlinu, s frekvenco 28 Hz. Po določitvi velikosti delcev z metodo laserske difrakcije, smo L-fukozo uporabili za tankoplastno oblaganje CPSC, ki je bila predhodno izpostavljena različnim pogojem relativne vlažnosti (54 % in 85 %). Odločili smo se za CPSC izpostavljeno 54 % relativni vlažnosti in izdelali CPE (koprocesirane pomožne snovi) z eno, dvema in tremi plastmi L-fukoze. CPSC delce smo obložili s plastmi L-fukoze z uporabo istega mlina kot v fazi mikronizacije, le da smo tu uporabili nežnejše pogoje; mletje je potekalo 5 minut na frekvenci 10 Hz, brez uporabe kroglice v posodici za mletje. Po vsakem dodatku L-fukoze smo mešanico za kratek čas izpostavili povišani temperaturi (150 ˚C), le rahlo nad temperaturo tališča sladkorne komponente. Za referenco smo v nadaljnjih testiranjih uporabili sledeče materiale: L-fukozo, CPSC in CPSC s 3x simuliranim oblaganjem (enak način priprave kot pri CPE, le brez dodatka fukoze). Vse referenčne materiale smo predhodno mleli 30 minut. Mikrokristalno celulozo in laktozo smo uporabili kot dodatni referenci v zadnjih testih po pripravi tablet. Temperaturo tališča sladkorja smo določili z uporabo diferenčne dinamične kalorimetrije (DSC), lastnosti trdnega stanja in prisotnost L-fukoze v koprocesiranih pomožnih snoveh pa z metodo rentgenske difrakcije prahov (XRPD). Pretočnost prahov smo merili z uporabo metode pretoka prahov skozi odprtino. Za podrobnejše informacije o površini delcev, obliki in velikost, smo uporabili vrstični elektronski mikroskop (SEM). Za tem smo tablete stisnili s pomočjo tabletirke na udarec (Korsch EK-0). Kompaktibilnost koprocesiranih pomožnih snovi smo ovrednotili s

primerjavo razmerja med silo zgornjega pečata med stiskanjem in silo, ki je potrebna za lom tablete.

Rezultati in diskusija: V prvi stopnji smo z zmletjem delcev poskušali dobiti ustrezne velikosti posameznih komponent koprocesiranih pomožnih snovi. Delci CPSC so se manjšali premo sorazmerno s časom mletja. Pri L-fukozi in D-manozi pa smo opazili, da dalj časa ko smo mleli delce L-fukoze in D-manoze, bolj so izkazovali neželeno lastnost občutljivosti na zračno vlago. Pri laserski difrakciji smo zato še dodatno upoštevali rezultate pridobljene po obdelavi z ultrazvokom. Upoštevali smo le meritve po 5, 15 in 30 minutnem mletjem, ker je bila po daljšem mletju distribucija velikosti delcev pri L-fukozi in D-manozi prevelika. Po 30-minutnem mletju CPSC in obdelavi z ultrazvokom je bila mediana volumske porazdelitve (Dia) 46.51 μm. Dia L-fukoze po 30-minutnem mletju in sledeči obdelavi z ultrazvokom je bila 5,99 μm, D-manoze po isti obdelavi pa 9.07 μm. Slike pridobljene z vrstičnim elektronski mikroskopom so nam predstavile jasne razlike v velikosti med CPSC in CPE s 3 in 5 oblogami sladkorja, medtem ko po 1 oblaganju ni bilo opaziti bistvene razlike. Pod večjo povečavo (1000x) smo opazili, da je površina delcev z dodatkom L-fukoze postala manj celostna, saj so se delci L-fukoze naključno zlepili na površini CPSC delcev. Kljub temu, da smo vrstični elektronski mikroskopom v osnovi uporabili za preučevanje površine delcev, smo z njim prav tako pridobili informacije o velikosti delcev. V XRPD grafih koprocesiranih pomožnih snovi smo v primeru oblaganja s 3 in 5 plasti L-fukoze opazili spremembe pri istih 2θ kotih, kot pri referenčnih XRPD grafih L-fukoze. Pri merjenju pretočnih lastnosti prahov je najboljši rezultat izkazovala toplotno obdelana CPSC (53.7 $\frac{\mathrm{mg}}{\mathrm{s}}$ oz. 145.3 $\frac{\mathrm{mm}^3}{\mathrm{s}}$). Koprocesirane pomožne snovi z eno, dvema in tremi oblogami sladkorja so imele slabše pretočne lastnosti. Prav tako so se pojavile težave s ponovljivostjo rezultatov, kot posledica tvorbe grudic.

V zadnji fazi smo pripravili tablete referenčnih materialov in koprocesiranih pomožnih snovi z uporabo direktnega tabletiranja. Takoj smo izmerili njihovo debelino in rezultat uporabili za izračun faktorja elastičnosti, skupaj s podatkom o maksimalni višini tablete med maksimalno silo stiskanja. Pri tabletah izdelanih iz mikrokristalinične celuloze in laktoze smo dobili najvišjo in najnižjo vrednost sile potrebne za lom tablete. Vsi vzorci koprocesiranih pomožnih snovi so imeli skoraj identične profile tlak stiskanja-sila potrebna za lom tablete. Le-ti so bili dokaj podobni pri referenčni CPSC.

Sklep: Koprocesirane pomožne snovi, pripravljene s tankoplastnim oblaganjem s sladkorji in metodo za površinsko obdelavo delcev spodbujeno s toploto, predstavljajo potencialne pomožne snovi za metodo direktnega tabletiranja v industrijskem merilu. Nadaljnji poskusi bi potrebovali strožji nadzor izpostavljenosti sladkorja atmosferski vlagi ter hkrati pripravo bolj homogene binarne mešanice.

Ključne besede: direktno tabletiranje, koprocesirane pomožne snovi, katalitično predhodno obdelana celuloza iz mehkega lesa, fukoza, metoda za površinsko obdelavo delcev spodbujena s toploto.

LIST OF ABBREVIATION

API	active pharmaceutical ingredient
CMC	carboxymethyl cellulose
CPE	co-processed excipients
pre-CPE	preliminary co-processed excipients
CPSC	catalytic pretreated softwood cellulose
DPI	dry powder inhalers
DSC	differential scanning calorimetry
EF	elasticity factor
FDA	Food and Drug Administration
GMP	Good Manufacture Practice
GRAS	generally regarded as safe
HPMC	hydroxypropyl methylcellulose
ICH	International Conference on Harmonisation
IPEC	International Pharmaceutical Excipients Council
MCC	microcrystalline cellulose
MV	mean diameter in microns of the volume distribution
ODT	oral disintegrating tablets
PITs	process-induced phase transformations
PM	physical mixture
PVP	polyvinylpyrrolidone
RH	relative humidity

SEM	scanning electron microscopy
SMCC	silicified microcrystalline cellulose
Tg	glass transition temperature
US	ultrasonic treatment
XRPD	X-ray powder diffraction

1. INTRODUCTION

1.1. Tablet as a dosage form

Tablets are the most preferred solid dosage form. They represent 80 % of all administrated dosage forms (1), due to their safety and convenience in administration, very versatile means of preparation and accurate dosing. Tablets have better chemical and physical stability compared to liquid dosage forms, are safer compared to parenteral administration and likewise tamper-proof compared to capsules. Moreover they can be produced by relatively low cost, using cheap mass production with robust and quality controlled production procedures (1). Three methods can be used for preparation of tablets: wet granulation, dry granulation and direct compression (2). Among all the techniques available, during the past years, the greatest breakthrough has been made in tableting toward direct compression and high speed manufacturing (3). Today direct compression is considered as the most convenient technique for manufacturing tablets due to simplicity of the process and cost effectiveness.

1.2. Direct compression

Direct compression can be defined as a process by which the tablets are pressed directly from powder blend of active pharmaceutical ingredient (API) and suitable excipient. Several stages of powder deformation can be detected during direct tablet compression. Firstly, when the punch starts to penetrate into the die, the smaller particles are forced to move into the voids between bigger particles, thereby the denser form of the powder is obtained. Then particles start to rearrange and form bonds, which provide coherence and elastic deformation takes place. Once the elastic limit of the material is exceeded the particles will start to deform irreversibly. The material can deform plastically or it can fragment. If the compression force is still increasing even the plastically deforming material start to fracture (1, 2, 4).

Pharmaceutical materials vary in their direct compression behaviour. For instance, it is well known that microcrystalline cellulose (MCC) goes through plastic deformation during compression. Dibasic calcium phosphate dihydrate, however, is reported to deform mainly

by fragmentation. Most of the materials are somewhere between, thus the ideal mixture of excipients has to be found, in order to get the right balance between brittle fracture and plastic deformation (1).

Direct compression is a preferred method for the preparation of tablets, due to:

- ✓ Economic advantage; it requires fewer unit operations than wet granulation, including less equipment, lower power consumption, less space, time and labour (5).
- ✓ It is more suitable for heat or moisture sensible active ingredients (5), and it further increases the stability of API.
- ✓ Faster dissolution rate due to the fact that tablets prepared by direct compression disintegrate into particles instead of into granules, which directly come into contact with fluid.
- ✓ Fewer changes in dissolution profiles make the specification easier (5).
- ✓ Less contamination (shorter time of production) including minimal possibility of microbial growth, due to absence of water.
- ✓ Less wear and tear at punches and dies.
- ✓ Reduced documentation and simplified validation (2, 3, 5).

However, less than 20 % of pharmaceutical materials can be compressed directly into tablets (2, 4). The main reason is the poor compressibility of active ingredient, which effects the final content of the whole dosage form. A lot of active ingredients also possess low flowability, lubricant and cohesion properties. Acetaminophen as the poorly compressible active ingredient can represent only 30-40 % of the final product (3). This means, to achieve the specific amount of active ingredient, larger tablets should be produced, which raise a problem of swallow difficulties (5). In addition, segregation, weight variation and content uniformity could appear as a result of density difference of the API and excipients as well as induced static charge (while mixing materials in the dry state). The solution is to design the excipients with particle size as close as possible to the active ingredient (2).

1.3. Excipients role in direct compression manufacture

International Pharmaceutical Excipients Council (IPEC) defines excipients as: “Substances other than the API in finished dosage form, which have been appropriately evaluated for safety and are included in drug delivery system to either aid the processing or to aid manufacture, protect, support, enhance stability, bioavaibility or patient acceptability, assist in product identification, or enhance in any other attributes of the overall safety and effectiveness of the drug delivery system during storage use” (6). Also solvents which might be dried off later are considered as excipients, so they should meet standards of pharmacopoeia unless adequately justified.

There are around 8000 “non-active” ingredients that are used in food, cosmetics, and pharmaceuticals worldwide. Although the FDA maintains a list of inactive ingredients, the EU Pharmacopoeia and other texts of other European countries do not have officially published list. For the use in pharmaceuticals, additional quality, functionality and safety requirements have to be made. The manufacturer of the excipient must follow GMP (7). This is the place where IPEC enters. IPEC is an industry association that comprises three regional organisations, located in US, Europe and Japan, which gives the guidelines following the ISO 9000 structure. They provide a way to assess whether system is in place, provide a means for evaluation of the effectiveness of the system, provide guidance on how to conduct an audit of a manufacturing operation that produces excipients and in turn, give guidelines on auditing their distribution and repackaging (7). Their goal is to standardize the requirements for purity and functionality testing.

The main groups of excipients are: diluents, binders and adhesives, glidants, disintegrants, lubricants, antiadherents, colorants, flavours, and sweeteners (7). There are a lot of excipients owning different function; some of them have more than two. For instance, microcrystalline cellulose (MCC) could function as diluent and disintegrant, while sugars can be diluents, binders and adhesives, flavours and sweeteners. The other example is starch, which can work as a binder and disintegrant.

In the light of tablet manufacturing changes due to direct compression technology progress and appearance of high speed machines, excipients with better flow and compression properties are desirable (5). Faster and high efficient tableting machines have started to run in the production (100.000-200.000 tablets/h). Such a machine requires excipients with

good compressibility at short dwell time, and low weight variation. Moreover, there are other important properties that should be taken into account, especially for excipients used in direct compression. They should have high bulk density, similar particle distribution as the active ingredient as well as minimum batch-to-batch variation (1). Other critical parameters concerning all kinds of excipients, not only those for direct compression are: compatibility with packaging components, physical and chemical stability, physiological inertness, microbiologically pureness and commercial availability at the reasonable price, and suitability to agency requirement (7).

Since excipients represent one of the major part/active support of the final dosage form, the term Functionality is used. Functionality means the desired activity. The equivalent term of the active ingredient is called efficiency (2, 6). It is important to control the functionality by purity and identity, due to multiple functions of excipients and different behaviour, depending upon the vendor (2). Tablets could also have different behaviour after compression, so the batch-to-batch variation due to different vendor could appear (2, 3, 5). Many changes have also been made in the regulation of the materials. Every new medical component that appears on the market had to meet new stability requirements and also some stringent regulatory requirements.

The truth is that there is no excipient that would meet all the criteria and standards. Industry is in deficit of excipients with suitable functionalities, especially in terms of better flow properties and compression properties (3). However, the production of new excipients is not cost effective. There are a lot of fundamental solid-state properties like particle shape, size, morphology, surface area and porosity that have to be tested. These physico-chemical properties can influence on excipient functionality and solid-state characteristics like flowability, compressibility, dilution, disintegration, and lubricant potential. Companies have to do additional safety and toxicity tests. In the recent years no new chemical excipient has been sent onto market (3). The companies rather stay with the well-known materials and make new grades of existing excipients (e.g., pregelatinized starch, croscarmellose, and crospovidone) or design a new combination of existing excipients.

1.4. Particle surface engineering as source of new excipients

Particle engineering is a concept involving the manipulation of particle parameters (particle shape, size and size distribution, surface area, and porosity) (5). This treatment can lead to changes in powder bulk and flow properties, compressibility, moisture sensitivity, and machinability (3).

One of the possibilities to improve physico-chemical properties is the effective treatment of particle surfaces. This can lead to fewer tendencies of interactions between the particles and finally better flow properties. Moreover, usage of the process of particle surface engineering is cost and time effective as it avoids the granulation step and use direct compression method instead. There were a lot of research studies made for particle surface engineering in the field of dry powder inhalers, tablets and capsules (8).

Two methods of particle surface engineering can be used: particle surface smoothing or powders particles coating. The process of particle smoothing may decrease attraction forces between particles and reduces the tendency to interlock. As the result, compound with better flow and packing properties can be obtained. Modified in this way, α-lactose monohydrate is very popular as a carrier for dry powder inhalers (DPI). There is a various selection of smoothing procedures, for example controlled crystallisation from the different solvents, wetting in a water-ethanol mixture (with or without a ternary component) followed by drying in a high-shear mixer, and high temperature experiments with alcohol solutions. The changes in particle morphology occur as the result of Ostwald ripening phenomena, which appears due to solubility differences between smaller and bigger particles. Smaller particles are dissolved and recrystallized in the non-round areas of the larger ones, giving rise to smooth particle surface. Generation of fine particles on the surface of lactose during milling could give the result of better performance of DPIs. What is more, as the smoothing technique, supercritical fluid technology and aerosol flow reactor method can be carried out.

The other approach, coating of the powder particles, may improve the dispersion, dissolution and flow rate, as well as the bad taste, environmental protection and could control release properties. The whole process needs careful consideration due to small size of the particles, irregular shape and high level of adhesion/cohesion and therefore possibility of agglomeration. Coating of powder particles can be carried out with liquid by using top-spray fluidized bed system or spray drying technique, coacervation of the coating

agent in a stirred suspension followed by filtration of the suspension and drying the wetted material, and the physical dry blending of the particles (8).

Particle engineering in a term of using a single material can provide only a few functionality improvements. In further cases it is better to use co-processing or particle engineering of a two or more existing excipients (3).

1.5. Co-processed excipients

Co-processed excipients can be described as a mixture of two or more well-known excipients on a subpartical level with synergistic benefits and minimization of drawbacks. Process includes special modification without altering the chemical mixture. One particle could be incorporated into the particle structure of the other, by using co-drying method (drum drying or spray drying) or co-precipitation (5, 9). The process combines the advantages of wet granulation with direct compression (3). By embedding powder in minigranules, a homogenous distribution without segregation can be achieved. This treatment can also minimize anisotropic behaviour of the particles, so that the deformation can occur along any plane. As a result, multiple clean surfaces are formed during the direct compression.

For instance, if a filler-binder has low disintegration properties, it can be co-processed with the excipient possessing good wetting properties and porosity (disintegrant) in order to increase the water uptake (5). Also two disintegrants acting on a different way (crospovidone by wicking action, croscarmelose sodium by swelling action) can be co-processed in order to achieve synergistic effect (10).

Methods for preparation of co-processed excipients are: physical modification, grinding, crystallization, spray-drying, agglomeration and dehydration (5). Nowadays there are already some available co-processed excipients on the market. They are mentioned in Table I, accompanied with their way of application and main advantages.

Table I: Some examples of the commercially available co-processed excipients for direct compression (2), (3), (5), (11), (12), (13).

Brand name	Adjuvant/s	Application	Advantages	Manufacturer, country
Advantose FS-95	Fructose 95 %, starch 5 %	Nutraceutical and chewable vitamin application	Excellent flow, good compressibility, improved tablet hardness, superior sweetness	SPI Polyols, France
Avicel CE-15	MCC, guar gum	Chewable tablets	Less grittiness, minimal chalkiness, overall palatability	FMC Biopolymer, Philadelphia, USA
Barcroft CS 90	Calcium carbonate, starch	Diluent for direct compression		SPI Pharma, Wilmington, USA
Barcroft Premix St	$Al(OH)_3$, $Mg(OH)_2$, sorbitol	Diluent for direct compression		SPI Pharma, Wilmington, USA
Cellactose 80	75 % α-lactose monohydrate , 25 % cellulose powder	High-dosage tablet, herbal formulation	Good flowability, highly compressible, good mouthfeel	Meggle AG, Germany
DI-PAC	Sucrose, dextrin	Diluent for direct compression	High flowability, low hygroscopicity, sweetness and nonreactivity with other tablet components	American sugar, USA
F-Melt (type: C, M, F1)	Carbohydrates, disintegrants, inorganic ingredients	For manufacturing oral disintegrating tablets (ODT)	Fast oral disintegrating time, high tablet strenght, high API loading, highly flowable with minimum or no sticking/capping	Fuji Chemical Industry Co., Ltd (Japan)
Ludiflash	Mannitol 90 %, Kollidon CL-SF (crospovidone) 5%, Kollicoat SR 30 D (polyvinyl acetate)	Direct compression, high speed tableting	Good flowability, less water absorption, no segregation of the active ingredients	BASF, Germany
Ludipress	Lactose, PVP, crospovidone	Chewable tablets and lozenges, for effervescent tablets and as bulk agent for modified release formulation	Low degree of hygroscopicity, good flowability, tablet hardness, independent of machine speed	BASF, Germany
MicroceLac 100	α-lactose monohydrate 75 %, MCC 25 %	Filler/binder for direct compression	Superior flowability and binding properties, less lubricant sensitivity	Meggle AG, Germany
Pharmatose DCL 40	95 % β-lactose, 5 % lacitol	Direct compression	Good flowability, high dilution potential(better binding properties), low water uptake	DMV, Netherlands

Prosolv	MCC, colloidal silicone dioxide	Direct compression of tablets containing poorly compressible ingredients	Better flow, hardness, reduced friability	Pen west USA, JRS Pharma, Germany
Plasdone S-630 copovidone	Vinyl acetate, N-vinyl-2-pyrrolidone	Tablet binder, matrix polymer for solid dispersions	More flexible, less brittle films compared to films of PVP homopolymers	ISP, USA
RetaLac	Equal parts of α-lactose monohydrate and hypromellose [HPMC]	Direct compression, especially for sustained release formulation; replacement of formulations using wet granulation	Improves wettability of HPMC, minimises friability	Meggle AG, Germany
Starlac	80 % α-lactose monohydrate, 15 % maize starch	In low dosage and fast dissolving formulation, cores for coating, homeopathic formulation	Good flowability, low lubricant sensitivity	Roquette, France/Meggle AG, Germany
Xylitab	Xylitol, Na CMC			Danisco, USA

Steps in the design of co-processed excipients are:

1. Studying the physico-chemical properties of the materials and requirements about their functionality.
2. Selection of proper amount of each material to get desirable mechanical properties during tablet compression.
3. Find the proportion of the excipient in order to get the homogenous dispersion solution (additional step in preparation by spray drying).
4. Selection of optimal particle size.
5. Selection of the best method of preparation co-processed excipients (5).
6. Optimization of the process in order to avoid batch-to-batch variation.

Likewise the chosen material should be at the reasonable price (3).

Advantages of co-processed excipients**:**

- ✓ Absence of chemical changes, resulting in fewer regulatory problems. If the parent excipients are generally regarded as safe (GRAS), the same can also be considered about the co-processed excipients. On top of, they do not require additional toxicology studies (5).
- ✓ Improved compressibility, because only few excipients show no elastic recovery after compression, which means we have to combine them with good compressible dilutant (2). Example: MCC loses compressibility after the water uptake, so it is better to co-process it in SMCC (Silicified Microcrystalline Cellulose).
- ✓ Better dilution potential- this means the highest amount of an active ingredient that could be mixed with excipient and still obtain good compressibility on the way to the final dosage form. A directly compressible excipient should have high dilution potential, so the final dosage form has a minimum weight (2).
- ✓ Improved flow properties as a result of impregnation of one particle into another and therefore optimal particle size, particle-size distribution and spherical shape after spray drying (5). In addition there will be less fill weight variation, so the process should be running well also on high speed compression machines that have short dwell-time (milliseconds). The reproducibility of the transferred powder blender could fulfil the requirements for variation (± 5 %) (2).
- ✓ Reduced lubricant sensitivity; e.g.: the combination of lactose-cellulose in Cellactose, where we use a lot of lactose monohydrate (brittle material), and less cellulose (plastic material). The latter is fixed between or onto the particles of lactose and provides continuous matrix with large surface area for bonding. At the same time lactose has a low lubricant sensitivity, due to newly exposed surfaces upon compression and breaking up the lubricant network (3).
- ✓ Improved organoleptic properties.
- ✓ Decrease of cost because fewer tests and improved functionality.

- ✓ In house procedure which promotes the collaboration of excipients and pharmaceutical manufacturers to develop tailor-made innovative excipients (e.g. Cydex and Pfizers) (3).

Limitations of co-process excipients are linked to a requirement, that every new formulation needs its optimum ratio between API and excipient. The mixture of co-processed excipients is fixed and there is a lack of official acceptance in Pharmacopoeia. That is why in documentation the significant advantages compared to the physical mixtures should be shown (5).

1.6. Catalytic pretreated softwood cellulose (CPSC)

Cellulose is the most abundant high molecular weight linear biopolymer in the world. Cellulose derivates are widely applied in medicine for tissue engineering, controllable drug delivery, and tablet coating. They are chemicaly inert, biocompatible, have good tablet forming properties, including good disintegration properties. Microcrystalline cellulose (MCC) is the most popular excipient used in tablet compression (4). It has both, crystalline and amorphus regions (10). MCC is prepared by hydrolysis of α-cellulose with mineral acids. As a result, partially depolymerized cellulose is formed (10), followed by a formation of aggregates of smaller cellulose fibers using spray drying technique. Two main parameters can be changed using differend kind of cellulose and preparation method: degree of crystallinity and particle size (4). MCC is used as diluent in tablets prepared by wet granulation, as filler in capsules and for the production of spheres (2), and as an anti-adherent, or disintegrant in direct compression (1). It is quite often used excipient in pharmaceutical applications because of good compressibility at low compression pressures, high dilution potential and good flow properties (1).

The main disadvantage of MCC is its high hygroscopicity. The process of solubilising hemicellulose disrupts cellulose crystallinity and/or increases pore volume (75 % of water uptake in 1 week) (3). That can be the reason for capping at the high compression speeds. Also, MCC loses compressibility after the water uptake. The problem can be solved using the co-processed state, for example with silicium dioxide in SMCC (silicified microcrystalline cellulose) (2).

The other solution is to use catalytic pretreated softwood cellulose (CPSC) and hereby to increase the accessible surface area, which is usually sealed with lignin (31%). CPSC was isolated by Hakola et al., from pine soft wood *(Pynus sylvestris)* by using a catalytic oxidation and acid precipitation method. The method was based on theory of a new catalytic pre-treatment method for the separation of cellulose and lignin from lignocelluloses biomass for enzymatic hydrolysis (14, 15). Final product (CPSC) could easily undergo hydrolysis; avoid the loss of hydrolysable carbohydrates and the formation of toxic compounds.

1.7. Rare sugars

There are only seven monosaccharides in the form of pentoses and hexoses that occur frequently in nature: D-Glucose, D-Galactose, D-Mannose, D-Fructose, D-Xylose, D-Ribose and L-Arabinose. Rare sugars are defined as monosaccharides and their derivates scarcely ever existing in the nature. They are usually prepared with enzymatic treatment, which has to be further investigated in the way of better efficiency (16).

Additionally, they have various well known biological functions and enormous potential for applications in pharmaceutical, cosmetics, food and flavour industries. For example, L-Ribose is used as a building block to synthesize the nucleoside analogues in a preparation of clevidine, an antihepatitis B virus drug (16).

The Tabel II. represents the main physico-chemical and organoleptic properties of sugars, taken into preliminary study. Fig. 1 presents selected sugars in Howard projection.

Tabel II. Preliminary study of sugar properties. Key: *99% purity, **98% purity.

	Melting point (˚C)			Physico-chemical properties					
	Merck index (17)	**Sigma Aldrich** (18)	**Alfa Aesar** (19)	**Formula** (17)	**Molecular weight** (17)	**Optical rotation** (19)	**Solubility (water)**	**Solubility(other)**	**Sensitivity**
L-Arabinose	**157-160**	160-163*	155-160*	$C_5H_{10}O_5$	150,13	+104° (c=10 in water, 24h)	100g/100 ml (17)	0,4g/100ml 90% alc. (17)	
D-Arabinose	/	**162-164***	154-158*	$C_5H_{10}O_5$	150,13	-104°(c=10 in water, 24h)	Very soluble (20)	Sligtly soluble in ethanol, insoluble in ether, acetone, MeOH (20)	
L-Fucose	**140**	150-153*	139-142*	$C_6H_{12}O_5$	164,16	-75° (c=10 in water)	soluble (17)	soluble in alc. (17)	hygroscopic (19)
D-Fucose	**144**	144-145**	135-141*	$C_6H_{12}O_5$	164,16	+76° (c=10 in water)	soluble (17)	moderately soluble in alc. (17)	hygroscopic (19)
L-Galactose	**/**	163-165**	/	$C_6H_{12}O_6$	180.16	/	/	/	/
D-Galactose	**167**	168-170*	164-168**	$C_6H_{12}O_6$	180,16	+80° (c=5 in water, 24h)	very soluble (24), finaly at 25˚C= 68% (17)	soluble in pyridine, slightly soluble in alc. (14), insoluble in ether, benzen (20)	/
L-Mannose	/	**129-131***	129-131*	$C_6H_{12}O_6$	180,16	-13,8° (c=10 in water, 20h)	very soluble (20)	/	hygroscopic (19)
D-Mannose	**132-133**	133-140*	163-165**	$C_6H_{12}O_6$	180,16	+13.8° (c=10 in water, 24h)	250g/100 ml (17)	Slightly soluble in EtOH (20), 0,4g/100ml abs. alc., 28,5g/100ml pyridine (17), Insoluble in ether, benzene (20)	hygroscopic (19)
L-Rhamnose	**82-92**	90-95*	89-94*	$C_6H_{12}O_5$	164,16	+8° (c=10 in water, 20h)	very soluble (20), 10g/100ml ?	Very soluble in EtOH (20)	sublimes at 105˚C and 2mmHg (17)
L-Ribose	/	**81-82*** **	85-88*	$C_5H_{10}O_5$	150,13	/	/	/	/
D-Ribose	**87**	88-92*	82-86**	$C_5H_{10}O_5$	150,13	-20° (c=10 in water, 28h)	Soluble (17)	slightly soluble in alc. (17)	hygroscopic (19)
L-Xylose	**153-154**	/	147-151*	$C_5H_{10}O_5$	150,13	-20° (c=10 in water, 10h)	125g/100 ml (17)	soluble in pyridine, hot alc. (17)	hygroscopic (19)
D-Xylose	**144-145**	154-158*	147-151**	$C_5H_{10}O_5$	150,13	+19° (c=10 in water + NH4OH)	Very soluble (17)	Soluble in EtOH, slightly soluble in ether (20)	hygroscopic (19)
D-Xylitol	**93-94,5**	94-97*	92-96*	$C_5H_{12}O_5$	152,15	/	64,2g/100 g (17)	1,2g/100g ethanol, 6,0g/100g methanol (17)	hygroscopic (17)

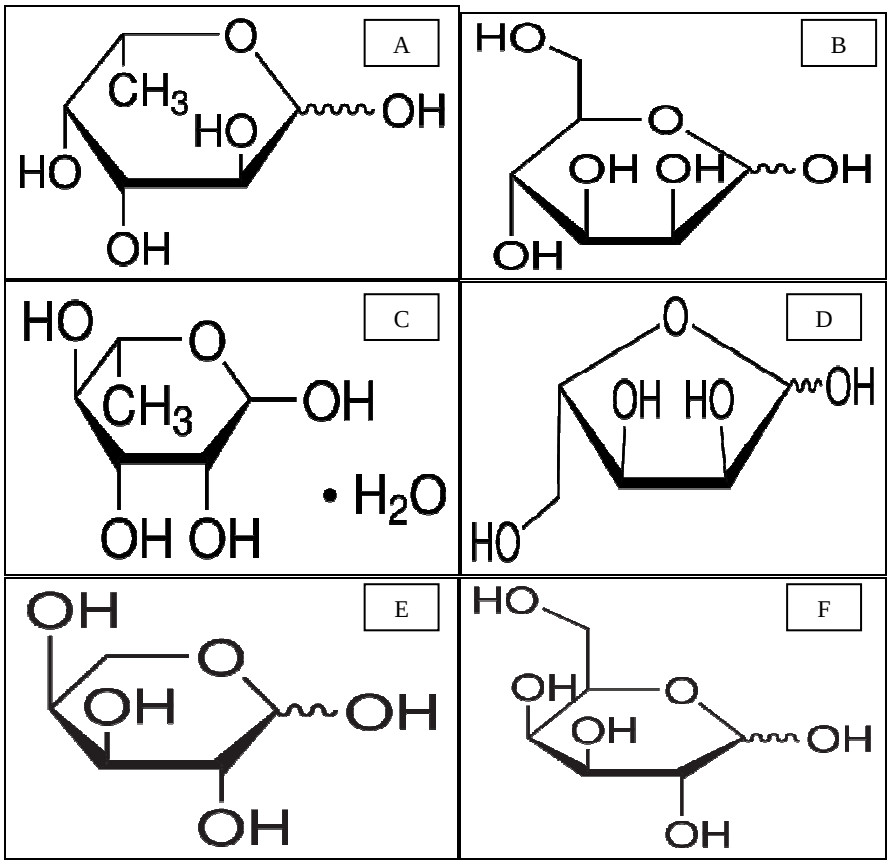


Figure 1. Selected rare sugars in Howard projection (18): L-Fucose (A), D-Mannose (B), L-Rhamnose monohydrate (C), L-Ribose(D), L-Arabinose (E), D-Galactose (F).

2. AIMS OF THE STUDY

The objective of the research will be to develop co-processed binary mixture of catalytic pretreated softwood cellulose (CPSC) and rare sugars, which would be suitable for the next step of direct compression. The research will be carried out to confirm the hypothesis: The development of new co-processed excipients of pretreated softwood cellulose and rare sugar significantly improves process of direct compression in comparison with the pure materials.

Questions concerning the research are:

1. What is the effect of dry ball milling (micronization) on the particle size and size distribution?
2. Can we apply sugar thin layering and thermally induced particle surface engineering of cellulose for preparing novel co-processed excipients?
3. What are the particle and powder properties of those co-processed excipients?
4. What kind of process-induced phase transformations (PITs) (or incompatibilities) are related to thermal treatment process?
5. What is a short-term physical solid-state stability of the present co-processed excipients under ambient room condition?
6. What is the practical relevance of new powder flow testing method at a small scale applied in this study?

3. MATERIALS AND METHODS

Raw materials

- Catalytic pre-treated softwood cellulose (CPSC) was isolated from pine soft wood (*Pinussylvestris*) and carried out according to the method described by Hakola et al. (15) with some modification.
- MCC (Avicel PH101 Ireland, LOT#6939C)
- L-Arabinose (CT 99, Resource #8134269, Lot#A121T9G01, Danisco USA, made in Finland)
- L-Ribose (Crystalline CT, RIB0809052, Danisco USA, made in Finland)
- L-Fucose (Crystalline CT, Material #4381141706, Danisco USA, made in Finland)
- L-Rhamnose (MC, Material #8131352, LOT# 1941089929, Danisco USA, made in Finland)
- D-Galactose (CT98, Material #8137770, LOT# 1941068582, Danisco USA, made in Finland)
- D-Mannose (CT Material #8135960, LOT #M120T10A30, Danisco USA Inc.)
- Lactose monohydrate (Pharmatose®80M; DFE Pharma, Germany)
- Acetone (E. Merck, Germany) solution of magnesium stearate (Ph. Eur.) 5% w/w
- White spirit (APChemical)
- Silica gel orange, with moisture indicator free of heavy metals (13767-1KG-R, LOT#SZBC348OV, CAS: 112926-00-8, Sigma Aldrich, Germany)

Apparatus

- Scale (Denver Instrument APX-200, USA)
- Laser diffractometer (MicrotracBluewave, USA)
- Laboratory-scale Retsch MM 400 Mixer Mill (Retsch GmbH, Germany)
- Gallenkamp Hotbox oven (size 1, UK)
- Desiccator (Polypropylene, Kartell, Italy)
- X-ray powder diffractometer (D8 Advance Bruker AXS GmbH, Germany)
- Scanning electron microscope (SEM) (Helios NanoLab 600, FEI Company, USA)

- Flow-Pro flow meter (SAY Group, Helsinki, Finland)
- Instrumented Korsch EK-0 eccentric tableting machine (ErwekaApparatebau, Germany)
- Digital micrometer (Sony DZ 521, Tokyo, Japan)
- Analytical balance (Sartorius CP 2245, Raute, Goettingen, Germany)
- Tablet hardness tester (Shleuniger 2E, Dr. Shleuniger, Pharmatorn AG, Solothurn, Switzerland)
- Differential scanning calorimeter (DSC 4000, Perkin Elmer Ltd., Shelton CT, USA)

Laboratory materials/equipment

- 25 ml volume stainless steel milling jars (Retsch GmbH, Germany)
- 12 mm diameter stainless steel balls (05.368.0037, Retsch Gmbh, Germany)
- Petri dishes
- Stainless steel micro spatula
- Vials
- Discs for XRPD samples
- Beakers
- Carbon tape
- Sieve Analysensieb 150 μm (Nr. 330539, ISO 3310-1, Edelstahl, Austria)
- Standard aluminium 40 μL pans and covers (02190041, Perkin Elmer Ltd., Shelton, CT, USA)
- Standard crimper press (02190048, Perkin Elmer Ltd, Shelton, CT, USA)

3.1. Micronization process of CPSC and rare sugars

Milling of CPSC was performed using Retsch MM 400 Mixer Mill (Retsch GmbH, Germany). CPSC was placed in a 25 ml volume stainless steel milling jar with one 12 mm diameter stainless steel ball and milled at 28 Hz frequency for 30 minutes. Sugars (L-Fucose, D-Mannose) were micronized by using the same apparatus and milling conditions. L-Fucose was chosen for further experiments.

3.2. Preparation of co-processed excipients (CPEs)

CPSC was kept under controlled temperature (23.4 ˚C) and two different humidity conditions (54 % RH (KCl) and 85 % RH ($Mg(NO_3)_2$) in desiccators, for 6 days before preparation of the pre-physical mixture (pre-PM). Pre-physical mixture (2.85 g) of pre-milled (30 minutes) CPSC and micronized (30 minutes) fucose (0.15 g) was blended without balls in a laboratory scale ball milling machine (Retsch MM 400 Mixer Mill) at frequency 10 Hz for 3 minutes. Afterward powder was placed into preheated oven (Galenkamp, UK) on 150˚C for 15 minutes.

Samples were then sieved manually with Sieve Analysensieb (Edelstahl, Austria) with sieve diameter 150 μm, due to their visible wide particle distribution in order to remove lumps. For further analyses the sieved powder (the powder with particle size under 150 μm) and also the unsieved powder were collected separately in vials and stored under controlled laboratory conditions (T=23.5 ˚C, 20 % RH) (Table III).

Table III. Composition of pre-test samples

	54 % RH	85 % RH
sieved	pre-CPE 1	pre-CPE 3
unsieved	pre-CPE 2	pre-CPE 4

Samples were prepared to determinate required moisture content in CPSC and L-Fucose percentage for further thermally induced particle surface engineering. Pre-tests with XRPD and SEM were performed. It would appear that the CPSC samples with higher moisture were already stuck together, forming lumps: some of those were too big to get through the mesh after sugar treatment. All in all, it was decided to prepare CPSC RH 54 % (pre-CPE 1/pre-CPE 2) for further investigations, to avoid moisture impact to be too high.

For CPE samples, pre-milled CPSC was kept under controlled temperature and humidity conditions at 23.5 ˚C and 54 % RH (prepared with ($Mg(NO_3)_2$) for at least one day before preparation of the physical mixture (PM). Physical mixture (3 g) of pre-milled (30 minutes) CPSC (2,85 g , 95 % w/w) and micronized (30 minutes) L-Fucose (0,15 g , 5 % w/w), was blended without balls in a laboratory scale ball milling machine (Retsch MM

400 Mixer Mill) at frequency 10 Hz for 3 minutes. During this process, the fine sugar particles were layered onto the surface of larger host CPSC particles.

Co-processed excipients (CPE) were prepared by thermally induced particle surface engineering of CPSC as it is shown in Fig. 2 and Table IV/ Table V.

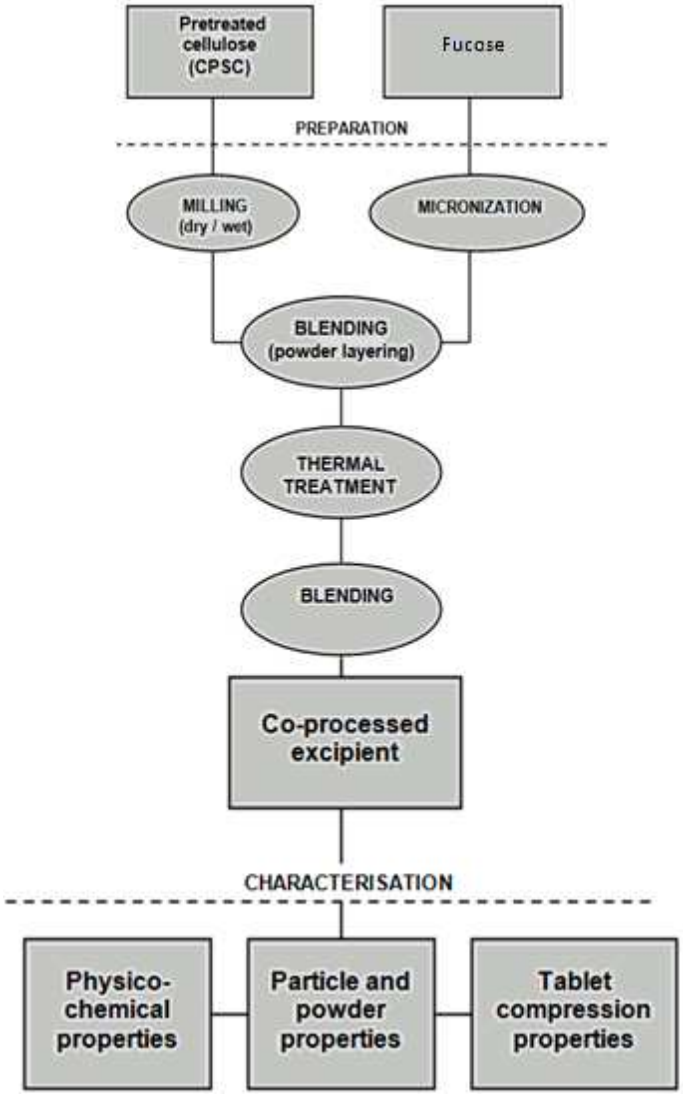


Figure 2. Preparation and characterization of co-processed excipients of pretreated cellulose and rare sugar (L-Fucose)

Due to the expected needs of the materials for further testing double amount of samples was made. Both parallels were made by using similar parameters and conditions.

Table IV. Composition of test samples, parallel 1 (key: number in bracket after fucose presenting number of fucose layers).

Material	PM1		PM2		PM3	
	Mass (g)	% (w/w)	Mass (g)	% (w/w)	Mass (g)	% w/w
CPSC	2.85	95	2.85	95	2.85	95
Fucose (1)	0.15	5	0.15	5	0.15	5
Fucose (2)			0.15	5.62	0.15	5.46
Fucose (3)			0.15	5.56	0.15	5.45
Fucose (4)					0.15	5.36
Fucose (5)					0.15	5.29
Total mass	2.67		2.65		2.82	

Table V. Composition of test samples, parallel 2 (key: number in bracket after fucose presenting number of fucose layers).

Material	PM1		PM2		PM3	
	Mass (g)	% (w/w)	Mass (g)	% (w/w)	Mass (g)	% w/w
CPSC	2.85	95	2.85	95	2.85	95
Fucose (1)	0.15	5	0.15	5	0.15	5
Fucose (2)			0.15	5.47	0.15	5.73
Fucose (3)			0.15	5.59	0.15	5.62
Fucose (4)					0.15	5.54
Fucose (5)					0.15	5.43
Total mass	2.67		2.65		2.85	

Sample Nr.1/CPE1 (thermally treated (1x) physical mixture of CPSC and L-Fucose (both milled for 30 min))

Fucose layered PM1 was prepared as it was described in the previous section. PM1 was exposed to a short-term (9 minutes) thermal treatment at elevated temperature conditions (150 ˚C) in oven, slightly above the melting temperature of L-Fucose (140 ˚C) (17). Afterward the sample was taken out of the oven, left for 10 minutes to cool down and relocated in a vial. Further the sample was kept under controlled laboratory conditions (T=23.5 ˚C, 20 % RH).

Sample Nr.2/CPE2 (thermally treated (3x) physical mixture of CPSC and L-Fucose (both milled for 30 min))

The procedure for the first part of treatment was the same as for the Sample Nr.1/CPE1. After cooling down the sample, 0.15 g (5.62 % for parallel 1 and 5.47 % of total mass for parallel 2) of L-Fucose was added, and the process continued with same blending and thermal treatment parameters as for the first blending set. Sample was placed in the preheated oven at 150 ˚C for 9 minutes and left for 10 minutes to cool down. Totally 3 heating circles were performed with 0.15 g of L-Fucose added each time.

Sample Nr. 3/CPE3 (thermally treated (5x) physical mixture of CPSC and L-Fucose (both milled for 30 min))

The procedure for the first part of treatment was the same as for the Sample Nr.1 and 2. After cooling down the sample, 0.15 g (5.46 % and 5.73 % of total mass) L-Fucose was added and the process continued with same blending and thermal treatment parameters as for the first blending. Sample was placed in the preheated oven at 150 ˚C for 9 minutes, left for 10 min to cool down. The whole procedure was performed 5 times with 0.15 g of L-Fucose added each time.

References

As the references, L-Fucose (milled for 30 minutes), CPSC (milled for 30 minutes) and CPSC (milled for 30 minutes) with 3 simulated layering treatment (the same thermal treatment as used for CPEs, without L-Fucose addition) were used.

3.3. Particle size, shape and surface morphology

3.3.1. Laser diffractometry

The particle size and size distribution measurements of CPSC and rare sugars after micronization were performed by means of laser diffractometer (MicrotracBluewave, USA). Samples of D-Mannose and L-Fucose were measured after different time of micronization (5, 30, 60, and 120 minutes). Before measurements samples were put into the vacuum oven Vaciotem-TV (990 bar, 50 ˚C, 24 h). White spirit was used as a solvent (fluid refractive index=1,426). Measurements were done in triplicate, after that the

ultrasonic treatment (120 seconds) was performed. Between different samples, system was rinsed with white spirit. Particle size and size distribution by means of number and volume distribution were calculated and graph representing percentage of each particle size passing through a broaded beam of monochromatic light (laser) was drawn.

3.3.2. Scanning electron microscopy (SEM)

Surface morphology of pure materials and CPEs were investigated with a high-resolution scanning electron microscope (SEM). Small amount of powder samples was fixed on carbon tape and then analysed at different magnification (100x, 200 x and 1000 x) by using Helios NanoLab 600 (FEI Company, Germany).

3.4. Powder properties

3.4.1. Powder flow

Flow rate of powders was measured by laboratory Flow-Pro flow meter (SAY Group, Helsinki, Finland). In general, flow meter measures the mass of a powder per time that flows through a container (funnel, cylinder or hopper) (21). In Flow-Pro flow meter, a powder sample is exposed to vertical oscillations, which break the cohesive forces responsible to form vault structure. Additionally, volume flow rate can be determined (21). The system included a frame, sample holder, orifice, and analytical scale. The volume of the sample holder (hopper) was 5.96 ml, while the diameter of the orifice was 3.0 mm. Analytical scale was connected to a computer that calculates the flow rate (milligrams per second); using millimetres per second data it was possible to investigate the shape of the mass function. Three parallel measurements were performed under controlled room conditions (21 ˚C/50 % RH). Relative humidity was also constantly detected by the apparatus. It was important to control relative humidity, since APIs and pharmaceutical excipients are in most cases electrical insulators. This could be the reason for possible variation of results between three different parallels. According to Seppälä et al. (22), even 1% changes in relative humidity affects the flow rate of MCC powder.

3.5. Physico-chemical characterisation

3.5.1. X-ray powder diffraction (XRPD)

X-ray diffraction patterns measurements of pure materials (rare sugars, CPSC), PMs and CPEs were performed. X-ray patterns of samples were collected using Bruker D8 Advance diffractometer using Cu radiation λ=1.5418 Å, operated at 40 kV and 40 mA.

3.5.2. Differential scanning calorimetry (DSC)

Thermal behaviour and melting temperatures of L-Fucose and CPEs were investigated using differential scanning calorimetry, DSC (DSC 4000, Perkin Elmer Ltd., Shelton, CT, USA). Apparatus used single furnace technology, where one large furnace contains both, sample and an empty pan as a reference. Energy change in the sample was calculated after measuring temperature difference between the sample side and reference side.

Samples were placed in desiccators (0 % RH) for 3 days before DSC run. For DSC measurements samples were enclosed in a sample pan in order to avoid the direct contact between sample and furnace and/or sensor and not causing any problems on the baseline. Covers were crimped on DSC pans by using Standard crimper press (02190048, Perkin Elmer Ltd, Shelton, CT, USA) and 3 pinholes were made in covers. The scans were obtained by heating from 30 ˚C to 205 ˚C at a rate 20 ˚C/min. Each sample was measured in triplicate.

3.6. Tablet compression of co-processed excipients

The final dosage form (tablet) was prepared using direct compression method at the University of Helsinki. Tablets were compressed with an instrumented Korsch EK-0 eccentric tableting machine, working on manual filling and equipped with 9 mm flat-faced punches. Samples were weighted out and poured into pre-lubricated die (acetone solution of magnesium stearate 5 % w/w). The operating speed of the tablet machine (36 rpm) and the height of the tablet (3 mm) were kept constant, so only the properties of the compressed materials caused changes in the shape of the force-distance profiles, and the changes in machine set up did not give any extra impact. Therefore, the upper punch was first adjusted to its lower position and the position of lower punch was adjusted by placing a calibration plate (3.0 mm) between the punches. The amount of powder (starting with

0,250 g) for every next tablet was raised for 0.01 g until we reached the highest advisable force on upper punch Fu, max (approximately 10kN).The thickness of the tablets was measured immediately after compression with digital micrometer (Sony DZ 521, Tokyo, Japan). Data about the height of the tablet during maximum compression force (H_{min}) and height of the tablet after removing it (H) were used to calculate elasticity factor (EF), with Eq. (1):

$$EF = \frac{H - H_{min}}{H_{min}} X\ 100\ \% \quad (7)$$

The crushing strength of tablets was determined using a tablet hardness tester (Schleuniger 2E, Dr.Schleuniger Pharmatorn AG, Solothurn, Switzerland).

4. RESULTS AND DISCUSSION

4.1. Micronization process of CPSC and rare sugars

Milling of CPSC and rare sugars was performed using Laboratory-scale Retsch MM400 Mixer Mill (Retsch GmbH, Germany). As a result, fine powder of CPSC was produced. However, micronization of D-Mannose and L-Fucose was challenging, since micronized powders got stuck on the jar walls. In both cases it made it difficult to remove the powders from the milling jar walls. Increasing the milling time produced harder compact on the milling jar walls. It can be due to the fact that both powders/sugars are prone for moisture absorption from the surroundings.

Those sugar properties also influenced further CPE preparation. Firstly, lower yields resulted as PM loss in blending process, because powder got stuck on the wall. Secondly, CPE with lower mass than was expected was weighted out after each thermal treatment, due to the loss of water.

4.2. Particle size, shape and surface morphology

4.2.1. Laser diffractometry

Particle size and particle size distribution characteristics are important for evaluation final dosage form of the drug. Particle size distribution was determined with a laser diffractometer (MicrotracBluewave, USA)

Mie theory of light scattering was used to calculate the particle size distribution (23). Characteristic parameters, such as MV (Mean diameter in microns of the “volume distribution”) and Dia (also known as D50, presenting the size of the particles which splits the distribution with half above and half below this diameter) were calculated (23). The measurements were done before milling, after 5 min and 30 minutes of milling. As it is shown in Table VI and VII, the mean diameter, of the volume distribution of L-Fucose and D-Mannose before milling was 225.8 μm and 148.7 μm, respectively. The results of particle size distribution for L-Fucose showed that 50 % of the particles were smaller than 217.7 μm (Table VI). The volume median diameter for D-Mannose was 134.3 μm (Table VII).

After the ultrasonic (US) treatment, MV for L-Fucose particles was 119.4 μm (reduced by 47.5 %) and for D-Mannose 38.88 μm (reduced by 73.9 %) (Table VI, VII). Obtained Dia results after US treatment for L-Fucose was 113.0 μm and 30.43 μm for D-Mannose, respectively (Table VI, VII). The difference among results (with and without US treatment) can be due to the fact that sugars have the tendency to form agglomerates after moisture exposure.

After 5 minutes of milling, MV for L-Fucose particles was 277.8 μm (Table VI). Obtained results confirmed some electrostatic interaction between smaller particles of L-Fucose; as a result, bigger particles (agglomerates) were formed. However, it seemed that milling had not induced the agglomeration of D- Mannose, as the increase of MV was insufficient (145.8μm). Obtained data after US treatment indicated that L-Fucose and D-Mannose particles became smaller after milling, with mean diameter for L-Fucose 67.62 μm and for D-Mannose 16.80 μm, respectively.

As the MV is always a result of range of all particle size, agglomerate formation can influence the final value. Ultrasonic treatment had proven to be more effective for obtaining more reliable results of particle size distribution. MV results after US treatment showed that MV for L-Fucose was 119.4 μm and for D-Mannose 91.34 μm, respectively. Milling for 30 minutes and further US treatment proceeded with 50 % L-Fucose particles smaller than 5.99 μm. Dia for D-Mannose after US treatment was 9.07 μm, which was only 0.18 μm smaller comparing to Dia after 5 minutes of milling with US treatment.

Further milling yielded bigger agglomerates, which could not be broken with US treatment. The rubbing of sugar grains created a static electric charge that repelled the grains, ejecting sugar in all directions. Sugar particles milled for 30 minutes became even more moisture sensible, due to their enhanced surface. As a result, distribution of particle size was too wide. Furthermore, strange phenomenon of particles getting stuck on the wall of laser diffractometer (where the samples were placed) could be observed. This can be due to non-polar properties of white spirit and potentially also the hydrophobic effect of particles.

The decision was made to work further on L-Fucose particles. There was higher yield after milling of L-Fucose, because powder got less stuck on the jar wall. Practically, more L-Fucose powder was available, which was another reason for choosing that sugar.

Table VI. Particle size distribution of L-Fucose

	Time; no US/US					
	0 min	**0 min US**	**5 min**	**5 min US**	**30 min**	**30 min US**
MV (μm)	225.8	119.4	277.8	67.62	239.8	119.4
Dia (μm)	217.7	113.0	177.6	8.81	3.91	5.99

Table VII. Particle size distribution of D-Mannose

	Time; no US/US					
	0 min	**0 min US**	**5 min**	**5 min US**	**30 min**	**30 min US**
MV (μm)	148.7	38.88	145.8	16.80	233.0	91.34
Dia (μm)	134.3	30.43	20.79	9.25	10.87	9.07

CPSC particle size distribution measurements were also performed. As shown it Table VIII, CPSC particles were getting smaller as the milling time was increasing. The time chosen for milling was 30 minutes, since it represented the optimal size of CPSC (MV of 92.68 μm) for further processing (thermally induced particle surface engineering).

Table VIII. CPSC size measurment.

	Time; no US/US					
	0 min	**0 min US**	**30 min**	**30 min US**	**180 min**	**180 min US**
MV (μm)	289.6	276.5	92.68	87.15	88.38	57.51
Dia (μm)	195.4	163.8	48.89	46.51	39.87	30.71

4.2.2. Scanning electron microscopy (SEM)

Particle size, shape, surface morphology and microstructure were studied by using high-resolution scanning microscopy, SEM. A little amount of sample was sputter coated with gold in argon atmosphere on carbon tape and examined with vacuum under high pressure. Three different magnifications were used; 100x and 200x magnification to get general

information about size of particles, and 1000x (1250x) to study details about surface morphology of selected particle. Considering there were no important differences between 100x and 200x magnification, only 100x magnification was used for further analysis.

The SEM particles size for L-Fucose (milled for 30 minutes) appeared to be in range of 0.17-1.84 μm (Fig. 3). Evidently, SEM data was not correlated with laser diffractometer data. This could be due to the reason that agglomerates were formed, especially during laser diffractometer analyses of L-Fucose.

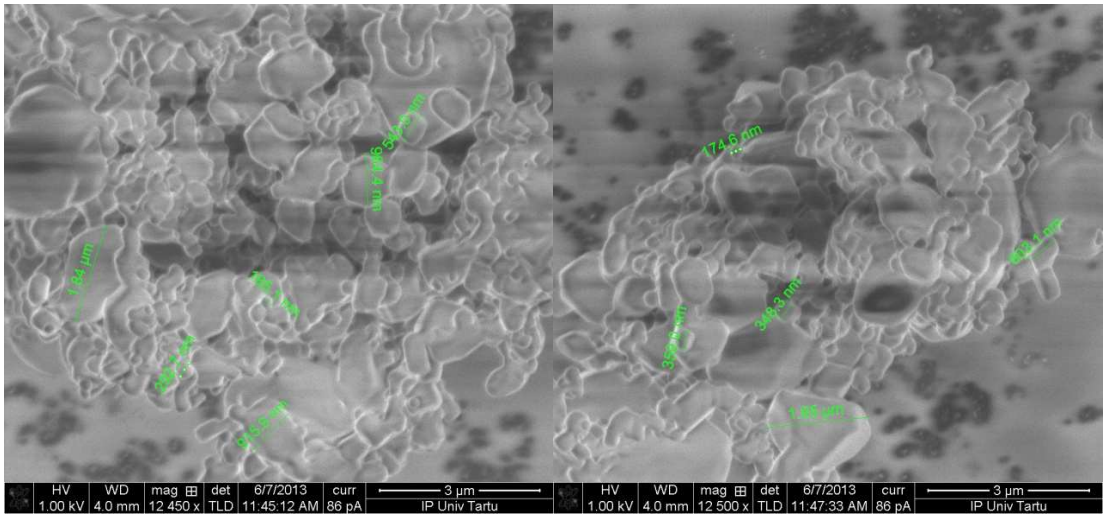


Figure 3. Scanning electron micrographs (SEMs) of micronized L-Fucose (30 minutes). Magnification 12500x.

CPSC powder consisted of relatively round but irregular particles. The particle size ranged from 50-100 μm (Fig. 4/A, 5/A). Fig. 4/B showed up a lot of particles in a shape of rods, while in figure 4/A particles were more rounded and symmetrical. Also some bigger agglomerates were formed, which can be attributed to a difference between CPSC which was thermally treated and the one that was not. Taken together, the data suggest some surface changes occurred during the thermal treatment; particles became larger and some of them were broken into asymmetrical particles.

In Figure 4/C it is shown that there were no significant differences between pure CPSC and thermally treated CPSC with L-Fucose. It can be argued that thermally induced particle surface engineering was unsatisfactorily after just one addition of L-Fucose. Differences seemed to appear between Figure 4/C, 4/D and 4/E. As it is shown in Figure 4 (C, D, E) the more L-Fucose had been layered onto CPSC, the bigger particles were formed.

Particles were also differently shaped and with less smooth surface. There is no doubt that some sugar was layered onto the surface of larger CPSC particles.

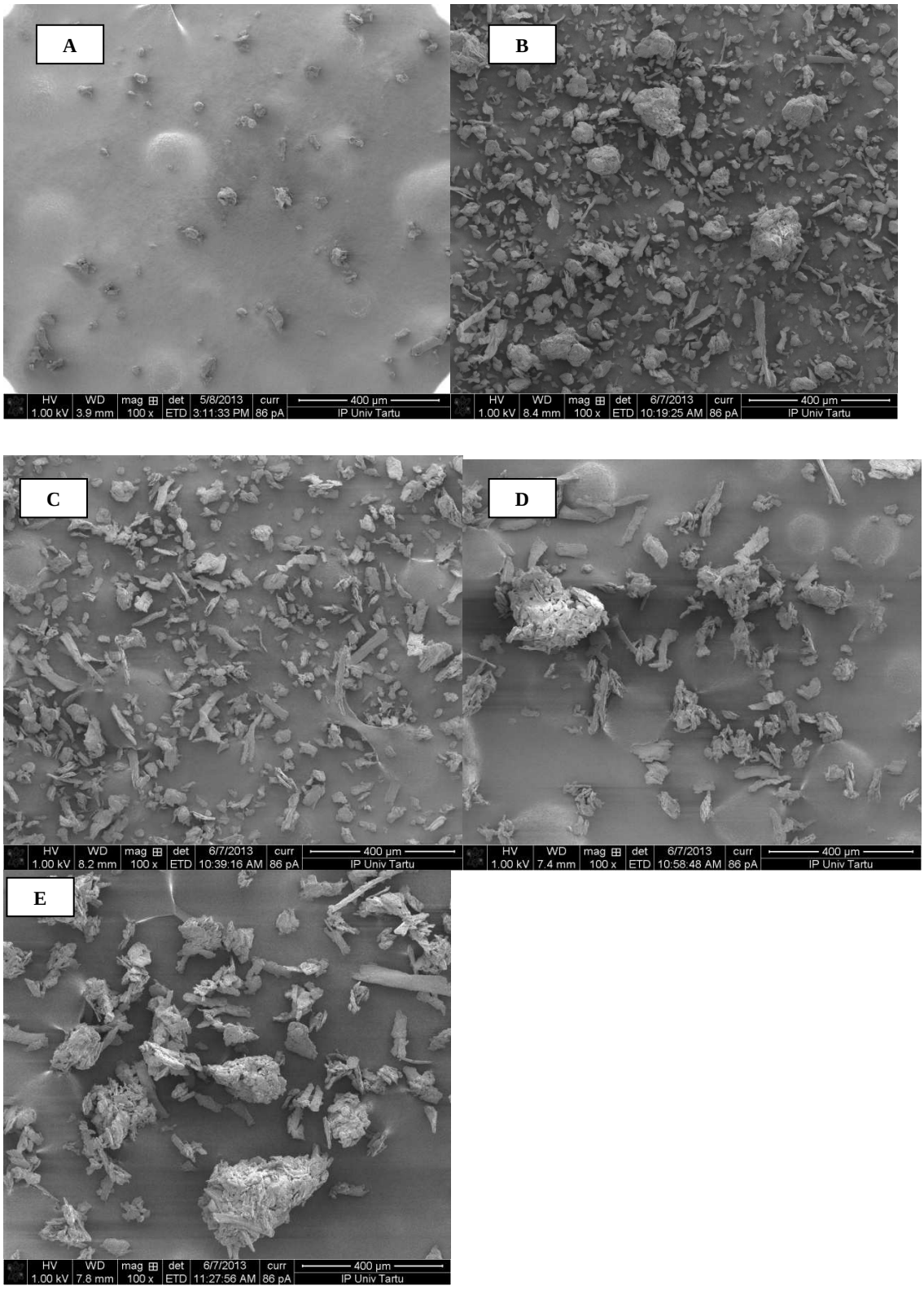
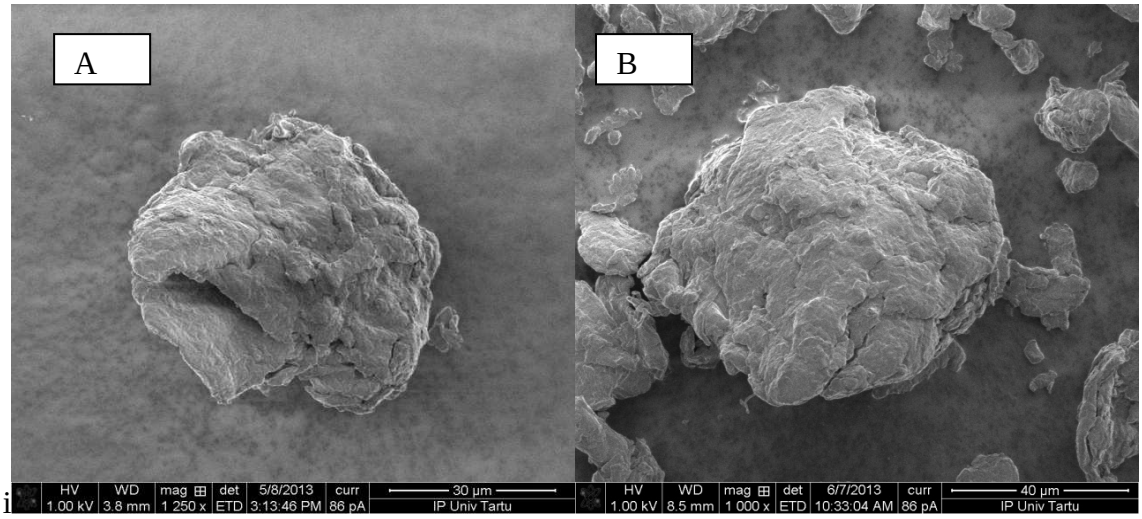


Figure 4. Scanning electron micrographs (SEMs) of differently treated CPSC by using the same magnification (100x). Reference pure CPSC (A), reference pure CPSC with 3 simulated layering treatments, thermally treated CPSC with L-Fucose 1x (C), 3x (D) and 5x (E) layering.

Large magnification was used to reveal differences in surface morphology between samples. The particles are larger in figure 5/C and 5/D compared with figure 5/B. What is more, it would appear that the surface became less integrated. It could suggest that sugar particles got randomly attached onto the CPSC surface. In the last sample (Fig. 5/E) it can be detected even more sugar was attached onto the CPSC surface.



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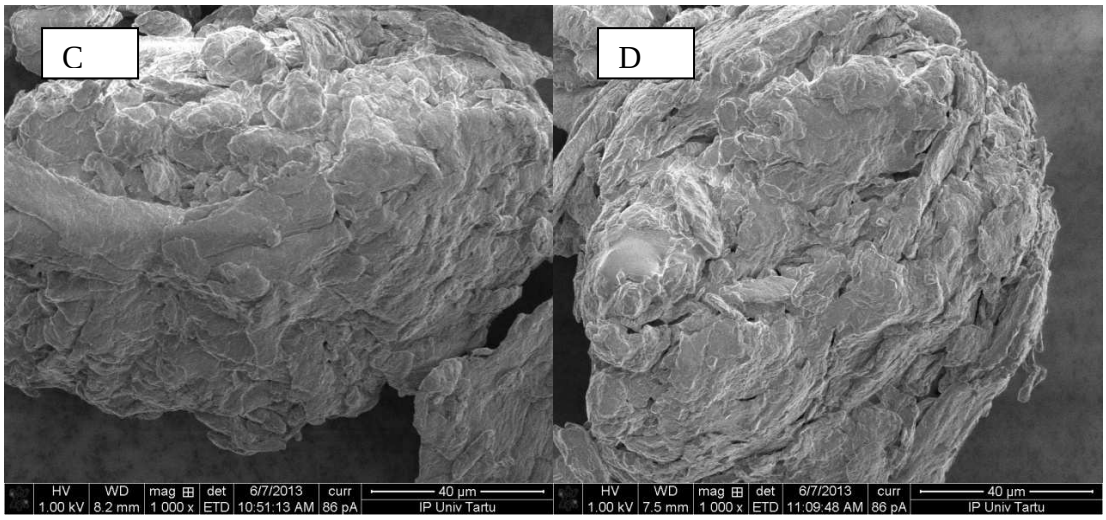

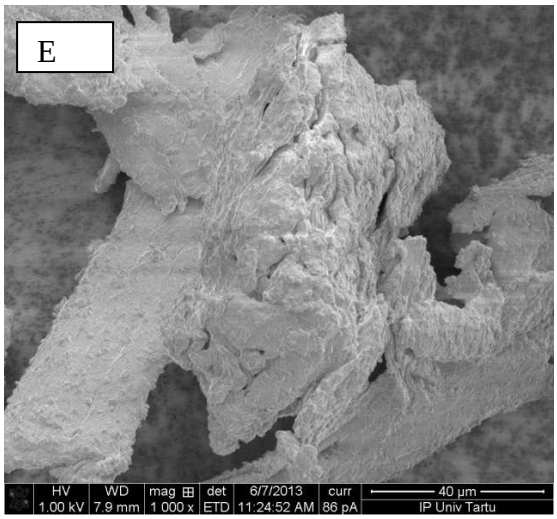

Figure 5 (also on previous side). Scanning electron micrographs (SEMs) of differently treated CPSC by using 1250x magnification for reference CPSC (A), 1000x magnification for CPSC with 3 simulated layering treatments (B), and for thermally treated CPSC with L-Fucose 1x (C), 3x (D) and 5x (E) layering.

The results above enhanced some visible differences between samples with different L-Fucose content. Nevertheless, the main disappointment was the particle shape, because it was random, without obvious pattern and form. More rounded particles were expected, with smoother surface and smaller size distribution.

4.3. Physical powder properties

4.3.1. Powder flow

The flow properties of a powder are essential in determining its suitability as a direct compression excipient. Flow through an orifice (2.9.36 Powder flow) could be found in European Pharmacopeia, but there is no general scale for flowability available, due to the different variation used (21). However, the experiment was carried out in the same container as the one that Seppälä et al. (22) used. According to Seppälä et al. (22) powders can be divided into three groups: freely flowing, intermediate flowing, and poorly flowing. Table IX provide information about those three groups that are classified by average flow rate in $\frac{\mathrm{mg}}{\mathrm{s}}$ and illustrated with examples.

Table IX. Powders grouped by their flowability (22).

	Freely flowing	Intermediate flowing	Poorly flowing
Average flow rate ($\frac{mg}{s}$)	More than 100	10-100	Below 10
Example	Sucrose, pregelatinized maize starch	MCCs	APIs (caffeine, carbamazepine, And paracetamol)

The results of flowability of pure materials and CPEs are presented in Table X. All powders can be classified as intermediate flowing materials. The best flow properties had thermally treated CPSC with flowing rate 145 $\frac{mm^3}{s}$. However, sticking of CPSC and MCC samples on the jar walls was still noticed.

Table X. Results of powder flow test.

	Avicel PH 101	CPSC, 3x thermal treatment	CPE1	CPE2	CPE3
Average flow rate ($\frac{mg}{s}$)	32.0	53.7	13.2	17.4	14.2
Average flow rate ($\frac{mm^3}{s}$)	131.6	145.3	74.4	74.4	50.1
Relative humidity (interval)	(38.9-39.3) %	(39.6-40.0) %	(43.1-44.2) %	(41.6-43.4) %	(40.5-41.9) %

Some problems with repeatability appeared also with the CPE3. It could be presumed that the reason was in lumps formation, due to the high relative humidity. Interestingly, the average flow rate for CPE1 and CPE3 was almost the same when focusing on $\frac{mg}{s}$ unit and totally different by using $\frac{mm^3}{s}$ unit. It would appear that in the case of CPE3, where lumps were formed, they were flowing through orifice one by one. One lump had in that case higher density than in the case of powder and therefore lower volume comparing to mass.

It could be argued that this method, or any other based on direct flowing of the powder through an orifice, should not be performed if powder sample forms lumps. Alternatively, indirect method, such as angles of repose, shear cell determination, or determination of a ratio of tap and bulk density could be used (22).

Maybe the problem that discontinuous powder flowing out of sample holder arises especially in our case, where in the first stage very fine/micronized powder of L-Fucose was used. Assuming small amount of sugar did not undergo thermally induced particle surface engineering and did not tend to bind onto CPSC surface. When interparticular cohesive forces dominated gravitational forces, a vault structure was formed. At some stage this should had been broken by using single upward motion, but anyway the flowability was not constant.

4.4. Physico-chemical characterization

4.4.1. X-ray powder diffraction (XRPD)

XRPD measurements were performed in order to determinate crystallinity changes occurred during thermally induced particle surface engineering of CPSC with L-Fucose. XRPD pattern (Fig. 6-10) shows a unique fingerprint of the crystallographic unit cell and provide information about actual atomic arrangement inside the crystallographic unit (24).

XRPD is an essential method to identify the presence and to determine the concentration of different phases in a mixture. In our case it was used as a qualitative phase analysis to identify the presence of L-Fucose in the final powder.

Pure materials

Firstly, pure reference materials, CPSC (Fig. 6) and L-Fucose (Fig. 7) were analysed. XRPD was also performed for CPSC sample after 3 simulated layering treatments (Fig. 6) and for L-Fucose sample after 30 minutes micronization (Fig. 7).

CPSC had the strongest peaks at 22.3˚ and 15.8˚ 2θ (Fig. 6). After 3 simulated layering thermal treatments one can see peak broadening at 15.8 ˚, as a result of sample contribution, a kind of crystal lattice distortion (micro-strain), due to dislocations and concentration gradients.

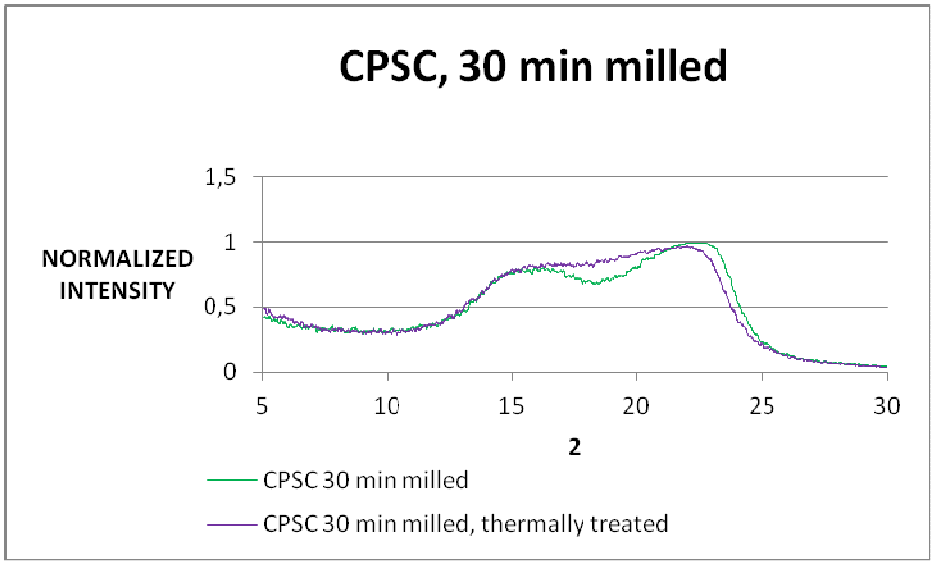

Figure 6. XRPD of CPSC after 30 minutes milling and CPSC after 30 minutes milling with 3 simulated layering treatment.

There were 9 peaks with higher intensivity obtained after 30 minutes of L-Fucose micronization, at 12.2˚, 14.6˚, 17.0˚ 2θ; two broader peaks not clearly separable between 17.6˚and 18.0˚; peaks at 21.6 ˚, 25.6 ˚ and 29.2, 29.9˚ 2θ. No significant difference in the structure was found between primary L-Fucose and L-Fucose after 30 minutes of miconization; it could just be presumed that intensivity of the peaks after micronization of L-Fucose was higher.

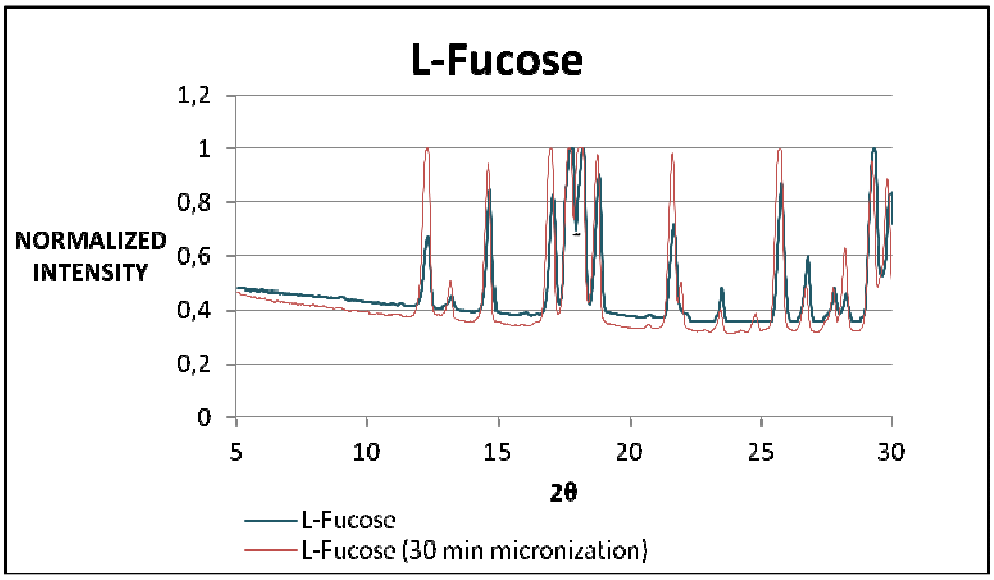

Figure 7. XRPD of L-Fucose and L-Fucose (30 min micronization)

Co-processed excipients (CPE)

Data indicated that the first sample (CPE1), after one treatment with L-Fucose, did not contain a significant amount of L-Fucose (Fig. 8). The line was thicker, which could point out to the presence mixture of L-Fucose and CPSC, but this evidence was not conclusive.

By comparing figures (Fig. 8-10), L-Fucose could be seen in the sample after 3 consecutive thermal treatments. Notable 2θ diffraction angles, determined by spacing between a particular set of planes, were 12.2˚, 14.6˚ and 16.9˚, 17.7˚, 25.6˚ 2θ, which was also characteristic of reference micronized L-Fucose. As expected, more L-Fucose peaks were seen in the last XRPD graph, after five consecutive thermal treatments. Those peaks were observed at 21.6˚and 29.2 ˚ 2θ.

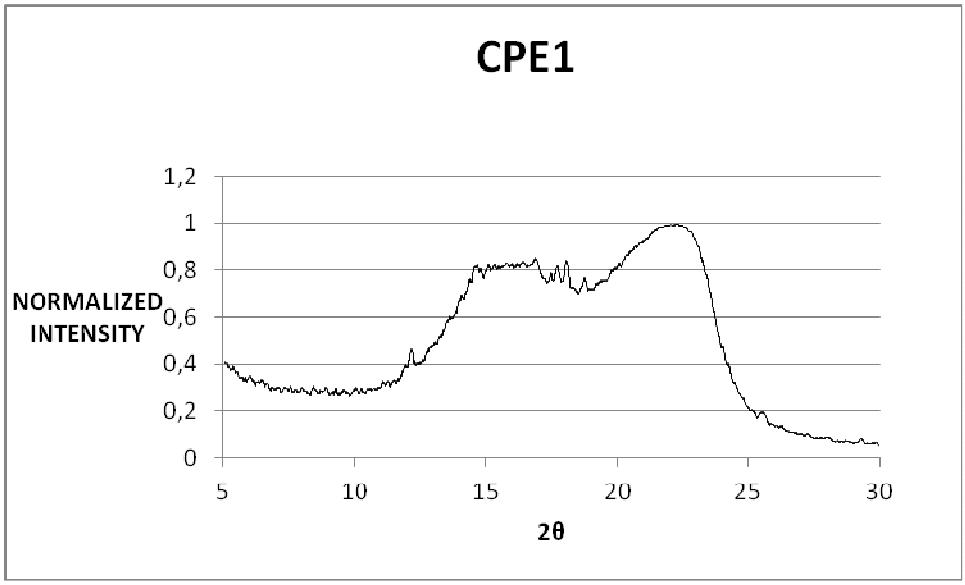

Figure 8. XRPD of CPE1.

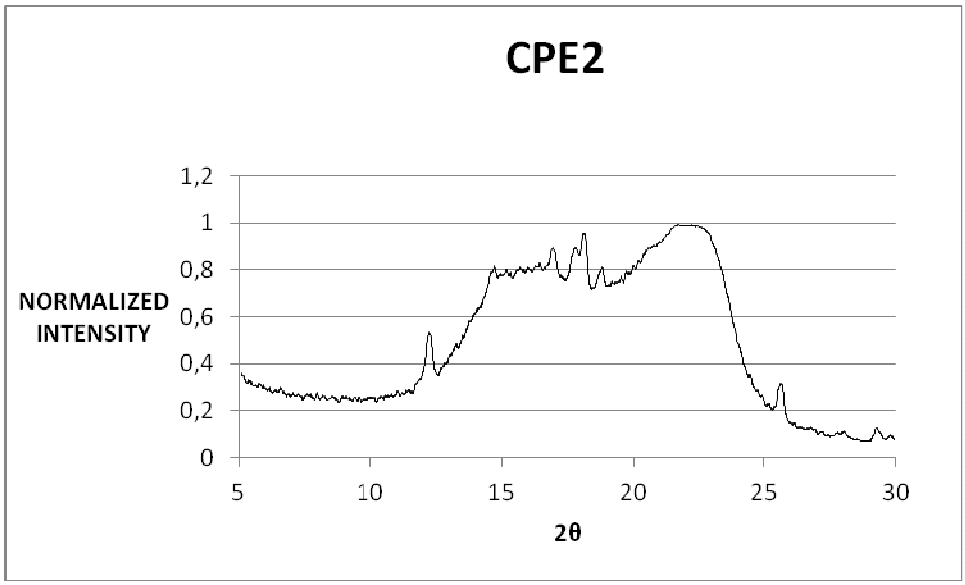

Figure 9. XRPD of CPE2.

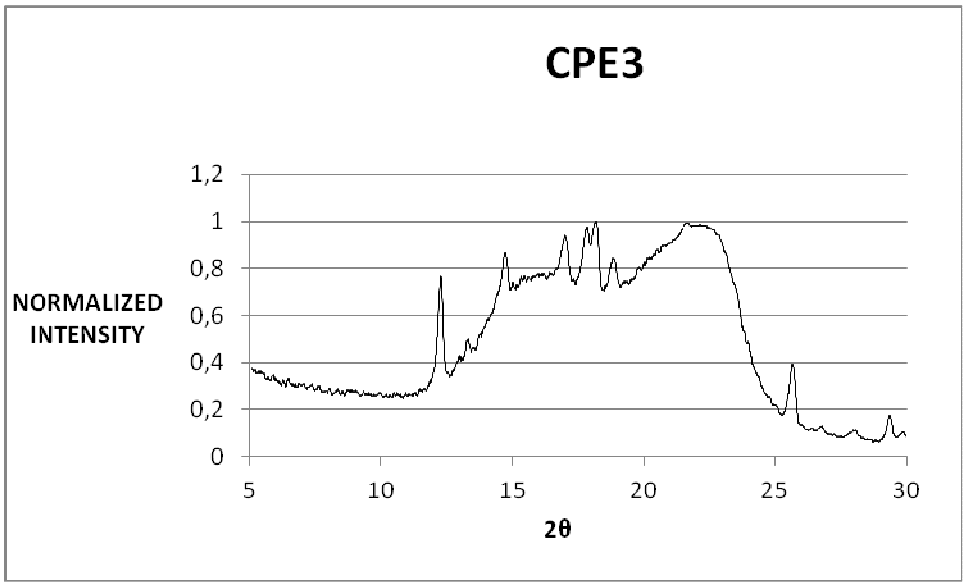

Figure 10. XRPD of CPE3.

4.4.2. Differential scanning calorimetry (DSC)

The melting point of the rare sugars and CPSC was measured using differential scanning calorimetry, DSC (DSC 4000, Perkin Elmer Ltd. Shelton, CT, USA) and compared to the data obtained from the literature.

Typical peak of L-Fucose was observed at 155.22 ˚C (Fig. 11). However, there was small difference with a previously published data; according to Merk's index (17) melting point of L-Fucose is 140 ˚C. Nevertheless, onset of the melt peak should be taken as the melting point; the whole melting region of the reference L-Fucose sample was in a range of 135-164 ˚C, hence the oven temperature used for CPEs preparation (150 ˚C) was high enough to melt L-Fucose within the exposition time applied. As the heat capacity increased in a sample some slope upward with higher temperature appeared.

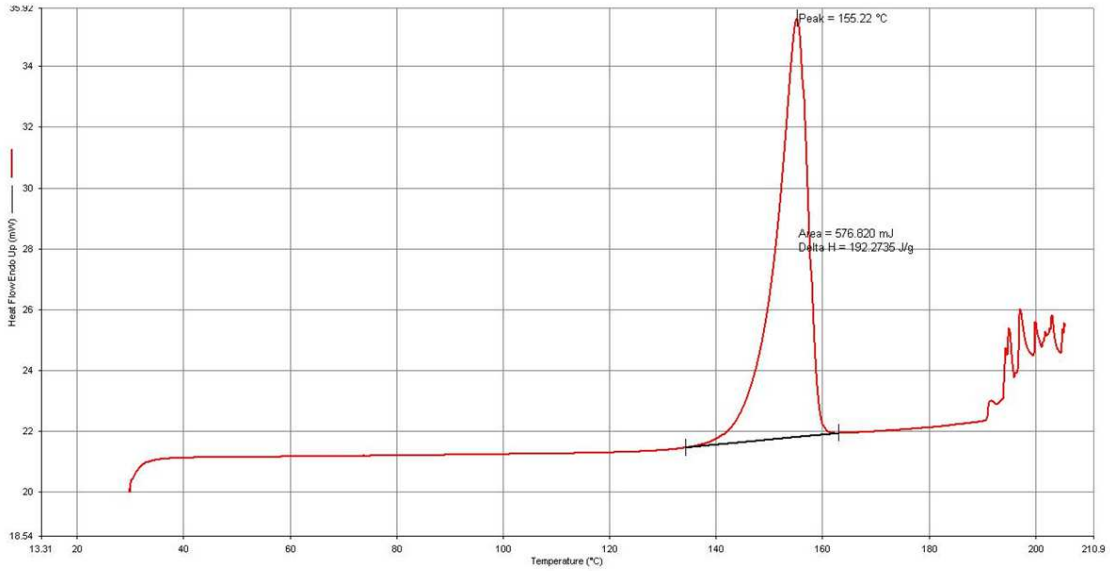

Figure 11. DSC of L-Fucose.

According to Penkina et al. (14), glass transition temperature (Tg) for CPSC was in the range of 169-171 ˚C.

Lower glass transition temperature (Tg) were obtained with CPE samples. There were peaks observed in lower temperature region; at 62.34 ˚C, 62.36 ˚C, and 60.50 ˚C. The shape of curve indicates the endothermic peak as the result of Tg change. It could be presumed the peak belongs to L-Fucose. According to the available literature (25), Tg for L-Fucose should be in the temperature range 313-328 K (39.58-54.85 ˚C).

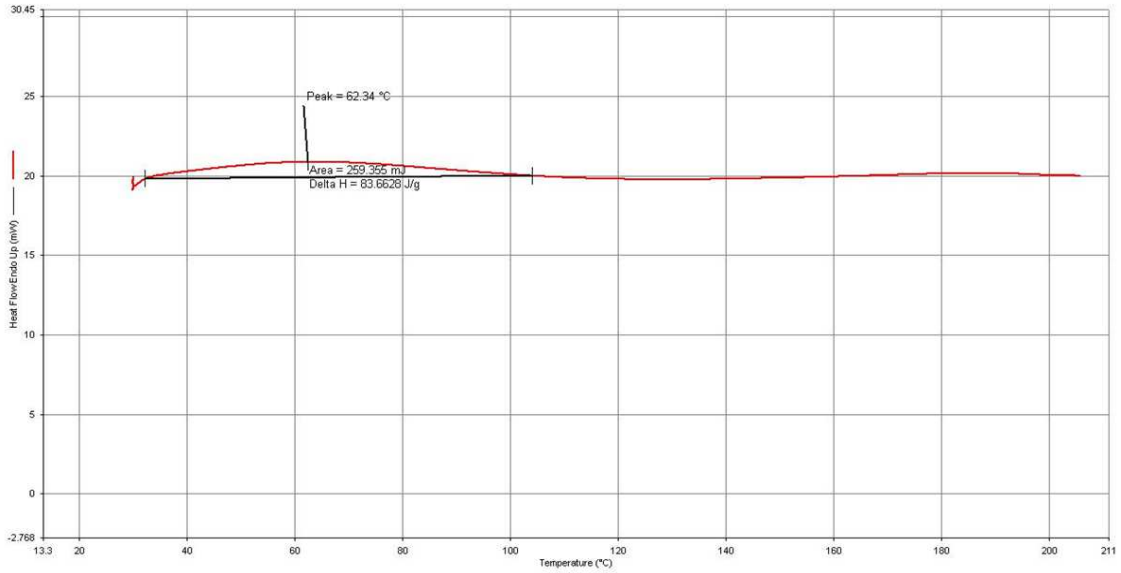

Figure 12. DSC of CPE1.

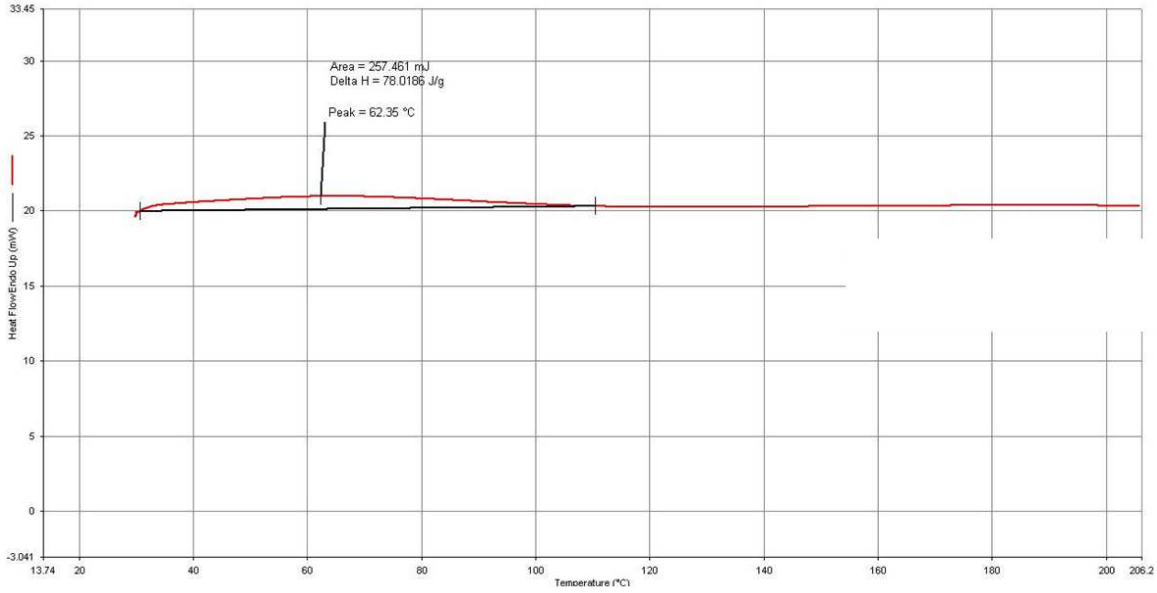

Figure 13. DSC of CPE2.

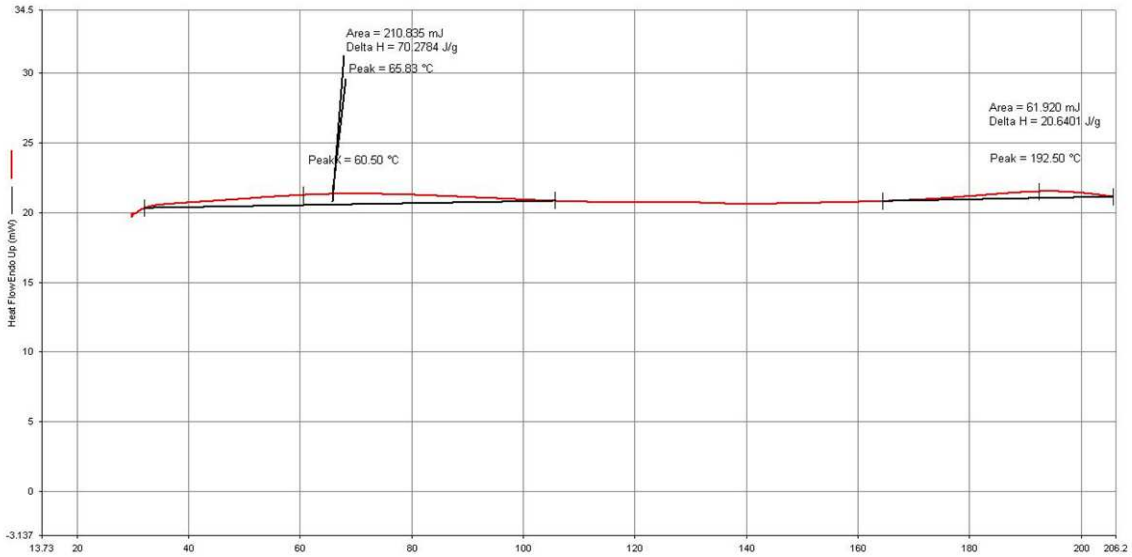

Figure 14. DSC of CPE3.

4. 5. Tablet compression of co-processed excipients

The compactibility of pure materials and CPEs was evaluated by determining the relationship between the upper punch compression force and tablet crushing strength (Fig. 15). As the amount of powder for direct compression had been increased in the next steps, also the upper punch compression force values raised. Two pure references (MCC and lactose) showed the highest and the lowest slopes of the curves for mechanical strength of the tablet. Graphs of CPEs with different amount of L-Fucose layering did not exhibit any significant variation. We could presume that CPE2 had slightly higher slope and perhaps better mechanical strength. Anyway, all CPEs exhibited higher crushing strength profiles than lactose, and as a result better compactibility.

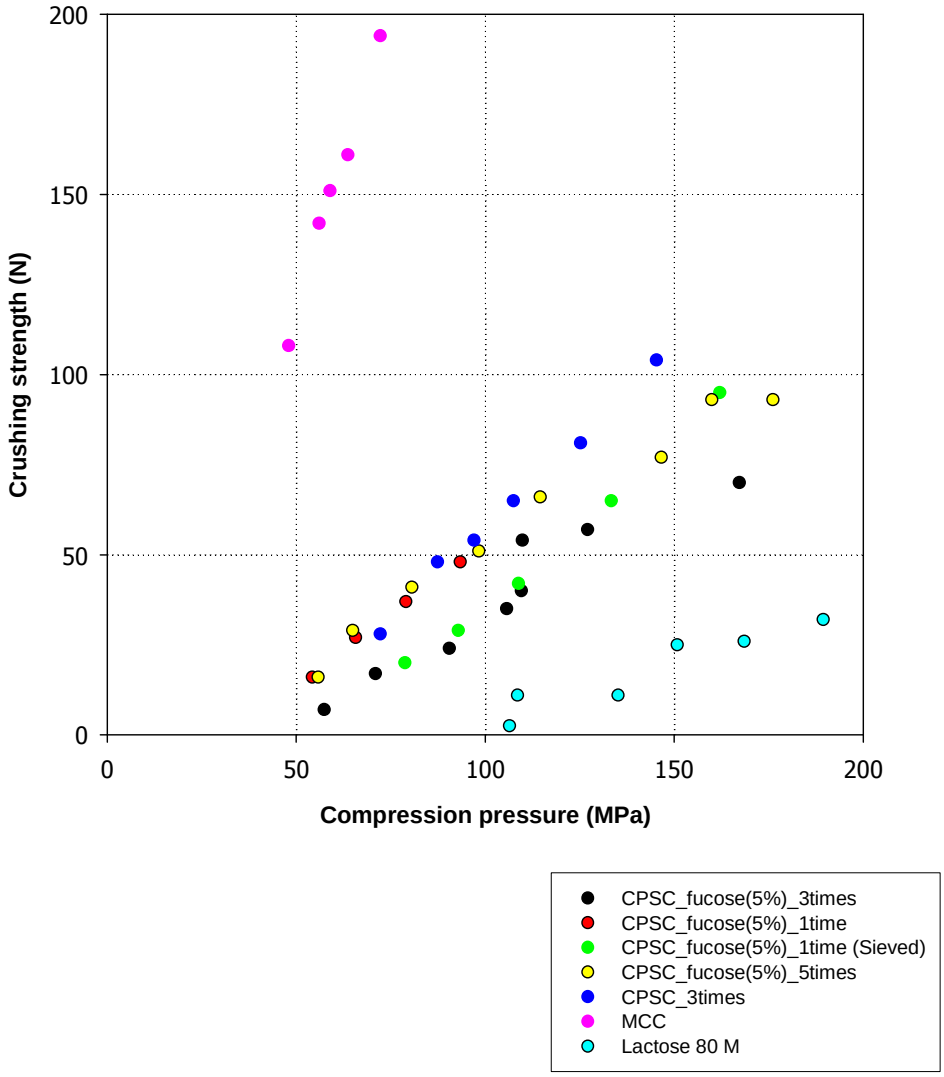

Figure 15. Effect of compression pressure on the crushing strength of the direct-compression tablets prepared from the CPE (CPSC with 5% L-Fucose), CPSC with 3 simulated layering treatments and from the two reference excipients.

The elasticity values (etc. elasticity factors=EF) of CPSC treated with sugars increased compared with untreated CPSC, especially in CPE3 sample. It could be seen that when the compression pressure was increasing, elasticity of CPE2 was also increasing reaching the same percentage as for CPE3 sample. This was actually not a desired result, because higher elasticity means tablet recovery after compression and possible capping/lamination. The highest elasticity was calculated for CPSC treated with L-Fucose 5 times (EF=23-26 %).

On the other hand, the elasticity values for CPSC (EF=17-22 %) were higher than the respective values for MCC (EF=12–18 %). This is in accordance with a finding of Penkina et al., who reported that EF for CPSC was 16-20 %, presenting higher elasticity values than the one for MCC (EF=12-14 %) (26).

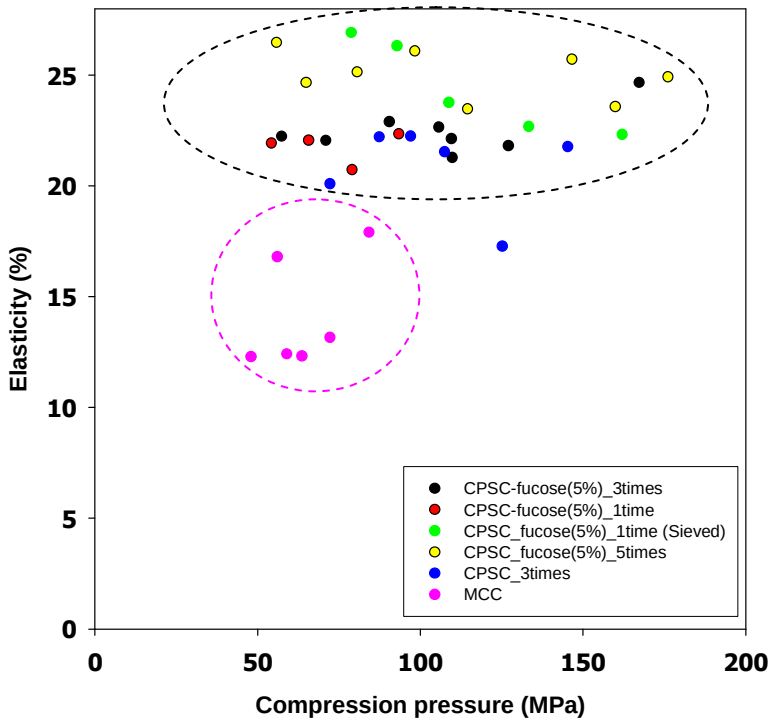

Fig. 16. Effect of compression pressure on elasticity of the direct-compression tablets, 4 of them prepared from CPE (CPSC with 5% L-Fucose), the reference pure CPSC with 3 simulated layering treatments and reference pure MCC.

5. CONCLUSION

The ball milling of L-Fucose, followed by milling in combination with CPSC was an effective method to get PMs and CPEs by thermally induced particle engineering. DSC confirmed that selected temperature from the literature for thermal treatment was sufficient for sugar layering. Laser diffractometry tests were performed in the order to get information about sizes of primary powders and final binary mixture. XRPD results were useful to quantify the approximate amount of L-Fucose in combination with CPSC after the preparation of CPEs. Also, SEMs gave important information about the particle size and their surface structure; as a result, bigger and less rounded particles were observed after every L-Fucose addition. Unfortunately, the flowability properties of the CPE mixture were not as good as it was expected, probably due to the lump formation. Finally, the compression of powders revealed that co-processed excipients did not exhibit better compactibility than CPSC and MCC reference. However, CPEs had better crushing strength profile than lactose, thus the better compactibility of CPE mixtures can make the latter a more favourable option when choosing between the two.

In the conclusion, sugar thin layering and thermally induced particle surface engineering of CPSC could be perspective method for preparation of new co-processed excipients for further direct compression into tablets. More experiments have to be carried out in order to control the moisture content in sugars and to get more homogeneous binary mixture.

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