CHARACTERIZATION AND EVALUATION OF MELIBIOSE AS NOVEL EXCIPIENT IN TABLET COMPACTION

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Tiivistelmä – Referat – Abstract

Lactose is probably the most used tablet excipient in the field of pharmacy. Although lactose is thoroughly characterized and available in many different forms there is a need to find a replacer for lactose as a filler/binder in tablet formulations because it has some downsides. Melibiose is a relatively unknown disaccharide that has not been thoroughly characterized and not previously used as an excipient in tablets. Structurally melibiose is close to lactose as it is also formed from the same two monosaccharides, glucose and galactose.

Aim of this research is to characterize and to study physicochemical properties of melibiose. Also the potential of melibiose to be used as pharmaceutical tablet excipient, even as a substitute for lactose is evaluated. Current knowledge about fundamentals of tableting and methods for determinating of deformation behavior and tabletability are reviewed.

In this research Raman spectroscopy, X-ray powder diffraction (XRPD), near-infrared spectroscopy (NIR) and Fourier-transform infrared spectroscopy (FT-IR) were used to study differences between two melibiose batches purchased from two suppliers. In NIR and FT-IR measurements no difference between materials could be observed. XPRD and Raman however found differences between the two melibiose batches. Also the effects of moisture content and heating to material properties were studied and moisture content of materials seems to cause some differences. Thermal analytical methods, differential scanning calorimetry (DSC) and thermogravimetry (TG) were used to study thermal behaviour of melibiose and difference between materials was found. Other melibiose batch contains residual water which evaporates at higher temperatures causing the differences in thermal behaviour. Scanning electron microscopy images were used to evaluate particle size, particle shape and morphology. Bulk, tapped and true densities and flow properties of melibiose was measured. Particle size of the melibiose batches are quite different resulting causing differences in the flowability.

Instrumented tableting machine and compression simulator were used to evaluate tableting properties of melbiose compared to α-lactose monohydrate. Heckel analysis and strain-rate sensitivity index were used to determine deformation mechanism of melibiose monohydrate in relation to α–lactose monohydrate during compaction. Melibiose seems to have similar deformation behaviour than α-lactose monohydrate. Melibiose is most likely fragmenting material. Melibiose has better compactibility than α – lactose monohydrate as it produces tablets with higher tensile strength with similar compression pressures. More compression studies are however needed to confirm these results because limitations of this study.

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melibiose, pre-formulation, tablet compaction, Heckel analysis

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Tiivistelmä – Referat – Abstract

Laktoosi on todennäköisesti eniten käytetty tabletoinnin apuaine lääkevalmistuksen alalla. Laktoosista on saatavilla lukemattomia laatuja, ja sen ominaisuudet ominaisuudet tunnetaan hyvin. Kuitenkin laktoosille on tarve löytää korvike täyte/sideaineena tablettiformulaatioissa, koska sillä on omat huonot puolensa. Melibioosi on suhteellisen tuntematon disakkaridi, jota ei ole vielä täysin karakterisoitu. Sitä ei ole myöskään aikaisemmin käytetty tabletoinnin apuaineena. Melibioosi koostuu samoista monosakkarideista, glukoosista ja galaktoosista, kuin laktoosi. Hypoteesina onkin, että samankaltaisesta rakenteesta johtuen melibioosin tabletointiominaisuudet olisivat lähellä laktoosia. Tämän tutkimuksen tarkoituksena oli karakterisoida ja tutkia melibioosin fysikokemiallisia ominaisuuksia. Myös melibioosin potentiaalia tabletoinnin apuaineena, ja jopa laktoosin korvikkeena on arvioitu.

Tässä tutkimuksessa spektroskopisista menetelmistä Raman, jauheröntgendiffraktometria (XRPD), lähialueen infrapuna (near infrared, NIR) – ja FT-IR (Fourier transform infrared) spektroskopioita käytettiin kahden eri toimittajan melibioosierien mahdollisten eroavaisuuksien selvittämisessä. NIR ja FT-IR – mittauksissa eroavaisuuksia materiaalierien välillä ei löytynyt. XRPD ja Raman mittauksissa eroavaisuuksia sen sijaan löytyi. Termisen analytiikan menetelmistä differentiaalista pyyhkäisykalorimetriä (DSC) ja termogravimetria käytettiin tutkittaessa melibioosin käyttäytymistä lämmityksen aikana ja eroavaisuuksia eri erien väliltä löytyi. Toinen melioosierä sisältää termogravimetria mittausten mukaan kiderakenteessaan jäännösvettä, joka haihtuu lähellä melibioosin sulamispistettä aiheuttaen osan eroista. Pyyhkäisyelektronimikroskooppia (SEM) käytettiin partikkelikoon-, muodon-, ja morfologian tutkimisessa, Myös melibioosin kaato-, täry-, ja todelliset tiheydet sekä valuvuusominaisuudet mitattiin. Melibioosierien valuvuusominaisuudet olivat hyvin erilaiset partikkelikoon erosta johtuen. Suhteellisen kosteuden muutoksen vaikutusta melibioosin ominaisuuksiin selvitettiin myös eri menetelmillä. Muutos suhteellisessa kosteudessa aiheutti osan mitatuista eroista materiaalien välillä.

Instrumentoitua tabletointikonetta ja puristussimulaattoria käytettiin tutkimuksissa, jossa melibioosin puristuvuutta ja puristettavuutta verrattiin α - laktoosi monohydraattiin. Heckelin analyysia ja puristusnopeuden vaikutusta määritettyihin kynnyspainearvoihin (Strain - rate sensitivity) käytettiin melibioosin muodonmuutoskäyttäytymisen selvittämiseksi. Melibioosin puristumismekanismi vaikuttaisi olevan samankaltainen laktoosin kanssa, ja melibioosi on todennäköisesti laktoosin tavoin fragmentoituva materiaali. Melibioosista saadaan, laktoosiin verrattuna, puristettua murtolujuudeltaan hieman vahvempia tabletteja samoilla puristusvoimilla. Melibioosin tabletoitavuuden arvioidaankin olevan jopa hieman parempi kuin laktoosin. Tabletoinnin apuaineena melibioosi vaikuttaa ominaisuuksiltaan lupaavalta, joskin lisätutkimuksia täytyy vielä suoritta ennen kuin lopullisia johtopäätöksiä voidaan tehdä.

Avainsanat – Nyckelord – Keywords
melibioosi, preformulointi, puristus, tabletointi, Heckel -analyysi

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1. INTRODUCTION

Lactose is a disaccharide that is probably the most used excipient in the field of pharmacy (Vromans et al. 1985). Lactose has many advantages in tablet formulations. It is inexpensive and it also has excellent solubility, low hygroscopicity, mild taste and good tableting properties (Bolhuis and Zuurman 1995). Although lactose is thoroughly characterized and available in many different forms, there is a need to find a replacer for lactose as a filler/binder in tablet formulations, because it has some downsides. Prevalence of lactose intolerance is for example up to 23% in central Europe and even 100% in some Asian populations (Patel and Minocha 2000). Also awareness of lactose intolerance is constantly rising in western societies. Even if the amount of lactose is relatively small in tablets, it can cause symptoms to very sensitive persons. In any case lactose free formulations have market benefits. Further, lactose is incompatible with primary amines due to Maillard reaction (Wirth 1998).

Melibiose is a disaccharide consisting of the same two monosaccharides, glucose and galactose, than lactose. According to European, United States and Japanese pharmacopeias melibiose have not been used as pharmaceutical excipient as they do not have monographs for melibiose. Some patents found from US and European patent office's mention melibiose as an ingredient in some pharmaceutical dosage forms among other common disaccharides (Daisy et al. 1998; Zalit et al. 2007). But in these patents examples of tableting properties of melibiose are not presented. It seems that tableting properties of melibiose have not been studied yet, because there are no any published research articles about compression properties and tabletability of melibiose to be found.

Aim of this work is to characterize and to study physicochemical properties of this relatively unknown sugar. The potential of melibiose as a pharmaceutical tablet excipient, even as a replacer for lactose is evaluated. Hypothesis is that being structurally so close to lactose melibiose would have similar tableting properties than lactose. Before any assumptions about suitability of melibiose as a pharmaceutical excipient, both physicochemical and mechanical properties of melibiose must be studied. Therefore, preformulation studies for melibiose are conducted and compactibility and

deformation behaviour of melibiose are studied and compared with lactose. Existing literature of melibiose and current knowledge about fundamentals of tableting and methods for determining the deformation behaviour and tabletability are reviewed.

2. MELIBIOSE

Melibiose is a disaccharide composed from monosaccharides galactose and glucose (Fig. 1). Melibiose is formed with an alpha linkage between galactose and glucose (α-D-Galactopyranosyl-(1α6)-D-glucose). Chemical Abstracts Service (CAS) name for melibiose is 6-O-α-D-Galactopyranosyl-D-glucose.

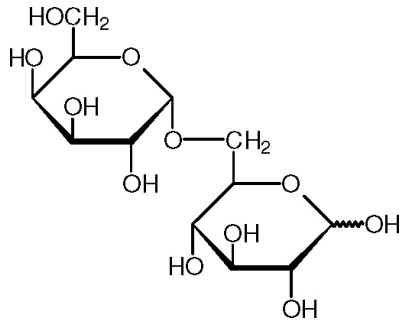

Figure 1: Structure of melibiose

2.1 Physicochemical properties of melibiose

Melibiose has two polymorphs, α- and β-melibiose, and also an amorphous form (Fletcher and Diehl, 1952). α- and β-melibiose are in crystalline form, which means that molecules are arranged in orderly repeating patterns also called the crystal lattice. In amorphous form molecules of the material do not form ordered crystal lattices and the molecules have more mobility than in crystal lattices. Polymorphism is related to chances in internal structure of crystals. Different polymorphs of crystalline solids and amorphous form of material usually have quite different physicochemical properties.

For example melting point for β-melibiose dihydrate is about 85 - 86 °C as for the α-melibiose monohydrate melting point is exceedingly higher, being in a range of 179 - 181 °C (Fletcher and Diehl, 1952). On the other hand both forms of melibiose have excellent solubility. According to Merck Index, water solubility of melibiose dihydrate and α-melibiose monohydrate is 2500 g/l. Crystalline solids are able to incorporate water molecules inside crystals upon crystallization process. During crystallization α-melibiose forms monohydrate and β-melibiose is usually in dihydrate form (Fletcher and Diehl, 1952). As free sugar, melibiose is a mixture of α-melibiose and β-melibiose and the composition is 4 α-melibiose-1 β-melibiose. Fletcher and Diehl (1952) have stated that α-melibiose monohydrate has superior crystallizing properties compared to β-melibiose dihydrate, and that it is the preferable form in which to isolate the sugar.

Different polymorphs of disaccharide can be identified by studying mutarotation. Mutarotation is a time-dependent change in optical rotation when optically active compound is dissolved in solution. For α - melibiose change of optical rotation after dissolution in water is from +166° to +142.3° (Fletcher and Diehl 1952). For β-melibiose anhydrate the change in optical rotation is from + 123.5° to +143.l°. Comparing to these measured values, it could be possible to determine the polymorphic form of melibiose, when same methodology is used.

Some estimates of polymorphic form can also be made from shape of crystals. α-Melibiose monohydrate crystals has been found to be orthorhombic (Kanters et al. 1976). β-Melibiose dihydrate crystals are monoclinic (Merck Index).

One disadvantage of lactose is that it undergoes heat-induced Maillard reaction with amino acids. Therefore, it can have incompatibility with some drugs in pharmaceutical formulations. Maillard reaction is a chemical reaction between the carbonyl group of the reducing sugar and the amino group of the amino acid (Maillard 1912). Unfortunately, melibiose is also a reducing sugar and therefore can undergo Maillard reaction. In fact melibiose have been shown to be more reactive than lactose in some conditions (Kato et. al 1989).

2.2 Pharmacokinetics, metabolism and toxicology

Although there is no toxicological data available for melibiose, it should be safe in assuming that it is not toxic or even harmful for humans as it is a component of food. For example soybeans and honey contain small amount of melibiose. Melibiose has also been used in clinically for intestinal mucosa barrier testing for paediatric patients (D'Antiga et al. 1999).

On the other hand, it should be taken in to account that humans cannot digest melibiose due to absence of α-galactosidase (Ramalingam et al. 2010). Some bacteria in gut microbial flora can break down melibiose to galactose and glucose (Van Laere et al. 1999). Therefore, as all indigestible disaccharides, melibiose can also cause flatulence and other GI –symptoms to some people (Ramalingam et al. 2010). Melibiose is also known to promote growth of some oral bacteria, and therefore, it can cause oral caries (Csáky 1965).

2.3 Sources and manufacturing

Most common natural source for melibiose is raffinose (Fletcher and Diehl 1952). Raffinose is a trisaccharide composed from galactose, glucose and fructose. Raffinose is hydrolysed to melibiose and fructose enzymically. Melibiose can also be found in small amounts in honey (Doner 1977).

Melibiose is produced traditionally from raffinose by fermentation with top yeast. Hudson and Harding (1915) describe a method that produces 175-200 grams of pure melibiose from 500 grams of raffinose. Melibiose can also be produced by hydrolyzing raffinose with a weak acetic acid. Sources of raffinose include sugar beet, cotton seeds and soya beans.

Recently, new methods for manufacturing melibiose have been presented (Kazumasa and Shinichi 2009; Kazumasa 2009). Melibiose can be produced synthetically from glucose and galactose in condensation reaction using *Geobacillus thermocatenulatus*

strains capable of sugar synthesis. Manufacturing melibiose from glucose and galactose is more cost efficient and it provides a new way to produce pure melibiose from inexpensive raw materials.

2.4 Use of melibiose

As mentioned before, melibiose is used for a clinical melibiose/rhamnose permeability test for noninvasive intestinal mucosa barrier testing (Barboza et al. 1999). Melibiose has also been found to increase the permeability of intercellular passage for calcium, magnesium and zinc (Mineo et al. 2004). It have been discovered that melibiose can also have effect on an immune system. Indigested melibiose strongly affected the Th cell responses to an ingested antigen and enhanced the immunological tolerance induced by the oral administration of an antigen (oral tolerance) in mice (Tomita et al. 2007). Also it has been found that melibiose may well be potential treatment for atopic dermatitis (Kaneko et al. 2003).

Melibiose can also be used as a sweetener in low calorie foods (Shigemura et al. 2009). When used as a sweetener some problems may occur from flatulence causing properties of melibiose and from the fact that sucrose is 3.5 times sweeter than melibiose (Merck Index). Melibiose is slightly sweeter than lactose that has about 20% of the sweetness of sucrose. Examples for melibiose in a pharmaceutical use are quite limited. Melibiose has been used as an ingredient in topical cosmetic formulations as a hydrophilic skin permeation enhancing agent (Kung et al. 2002). Melibiose has been mentioned in few patents as a possible excipient in tablet formulation, but these patents do not give examples of tableting with melibiose (Daisy et al. 1998; Zalit et al. 2007).

3. TABLETING

Tablets are the most common dosage form in a field of pharmacy (Rubenstein 2000). Even though pharmaceutical research is increasingly turning to more complex dosage forms, tablets are most likely to remain as important dosage forms in the future. For pharmaceutical industry, tablets have many benefits over other dosage forms including versatility, ease to develop and to manufacture in large scale, good stability and shelf life. Tablets are also easy to pack and handle. For patient's point of view, tablets are easy to administer and are generally well accepted.

Even though being the most used dosage form and sometimes even regarded as the most simple, tablet is a complex system consisting of an active pharmaceutical substance and a variety of excipients. Producing tablets that fill all quality requirements reproducible from batch to batch is also a complicated procedure. Tablets must have adequate mechanical strength to withstand handling but also good disintegration to achieve a desired drug release profile.

3.1 Pharmaceutical powders

A powder is defined as a heterogeneous mixture of solid particles as well as air existing both inside and outside of the particles (Nyström 1993). Having physical properties of solids, liquids and gases, powders have complicated rheological behaviour. Pharmaceutical powders are usually mixtures of an active pharmaceutical ingredient and variety of excipients. Most pharmaceutical powders consist of particles with a high crystallinity and a nonuniform particle shape and size distribution (York 1973).

3.1.1 Flow properties of powders

Flowability is an essential property of powders used for tableting (Prescott and Barnum 2000). Good flow properties are essential in producing tablets with consistent weight and strength. Flow properties of powders are affected mainly by particle surface, size and shape as well as particle size distribution (Carr 1965; Staniforth 2002). External conditions such as air content and relative humidity (RH) have a marked effect on flowability of powders (Hiestand 1966). Powder flowability studies and optimization of flow properties should be conducted during preformulation studies to minimize the effect of flow variations in production scale (Lewis and Simpkin 1994).

Several methods for studying flowability have been developed. The most common techniques include measurements of packing and avalanching behavior or flow rate of powder through orifice or funnel (Kaye et al. 1995; Ph. Eur. 2002a). A single technique that would describe complexity of the powder flow still do not exist. Different methods can be used in parallel to gain better insight in flow properties of powders (York 1980; Lindberg et al. 2004).

The Carr index and Hausner ratio derived from densification behavior of powders are used to describe flowability of powders. Hausner (1967) used ratio between bulk density and tapped density to quantify powder flowability (Eq. 1).

$$H = \frac{\rho T}{\rho B} \tag{1}$$

Where H is the Hausner ratio, ρT is the tapped density and ρB is the poured density. The Hausner ratio over 1.25 is considered indicating poor flowability (Hausner 1967). The Carr index also uses the ration between bulk and tapped density (Carr 1965) (Eq. 2):

$$C = 100 \times \frac{1 - \rho T}{\rho B} \quad (2)$$

Carr index values over 25 indicate poor, and less than 15 good flowability. Both the Hausner ratio and the Carr index have been criticized for depending on the methodology used and being empirically established values without theoretical basis (Guerin et al. 1999).

3.1.2 Powder compaction

The compaction of pharmaceutical powders is a complicated process that requires deep understanding about fundamental properties of excipients, drugs and mixtures (Rippie and Danielson 1981). After the die of the tableting machine has been filled and before pressure is applied, the powder particles are loosely packed and the density of powder is close to its poured density (York 1978). When the punches of the machine move closer to each other pressure is increased and volume reduction begins. First, particles rearrange so that the smaller particle move into the voids between the bigger particles and the powder comes more tightly packed. Regularly shaped particles attend to rearrange more easily than irregularly shaped particles (York 1978). When the initial rearrangement of the particles is completed and the pressure is further increased, the volume reduction takes place by the reversible or irreversible deformation or fragmentation of particles to smaller units (Duberg and Nyström 1986). During the compression the particle surfaces are brought closer to each other and interparticular attraction or bonds are formed (Rumpf 1958). After the pressure is removed and the decompression phase begins, the compacts usually undergo some degree of elastic recovery (Nyström et al 1993).

Compressibility is the ability of the powder to undergo volume reduction under pressure. The volume reduction process and the degree of the volume reduction of powders depend on both mechanical properties and volume reduction mechanism characteristics of the material in question (Jones 1977). The volume reduction of powder during

compression cycle happens in multiple stages and the volume reduction mechanism varies during different stages (Duberg and Nyström 1986).

Compactibility is usually considered to be the ability of powder to form a compact with an adequate strength (Fell and Newton 1970). The compactibility can also be related to the mechanical strength of compacts so that the used compression pressure is related to the force needed to break the resulting compact diametrically (Fell and Newton 1970). As the term, compression is used to describe the volume reduction process, the term compaction covers the whole process of the tablet formation including the bond formation.

3.1.3 Mechanical properties of powders

Mechanical properties of materials determine success of the powder compaction and the deformation behaviour of material is a property that mainly affects the tableting of powders (Roberts and Rowe 1986). Generally three volume reduction mechanisms, plastic deformation, brittle fracture and elastic deformation, are recognized. Deformation mechanism and behaviour during compression are unique for every material used in tableting.

Plastic deformation is a permanent type of deformation of material. In particle level plastic deformation can be described as change of shape of the particles. In particles of crystalline solid material, plastic deformation involves breaking of a limited number of atomic bonds by movement or dislocation of parallel crystal planes (Saada 1999). Plastic deformation is controlled by applied stress. The stress is the force applied on a compact or powder divided by the surface area of a compact. The stress applied to the powder bed causes a change in dimensions of the compact. Magnitude of this dimensional change is called strain. Materials considered plastic are for example sodium chloride, microcrystalline cellulose and many starches (Hardman and Lilley 1970; Roberts and Rowe 1986). Many researchers have shown that ductile materials that deform mainly plastically have a compression rate dependency that affect the tensile strength of tablets (Roberts and Rowe 1985). The compression rate dependency

is caused by the fact that plastic materials are able to undergo some degree of stress relaxation during the compression cycle.

Elastic deformation is time independent and reversible. All materials undergo some degree of elastic deformation under pressure (Marshall 1986). Elasticity of materials used in tableting is important factor to take into considerations. A high degree of elasticity is not a desirable quality for materials because the elastic expansion of compact after pressure is removed can lead to weaker tablets due to breakage of bonds between particles (Roberts and Rowe 1996). The elastic deformation of material can be calculated using following equation (Eq. 3):

$$\sigma_d = \varepsilon E \tag{3}$$

Where E is the Young's modulus of elasticity of the material, ε is the deformation strain and σ_d is the deformation stress.

Young's modulus, also called tensile modulus, is a measure of the stiffness of an isotropic elastic material. The Young's modulus is related to the tensile stress and the tensile strain according to following equation (Eq. 4):

$$E = \text{Tensile stress/Tensile strain} \tag{4}$$

The Young's modulus can be calculated from the linear part of the stress-strain plot. For tablets a beam bending experiment can be used to determine the modulus of elasticity. Texture analyzer instruments can be used to determine the tensile strength and the elastic modulus of tablets (Fig. 2).

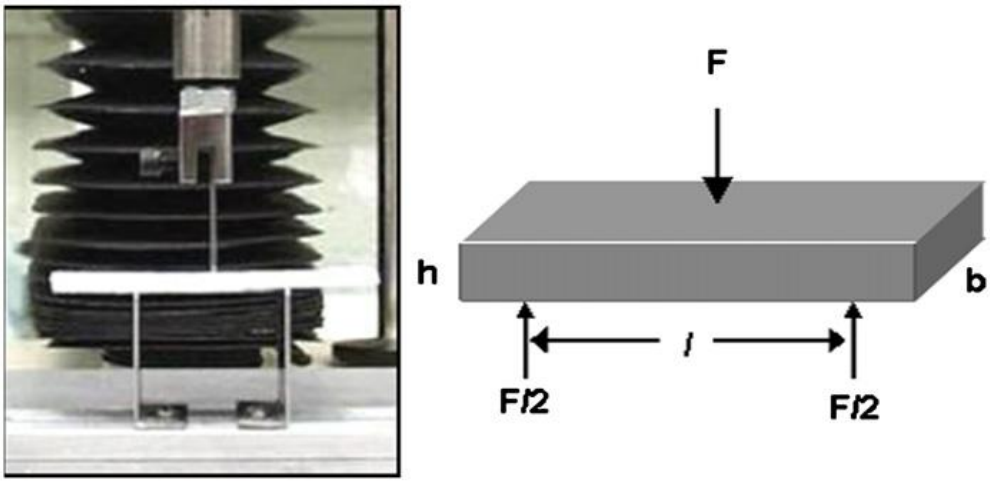

Figure 2. A texture analyser 3-point beam bending configuration for the tensile strength and the modulus (Hamad et al. 2009).

Fragmentation of particles means dividing of the crystalline particles to smaller secondary particles under pressure (Duberg and Nyström 1982). In particle level, fragmentation begins at the point called Griffith crack, where particle has surface flaws (Griffith 1921). Fragmentation occurs as applied pressure rises, when stresses inside the particle grow until the critical stress of the weakest flaw is reached (Mott 1945). The cracking of the particles is depending on the particle shape and size as well as the crystal structure (Duberg and Nyströn 1982). Typical examples of the brittle fragmenting materials are crystalline lactose and sucrose (Roberts and Rowe 1985). The fragmenting materials have some advantages in tableting over plastic materials. It has been shown that the fragmenting materials are less sensitive to the initial particle size, shape and texture (Alderborn and Nyström 1982a). Fragmenting materials are also insensitive to the compaction rate and they cause fewer problems in scaling up the manufacturing (Roberts and Rowe 1986). Fragmenting materials are also less sensitive to amount of lubricants used as well as the lubricant mixing time than the plastic materials (De Boer et al. 1978). Fragmentation of particles creates new clean surfaces for bonding that are not covered with a hydrophobic layer of the lubricant.

Materials are usually classified as brittle or ductile depending on their main deformation behaviour, although all materials undergo some degree of plastic, elastic and fragmentation during compression cycle (Duberg and Nyström 1986). The main

deformation mechanism also depends on material properties, such as particle size, and process parameters such as compression speed and compression pressures (Roberts and Rowe 1985).

Generally the plastic deformation is considered to be a desirable property for materials used in tableting as the plastic flow creates wide contact areas between particles (Benbow 1983). Materials deforming through plastic flow form stronger tablets with lower compression pressures than brittle materials (Tye et al. 2004). This is because dislocation and movement of parallel crystal planes consume less energy than breaking all atomic bonds at once during fragmentation.

Some degree of fragmentation of particles is also important because it creates new contact points for particles (Leuenberger 1989). In tablet formulations brittle excipients are usually needed when the drug is ductile and ductile excipient when the drug is brittle (Wells and Aulton 1988).

3.1.4 Physicochemical properties of powders

Physical properties including particle size, particle shape, specific volume and adhesiveness determine the compactability and flowability of powders (Carr 1965; Roberts and Rowe 1986; Kopp et al. 1989; Zuurman et al. 1994). The crystal structure and flaws together determine the deformation behaviour of materials during tableting process (Paronen and Pesonen 1986). Crystal habit can also influence tableting behaviour of powders (Shell 1963). Crystal habit refers to length, width, and thickness, in other words, external structure of crystals. Crystal habit influences particle orientation, and therefore, it has an effect on flowability, packing and compressibility of powders.

Existence of polymorphism and degree of crystallinity of the material determine its mechanical properties (York 1983). Different polymorphs can have significant difference in the compaction behavior, as it case with different forms of crystalline lactose (Vromans et al. 1985). Amorphous content of crystalline powders have been

shown to influence compaction properties of materials. For example adding amorphous lactose to crystalline α-lactose monohydrate increases tablet crushing strength significantly (Zillels and Steckel 2010). The molecular and crystal structure defines deformation mechanism for individual particles (Nesic 1987).

The particle size is connected to a bonding surface area so that the surface area is increasing when particle size is decreasing (Alderborn and Nyström 1982). Due to the increasing bonding surface area, tablet strength will generally increase when the particle size decreases. The magnitude of the effect of particle size depends on the compression mechanism of materials. Initial particle size has a smaller effect on the resulting tablet strength when compressing fragmenting materials (Alderborn and Nyström 1982). Materials also usually have a specific critical particle size, where volume reduction mechanism can change from fragmentation to plastic deformation (Roberts and Rowe 1987). The increase in surface roughness of particles increases possible contact points and bonding areas between particles (Alderborn 1988). Particle shape and geometry are also linked to surface area of particles. It can be concluded that plastically deforming material with irregularly shaped particles forms stronger tablets than the one with regularly shaped particles (Alderborn and Nyström 1982b). However, the particle surface geometry and roughness do not have an effect of same magnitude to compactibility of fragmenting materials.

Main bonding mechanism and the bonding surface area are factors for the compactibility of powders (Nyström and Karehill 1986). The bonding mechanism can be classified in five different categories (Rumpf 1958):

1. Solid bridges
2. Bonding due to movable liquids
3. Non - freely – movable binder bridges
4. Attraction between solid particles
5. Shape related bonding

According to Führer (1977), dominating bonding mechanisms affecting in compression of dry powders are the solid bridges formed by melting and the intermolecular attraction forces (e.g Wan Der Waals forces, hydrogen bonding and electrostatic forces). The mechanical interlocking is also important bonding mechanism when material consists of irregularly shaped particles.

Hygroscopicity and water content of powders are also important properties that can influence the flowability and the compactibility of materials as well as stability of the final product. It is known that different hydrate forms of materials have different compaction behaviour (Jaffe and Foss 1959). The moisture content also increases the tablet strength by increasing the tensile strength of the powder bed and decreasing density variations within the compact. Water acts as a lubricant by decreasing particle surface energy and adhesion to die wall (Rees and Hersey 1972).

3.2 Tableting properties and compactibility of lactose

Lactose is one of the most used filler/binder excipient in direct compression tableting. The commercially available forms of lactose include hydrous and anhydrous crystalline forms of α-lactose, β-lactose anhydrate and amorphous lactose (Lerk 1993). The α-lactose anhydrate can exist in stable or unstable form. The unstable form is very hygroscopic and can be produced, when α-lactose hydrate is heated at temperatures of 100 – 130 °C. The non-hygroscopic stable form can be produced with the heating temperature above 130 °C. The β-lactose can only exist in anhydrous form, because during crystallization process no water is incorporated to the crystal lattice. Spray-dried form of lactose contains crystalline α-lactose monohydrate or anhydrate and amorphous form of lactose (Vromans et al. 1986).

The different forms of lactose have different compaction properties (Bolhuis et al. 1995). The crystalline lactose is known to consolidate mainly by the brittle fracture as the degree of the plastic deformation is very low (Vromans et al 1985). The amorphous lactose has been shown to deform by plastic flow (Vromans et al. 1986).

The compactibility of the α-lactose monohydrate is depending on the powder surface area before compaction, because with increasing surface area the binding capacity increases (De Boer et al. 1986). The binding surface increases as the degree of fragmentation during the compaction increases. Different particle size fractions of the α-lactose monohydrate have different compactibility profiles. The compactibility is better for smaller median particle size fractions of lactose. The grades of ground and sieved α-lactose monohydrate used for tablet compression are usually compromises between flow and compression properties (Bolhuis and Zuurman 1995).

The anhydrous β-lactose fragments at higher degree than the α-lactose. Due to the more spherical particle shape and the rougher particle surface, it has better compactibility (Vromans 1987). The available forms of the β-lactose are not pure substances, but contain around 15% of the α-lactose monohydrate or anhydrate, and therefore, the compression behaviour is made up by both components (Bolhuis and Zuurman 1995).

4. METHODS FOR EVALUATING PROPERTIES OF POWDER MATERIALS

Preformulation studies for pharmaceutical active substances, excipients and mixtures used for tableting can be defined as the studies to investigate the physicochemical and the mechanical properties of the materials. Quality by design concept is becoming part of a pharmaceutical dosage form design (Leuenberger and Lanz 2005). Principle of the quality by design is that all factors influence designing, manufacturing and pharmacokinetic properties as well as the quality and safety of the dosage form are essential and must be thoroughly characterized (Hamad et al 2010). Therefore, the knowledge and scientific understanding about the critical material properties are becoming more and more important. The material properties determining the predictability and variability of manufacturing processes in particle, material and dosage form levels are shown in multi-scale in Figure 3. Preformulation studies must be designed so that these critical properties of the materials are defined.

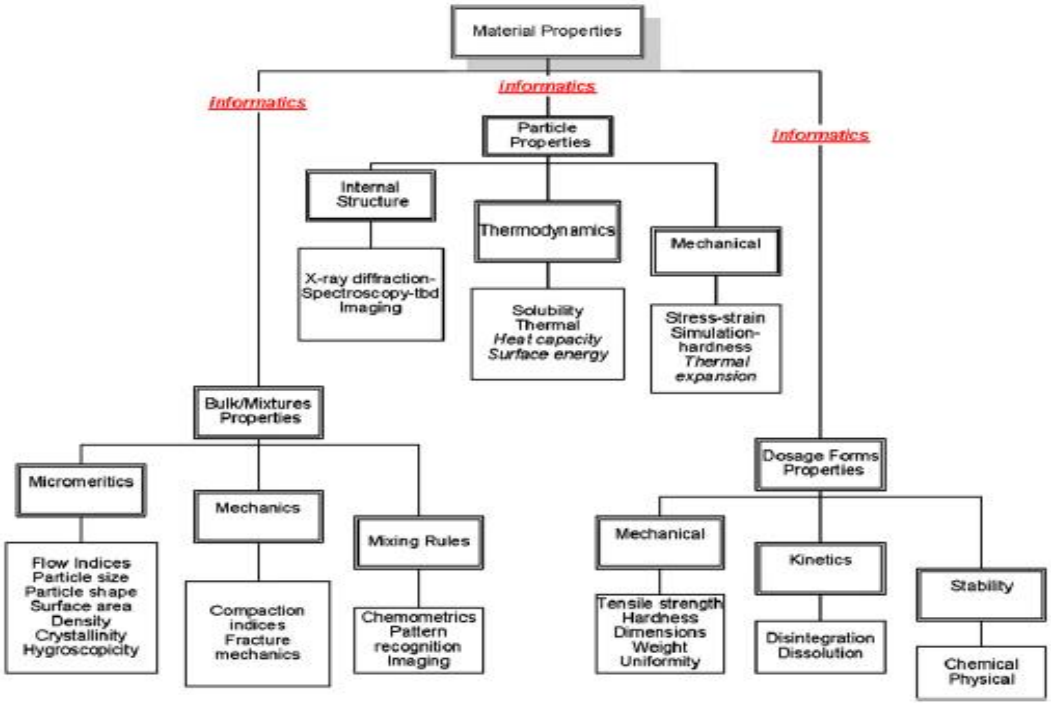

Figure 3. The pharmaceutical material properties at multiple scales (Hamad et al. 2010).

4.1 Physicochemical properties

The physicochemical properties of materials do not just affect the compaction properties but also other factors such as the stability of the products and the reproducibility of the manufacturing processes (Brittain et al. 1991). To study the solid state properties of materials, a number of spectroscopic methods, thermal analytical methods and methods for determination of hygroscopicity and solubility are used. Scanning electron microscopy is used to study particle shape, size and morphology. Optical microscopy also offers information about crystalline or amorphous state, particle size and morphology.

4.1.1 Spectroscopic methods

Spectroscopy is a study of interactions between the material and the electromagnetic radiation. The different spectroscopic methods can be used to derive information about the molecular structure and crystallinity of materials. Spectroscopy can also be used to detect, identify and quantify the materials. Every method has its unique features and limitations that must be taken in account when selecting them for each study (Gustafsson 2000). The most used spectroscopic methods in pharmaceutical powder research are X-ray powder diffraction (XRPD), solid-state nuclear magnetic resonance (NMR) and vibrational spectroscopy methods as infrared (IR) and Raman spectroscopy.

XPRD is a common technique for the study of crystal structure and atomic spacing in crystalline material. XPRD spectra are obtained by radiating materials with X-rays with wavelength similar to the interatomic distances (1Å) within a crystal lattice and recording diffraction, emission and absorption of the X-rays. By radiating the sample through a range of 2theta angles and recording diffraction, it is possible to attain all diffraction directions of the crystal lattice than can be observed as peaks in the XRPD spectra. 2theta refers to the angle of incidence and reflection of the X-rays based on the Braggs law. XRPD is best suited to the identification of the different polymorphic forms and the solvated and the anhydrous forms of a compound (Suryanarayanan 1995). Also amorphous material can be identified and quantified using XRDP as well as the degree of crystallinity of the material. Amorphous materials do not have long-range order and therefore the X-rays do not coherently scatter and peaks cannot be observed in the spectra. Sources of error in XPRD measurements include variations in particle size as well as sample orientation, height and orientation (Jenkins et. al 1986).

Infrared spectroscopy is based on the fact that molecules absorb specific frequencies of infrared radiation. Due the energy of the infrared radiation, bonds and groups in a molecule start to vibrate when they absorb radiation at the specific resonance frequency. IR spectrometer records the absorption of IR –radiation thought determined wavelength area. The vibrational modes of a molecule and can be used to deduce structural information of the material. IR spectrometry can be used to chemical identification as well as identifying different solid state structures of compounds. Three different spectral

intervals are commonly used, the far – IR (100 – 400 cm^{-1}), mid – IR (400-4000 cm^{-1}), near – IR (4000 – 14 000 cm^{-1}). The infrared methods are mostly used for identification of the organic compounds but it can be also used for the quantative measurements of polymorphs (Roston et al. 1993). IR methods are sensitive to the particle size and other disadvantage is that samples must be prepared in a suitable way. Typical way of preparing the sample is to mix it with alkali halide and compressing it to a pellet or tablet. Compressing can cause solid-state transformation of the sample material leading errors in the measurements (Bell et al. 1991). The near infrared spectroscopy (NIR) is a fast method for the material identification that does not require sample preparation. The NIR can also be used for quantitative determination of water content (Sinsheimer and Poswalk 1968).

The Raman spectroscopy is a vibrational spectroscopy that records inelastic scattering (Raman scattering) of photons caused by excitated molecules. In Raman measurements the spectra are measured by irradiating a sample with a powerful laser and recording the scattering. The difference in energy between the incoming and scattered photon, referred as the Raman shift, correlate to the differences between vibrational energy levels of the molecule. Therefore, the vibrational modes of the molecule can be recognized. Raman can be used to study crystal lattice vibrations associated with the molecule in the crystalline state. Differences between solid state forms of material can be identified studying these lattice vibrations from Raman spectra (Bellows et al. 1977). Raman spectroscopy can be used to study the polymorphic forms, crystallinity, multicomponent determination of dosage forms and in interaction studies (Bolton and Prasad 1984; Tudor et al. 1993; Quin and Kean 1998). On the contrary to the IR spectroscopy, the advantage of Raman spectroscopy is that water does not cause interference on the measurements, because water is a weak Raman scatterer. In addition Raman measurements do not require sample preparation (Gustafsson 2000).

4.1.2 Thermal analysis

Thermal analysis refers to the study of material properties as a function of temperature or time. In thermal analysis variety of different methods, including hot stage microscopy, microcalorimetry, dynamic mechanical analysis, differentitial scanning calorimetry (DSC) and thermogravimetry (TG), are used.

The DSC is probably the most used thermoanalytic method is a differential scanning calorimetry (DSC). DSC can be used to study both chemical and physical properties of pharmaceutical powders. In DSC the difference in the amount of heat required to increase the temperature of the sample and reference material, with well-defined heat capacity, are measured as a function of temperature. From the measurement of enthalpy changes during heating a physical transformation of the sample can be observed. Reaction in the DSC measurements can be divided in endothermic, such as melting and dehydration, and exothermic reactions, such as crystallization for example. The DSC can be used for example to study the stability, crystallization and glass transition temperature and polymorphism of materials (Giron 1995). Purity of materials can be also evaluated using DSC (Giron and Goldbronn 1995). Impurities in a material cause change in the melting points compared to pure material.

A thermogravimeter records the mass of the material as a function of temperature or time (Komatsu et. al 1994). It is mostly used to study the desolvation process and to quantify the volatile content in a solid. Decomposition of a compound can also be studied using TG. Most of the cases the TG study should be combined with the DSC to obtain most information about thermal behaviour of materials.

4.2 Mechanical properties

A variety of different methods for evaluating the mechanical properties of the powder materials have been developed as they are the main factor affecting the success of tableting (Appendix 2; Jain 1999). The modern instrumented tableting machines and

tableting simulators allow the parameters of the compaction process to be recorded. These parameters, including the displacements and forces of punches of the tableting machine, can be used to determine the deformation behaviour and other mechanical properties of the materials.

A range of techniques is used to assess the deformation behaviour and the compressibility of the pharmaceutical powders. These techniques include measurements of hardness, the Young's modulus and different tableting indices to estimate the brittleness, plasticity and elasticity of the materials (Hiestand and Smith 1984; Roberts and Rowe 1987). Attempts have been made to determine the mechanical properties of powders by single particle measurements (Duncan-Hewitt 1993). Compressibility and deformation behaviour have also been studied by measuring stress-strain relaxation or creep compliance, work involved in compaction, compaction force vs. time profiles and the powder density or porosity during or after compaction. The fragmentation tendency of materials has been evaluated by using scanning electron microscopy (Hardman and Lilley 1970) and by measuring the surface area changes during the compaction (Hardman and Lilley 1973).

The common traditional methods used to describe the compactibility and the compressability of the pharmaceutical powders are used in parallel to obtain more information about the material properties. Nowadays the computational multivariate methods allow combining data from the different methods (Haware et al. 2008). Multivariate method that combine the compression profile (time-resolved force and displacement data) to a generally accepted mathematical model such as the Heckel analysis has been also used for evaluation of the deformation mechanisms (Haware et al. 2008). Multivariate analysis can be used as a fast and straightforward method to differentiate, quantify and predict the compression behaviour of pure materials and mixtures. A three dimensional modeling allows combining the time, displacement and force information from one compression cycle to a one single data plot (Picker 1999). With the three dimensional analyses it is possible to identify and quantify the volume reduction mechanism of material due to the pressure and time simultaneously.

Empirical methods cannot sufficiently predict the distribution of material properties during different stages of powder compaction (Sinha 2010). For example porosity-axial

stress function traditionally used to describe tablet compression do not take account radial stress transmission or friction and density distribution in compacts with shapes different than flat and round (Cunningham and Sinka 2004). Numerical modeling of compaction processes for predicting compaction dynamics and resulting tablet properties has been adopted from soil mechanics and has been a rising trend in pharmaceutical technology during last decades. The computational simulations and models for powder compaction offer more understanding to the powder compaction process than traditional methods (Cunningham and Sinka 2004).

4.2.1 The mechanical strength of tablets

Comparing the force used for the compression and the mechanical strength of the resulting compact is a simple one point estimate and the most used method to study the tableting process (Fell and Newton 1970; Sonnergaard 2006). The effect of the compression force on the tablet strength is studied by using the tableting machine at a constant speed and varying the compression pressures. The crushing strengths and friability of resulting tablets are then evaluated. From slope of the compression force versus crushing-strength profile, it is possible to maintain qualitative information about the compactibility of materials (Fell and Newton 1970). The slope of the increase in the tablet strength is individual for each material. The sensitivity of material to changes of the compression pressures can be evaluated from the slope of compression pressure versus tablet strength. A very high slope value might indicate that the material can cause problems during the manufacturing, because very small chance in compaction force can cause significant increase in the crushing strength that can be seen in capping of tablets or variations in the disintegration and dissolution times (Chowhan 1982).

The crushing strength of tablet can be affected by tablet dimensions and a mode of fracture. The mode of the ideal fracture is that tablet splits in two halves with one diametrical fracture line (Fell and Newton 1970). Also a variety of process parameters, material properties and the amount of the lubricant used can have significant effect on measured crushing strength values (Davies and Newton 1996; Jones and Polderman

1977). Therefore, the crushing strength alone is a quite limited index of the compression properties of materials. It is also important to standardize the storage time and conditions for the tablets before the crushing tests are performed (Jones and Polderman 1977).

Because the crushing strength measurements do not take into account tablet diameters it is necessary to use the tensile strength measurement instead when the tablets with different sizes and shapes (Fell and Newton 1970). The radial tensile strength can be calculated from equation (Eq. 5):

$$\sigma_0 = \frac{2F}{\pi DT}, \tag{5}$$

where σ_0 is the tensile strength, F is the crushing strength, D is the diameter of the tablet, and T is the tablet thickness.

Tensile strength is less dependent on the tablet geometry than crushing strength but various factors such as the test conditions and compact properties can also cause errors in the measurement of the tensile strength (Fell and Newton 1970).

4.2.2 Tableting indices

Various tableting indices introduced by Hiestand and Smith (1984) are based on the ability of compacts to relieve shear stresses by plastic deformation to prevent fracture (Hiestand et al. 1977). These indices can be used to compare the relative tabletability of different materials. The Hiestand indices are calculated by using the indentation hardness and the tensile strength of cylindrical compacts.

Bonding index (BI) is used to estimate enduring of bonding areas formed during compression after decompression phase (Hiestand and Smith 1984). Bonding index can be calculated from the tensile strength of the tablet after the elastic recovery (σ_t) and

from the dynamic indentation hardness (*P*), which can be defined to be resistance of a material to plastic deformation, from following equation:

$$BI = \frac{\sigma_t}{P} \tag{6}$$

Materials with a high bonding index form stronger compact than the materials with low bonding index that may produce friable tablets (Venkatesh et al. 1998). The values of BI are usually at a range from 0 to 0.04.

Strain index obtained from the dynamic indentation hardness experiment is an indication of relative strain during the elastic recovery following the plastic deformation (Hiestand and Smith 1984). The strain index can be calculated with following equation (Eq. 7):

$$SI = \frac{P}{E'}, \tag{7}$$

where *P* is the dynamic indentation hardness and E' is the elastic (Young's) modulus given by equation 8:

$$E' = \frac{E}{1 - V^2}, \tag{8}$$

where E is the elastic modulus of the compact and *v* is the Poisson's ratio of the compact. SI values can vary from 0 to 0.04. A high SI value can indicate that the material may cause capping and lamination problems during the tableting cycle (Hiestand 1989).

Third indice, Brittle fracture index (BFI), is a measure of brittleness of compacts (Hiestand and Smith 1984). The BFI can be calculated from the tensile strength of a compact with ($\sigma_{t)}$ and without a hole ($\sigma_{t0)}$ at centre with following equation (9):

$$BFI = \frac{\sigma_t}{\sigma_{t0} - 1/2} \quad (9)$$

The BFI is an estimation of the compacts ability to relieve stress within the compact by the plastic deformation and value >0.2 can be considered as an indication of the capping and lamination tendency of materials (Hiestand 1989).

Hiestand states that these indices are useful in formulation development but not a single indice alone is sufficient to determine the tableting performance (Hiestand 1989). Number of factors, such as compact size and weight, differences in testing equipments, compact storage temperature and time and dwell time during compression, can cause variations in calculated Hiestand indices (Hiestand and Smith 1984; Hiestand 1989; Venkatesh et al. 1998).

4.2.3 Stress-strain relaxation and creep compliance

Time dependency of deformation under compression can be determined by stress-strain measurements. Stress relaxation can be measured as a function of time when constant compression pressure is maintained and decay of the upper or lower punch is measured. Plastically deforming materials show higher decay in the punch force than brittle materials because plastic deformation is more time dependent than brittle fracture (Cole et al. 1975). Plastic flow requires more energy for creating particle-particle contacts and bond formation and therefore the relaxation pressure is higher (Patel and Staniforth 1987).

Many models have been developed to analyze stress-relaxation data. For example the Maxwell model of the viscoelastic behaviour is commonly used (Armstrong and Cham 1986) (Eq. 10):

$$\ln \Delta F = \ln \Delta F_0 - kt \ , \tag{10}$$

where ΔF is the amount of the compressional force remaining in the viscoelastic region at time t, ΔF_0 is the total magnitude of this force at time zero, and *k* is the viscoelastic slope. Value of the viscoelastic slope can be used to classify materials according to the degree of plastic flow (David and Ausburger 1977).

Value of creep compliance (J) can be used to measure strain movement under constant stress (Warburton and Barry 1968). The creep in other hand means slow progressive deformation of material with time. The creep compliance can be derived from Equation 11:

$$J(t) = \varepsilon / \sigma \ , \tag{11}$$

where ε is the relative strain and σ is the constant stress. Creep compliance differs from the stress-relaxation tests, where the strain is kept constant. The apparent viscosity of materials can be determined by calculating the reciprocal of the slope from the linear part of the J(t) plot. Low apparent viscosity values indicate extensive plastic deformation of material (Malamataris et al. 1992).

4.2.4 Compaction equations

A compaction equation relates some parameter indicating state of the compaction of the powder with a function of the compaction pressure. Many compaction equations have

been developed and the purpose of fitting experimental data to an equation is to make it easier to compare different sets of data.

As the compression process is divided in different phases overlapping each other it is impossible to derive a simple equation that would adequately describe the whole process (Sonnergaard 2001). Generally mathematical models are considered to fit the data in the initial or the final stage of the consolidation process (Sonnergaard 1999). It is also very unlike that one equation will fit all deformation mechanisms (Denny 2002).

Measurement of the volume reduction and the porosity of the powder as a function of the compression pressure is a method widely used to describe compaction process (Walker 1923). The porosity of the compact can be measured when the dimensions and weight of powder column are known and compared to the true density of the powder. The porosity can be derived from Equation 12:

$$\varepsilon = \frac{1-\rho_A}{\rho_T}, \tag{12}$$

where ε is the porosity and ρ_A is the apparent density of the powder column and ρ_T is the true density of the powder.

The measurements of the porosity and the pressure are usually done with instrumented tableting presses or with a compaction simulator, where the displacements of the punches can be measured simultaneously with the compressive forces (Celik and Marshall 1989). Mostly uniaxial, one-sided compression is used. The accurate measurement of the dimensions of the compacts are needed to get the true values of the porosity, and machine looseness and machine part deformation must also be taken into account (Krumme et al. 2000). The precise value of particle density (true density) is also essential to obtain correct values of porosity (Gebaude et al. 1999). Helium pycnometry is the method of choice for measuring the true density of the powder (Sonnergaard 1999). In helium pycnometer a sample of known mass is loaded into a chamber of known volume. Then pressurised helium is let to flow into the chamber. The helium fills all empty space between particles as well as pores at the surface of the

particles. From the final pressure in the system the total gas and the true powder volume can be determined.

One of the earliest attempts to describe the powder compaction process was made by Walker (1923). Walker suggested, by examining metal powders, a logarithmic relationship between compaction pressure (P) and the relative volume (V) of the compact (Eq. 16):

$$V = C - K \times LogP\,, \tag{16}$$

where C and K are constants. The K values were found to be higher for the plastically deforming materials than for the fragmenting materials. Ratio of C/K can also be related to the compaction behaviour of materials, high ratios being related to materials producing weak tablets. Higher ratios of the C/K have been found to be characteristics for elastically deforming materials (Celik and Marchall 1989).

Equation developed by Kawakita (1956) is another well recognized in the field of the powder compaction. The Kawakita equation relates the volumes of the powder being compressed to the applied pressure (17):

$$\frac{P}{C} = \frac{1}{ab} + \frac{P}{a}\,, \tag{17}$$

where C is the degree of volume reduction($C=(V_i-V_p)/V_i$), where V_i is the initial apparent volume, V_P is the volume of the powder bed under applied pressure), P is the applied pressure, *a* and *b* being constants calculated from the plots of P_a / C vs P_a. The constant *a* represents the maximal degree of volume reduction (engineering strain) at infinite pressure. It has been claimed does not correlate to any physical property of the material being compressed (Ge 1993). The inverted constant *b* is related to the compression pressure needed to achieve the engineering strain of *a*/2. The constant *b*

has been estimated to correlate to plasticity of materials and is considered as the coefficient of compression (James 1972).

The Kawakita equation can also be used to study the flowability and tapping ability of powders by using following formula (Yamashiro et al. 1983) (Eq. 18):

$$\frac{N}{C} = \frac{N}{a} + \frac{1}{ab}, \qquad (18)$$

where N is tapping number and constant *a* is related to the powder fluidity and constant b to the the tapping ability of the powder.

The Kawakita equation has some limitations when used in compression studies. The equation can also be used for powdery materials, but not for granulated materials, in its original form (Van der Zwen and Sisken 1982). The equation can also only be applied to lower pressure levels because after certain pressures the equation becomes nonlinear (Ramberger and Burger 1985). In practise, the Kawakita plots show some level of nonlinearity and so the derived constants *a* and *b* do not correspond with the measured values. Initial packing state of the material can significally influence the degree of volume reduction so the shape and size of the die as well as the filling method can cause variations in the constants a and b derived from the equation (Sheik-Salem and Fell 1981). Physical meaning of the constants *a* and *b* is also being questioned (Kawakita and Ludde 1970). The Kawakita equation works only for a limited range of “soft fluffy” materials that produce nonlinear plots in the Heckel analysis.

The porosity/pressure functions are the most studied area of the tableting even though from the pharmaceutical industry point of view making tablets with specific strength is more important than to obtain specific volume reduction (Leuenberg and Rohera 1986). Therefore attempts have also been made to correlate tablet hardness and compression stress to relative density (Leuenberger et al 1982).

4.2.5 Heckel analysis

The Heckel equation is most used method for analyzing the deformation behaviors of the pharmaceutical materials (Roberts and Rowe 1986). Heckel (1961) developed the equation by examining plastically deforming metal powders. He suggested that the volume reduction of the metal powders is analogous to first-order kinetics of chemical reaction. Heckel also found empirical relationship between the yield strengths and the constant K from his equation (13) for a range of metal powders. From the relation between compression pressure and powder column density, identification of the phases of consolidation, deformation and compaction is possible. This type of equation was actually first proposed by Shapiro (1944), who also suggested that the reduction in porosity obeys a first-order type of reaction with applied pressure.

According to the Heckel equation (Eq. 13), change in the density of the powder column as a function of compression pressure is inversely related to the change in the porosity of the powder column (Heckel 1961a). Heckel equation gets following form:

$$\ln \frac{1}{1-D} = K \times P_{compression} + A \qquad (13)$$

In the Heckel equation D is the relative density of the powder column divided with true density of powder in compression pressure P. Constants A and K are determined from the extrapolated linear portion of the plot of ln(1/1-D) versus $P_{compression}$, A being the intercept and K being the slope (Fig. 4).

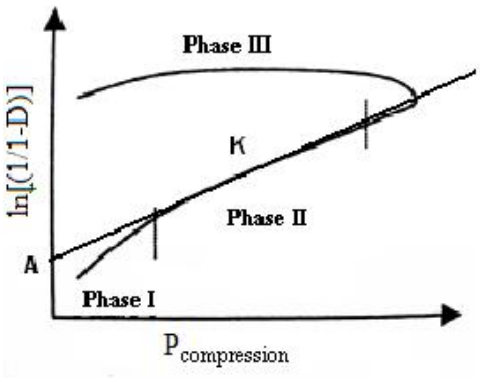

Figure 4. A typical example of a Heckel profile during compression and decompression of a powder (Duberg and Nyström 1986).

Heckel profile can be divided in three phases (Fig. 4.). At phase I volume reduction is caused by the particle rearrangement as the smaller particles fill the voids between the bigger particles (Heckel 1961a). Other explanation of the nonlinearity of this phase is the fragmentation of the primary particles at lower pressures or presence of agglomerates of the primary particles (Denny 2002). Phase II is the compression phase, where volume reduction is a result of the material deformation by plastic flow or fragmentation. The phase III is representative of the elastic expansion of material, when the compression pressure is relieved (Duberg et al. 1986, Paronen 1986).

Value of the constant K gives information of the plasticity of powder (Heckel 1961a). Plasticity increases as the value of the slope K increases. Mean yield pressure (P_y) is a value describing materials resistance for deformation (Hersey and Rees 1971). The mean yield pressure is related to the constant K by Equation 14 (Hersey and Rees 1970):

$$P_Y = \frac{1}{K} \tag{14}$$

The yield pressure values are usually lower for the plastically deforming materials that have lower resistance for deformation. For microcrystalline cellulose P_y –values from 47,6 to 104 MPa have been obtained (Roberts and Rowe 1987, Paronen 1986). For brittle materials yield pressure values are higher. For example the measured P_y values for α-lactose monohydrate are typically between 150 and 200 MPa (Ilic et al. 2009).

For the determination of the value of the K, it is important to precisely define the linear part of the Heckel plot. Usually the linear part is defined by taking first and second derivates of the plot (Roberts and Rowe 1985). For linear part first derivate is constant and second derivate is zero. Curvature nature of the Heckel plot in some materials prevents the determination of the linear part of the plot (Roberts and Rowe 1985).

Two methods, the tablet-in-die-method (at pressure) and ejected tablet method (at zero pressure), can be used to obtain data from the Heckel analysis (Heckel 1961a; Fell and Newton 1971). In the tablet-in-die-method the applied pressure and the packing fraction of the powder column are determined at several points during one compression cycle. In

the ejected tablet method the maximum upper punch pressures and the packing fractions are also used but the packing fraction is determined by measuring the dimensions of tablets after ejection from the die. The Heckel profiles obtained by at zero pressure -method usually contain elastic component that can lead to determination of falsely low yield pressure values (Krycer and Pope 1982). Paronen and Juslin (1983) have suggested that the elastic component can be excluded when the Heckel profile from “at zero pressure” -method is subtracted from the profile from “at pressure” -method.

Moisture contents of materials have an effect on calculated values of Heckel analysis. Increasing moisture content is linked to lower yield pressure values (Esezobo and Pilpel 1976). Particle slippage and rearrangement at the lower pressures increase with increasing moisture content resulting better compressibility for the material (Nokhodchi et al 1996). Overall moisture has a plasticizing effect on materials (Garr and Rubenstein 1992).

Heckel analysis has some limitations when used for pharmaceutical materials. Heckel made his research with metals, and the deformation behaviour of organic solids is quite different than that of metals (Duberg and Nyström 1986). The region where Heckel plots are linear occupies only a small part of the total densification, and the linear part of the plot is impossible to define for some materials. Sonnergaard (1999) states that the parameters derived from the Heckel equation are not reliable and reproducible material constants. It is also claimed that the yield pressure value does not necessarily represent the plastic deformation of materials, and is in any case overestimated, because of the elastic component. Heckel analysis is dependent on the experimental conditions and small errors can give great variations in the calculated Heckel parameters (Sonnergaard 1999). For example values of the yield pressure measured by different authors differ significantly. For example calculated P_y values for microcrystalline cellulose (Avicel PH 101) are from 47.6 MPa to 104 MPa (Roberts and Rowe 1987; Paronen 1986)

Correct determination of true density values used in the Heckel analysis has great importance. One percent error in the true density values can cause 10% error in the determined yield pressure values (Gebaude 1999). Accurate measurement of the punch displacement and corrections of machine deformation are important. The maximum compression pressure has an influence on the yield pressure values (Paronen 1986; Rees

and Tsardaka 1994). For the compression pressure values used to calculate the Heckel profile Ragnarsson et al. (1984) have recommended that the mean of upper and lower punch forces should be used.

Attempts have been made to improve methods for the determination of the Heckel plots. For example Krumme et al. (2000) suggest some critical matters to be taken in consideration when using the Heckel analysis. For correct determination of the relative density, the accuracy of height of powder bed under all circumstances should be better than 10 µm (+-5 µm). The determination of the diameter of the die should also be better than +-5 µm. True density of material should be measured using very high-pressure experiment and the accuracy of the measurements should be better than +- 0.01 g/ml. The correction of machine deformation by compression of solids is recommended, but is less critical.

The determination of the deformation behaviour of materials using the Heckel analysis can be done simply by calculating the yield pressure values but some other methods have also been proposed. Particle size has been shown to have an impact on the determination of Heckel profiles (Hersey and Rees 1970; Roberts and Rowe 1986). When different particle size fractions are compressed, different behaviors in Heckel plots are seen for fragmenting and plastically deforming materials (Fig. 5). Heckel plots for plastic materials for different size fractions of the same material remain parallel for the whole compression pressure range (Type 1). For fragmenting materials (Type 2) the Heckel plots show coincident linear relation when the initial structure of the powder bed is destroyed.

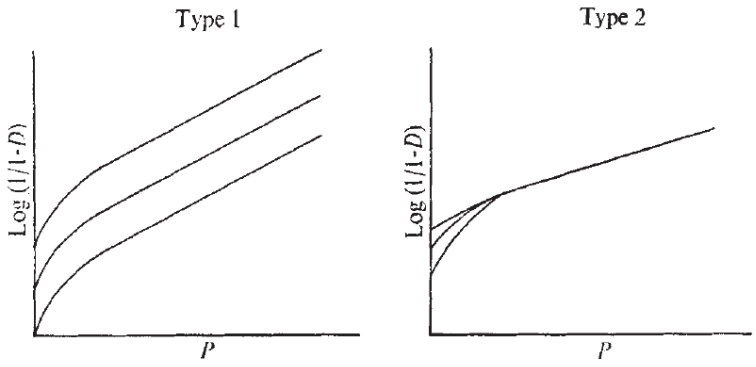

Figure 5: Heckel plots for different size fractions of plastic (Type 1) and fragmenting materials (Type 2) (Hersey and Rees 1971)

The Heckel parameters are also sensitive to the compression speed. Roberts and Rowe (1985) used the yield pressure values derived from the Heckel plots and introduced strain rate sensitivity index (SRS). The SRS describes the percentage increase in the yield pressure at two different punch speeds. It is given by the difference in the yield pressures at the lowest (P_y1) and at the highest punch velocities (P_y2) divided with yield pressure at the highest compression speed (Eq. 15). In the original research, the lowest punch velocity used was 0,033 mm/s, the highest being 300 mm/s

$$SRS = \frac{P_{Y2} - P_{Y1}}{P_{Y2}} \times 100 \tag{15}$$

Using the strain rate sensitivity as indicator it is possible to compare the deformation mechanism of different materials. Materials that deforms by fragmentation have SRS –values under 2% (Roberts and Rowe 1985). Plastically deforming materials have higher SRS –values. Table 1 shows SRS –values for materials with different deformation mechanisms (Roberts and Rowe 1985).

Table 1. Values of the strain-rate sensitivity (SRS) of the materials with different deformation mechanisms. (Roberts and Rowe 1985)

Material (Trade name)	**SRS%**
Calcium phosphate	-
Calcium phosphate	-
Heavy magnesium carbonate	-
Paracetamol for direct compression	1.8
Paracetamol drug	10.6
Crystalline lactose	16.2
Agglomerated α-lactose-monohydrate (Tablettose)	19.2
Anhydrous lactose	20.3
Microcrystalline cellulose (Acicel PH101)	38.9
Sodium chloride	39.9
Mannitol	46.4
Maize starch	49.3
Poly Vinyl Chloride (Corvic)	54.1

Curvature nature of the Heckel plot for materials undergoing high degree fragmentation prevents the determination of linear part of the plot, and therefore the SRS –index cannot be calculated (Roberts and Rowe 1985).

5. EXPERIMENTAL

5.1 Aims of this study

The purpose of this research was to characterize the physicochemical properties of melibiose with different methods commonly used in pharmaceutical preformulation studies. Compression studies were also done to determine compactibility, compressibility and deformation mechanism of melibiose compared to α-lactose monohydrate and other well-known materials. Potential of melibiose as a replacer of lactose in tablet formulations was also evaluated. Crystalline lactose is a fragmenting material and hypothesis was that, as crystalline material, formed from the same two monosaccharides, melibiose would also be fragmenting. Two different batches of melibiose from two different suppliers were used in this research to determine if there are differences between them and to find the best quality of material for tableting on the market at the moment. Effect of the moisture content on the material properties was also under examination.

5.2 Materials

Melibiose was purchased and used from two different manufacturers. Melibiose monohydrate >98% (HPLC) was obtained from Sigma-Aldrich (St Louis USA) and other batch of melibiose monohydrate from Senn Chemicals (Dielsdorf Switzerland). These different batches of melibiose are referred in this study as melibiose (Sigma) and melibiose (Senn), respectively. Milled α-lactose monohydrate (Pharmatose 80M and Pharmatose 200M; DMV-Fonterra Excipients, Veghel Neatherlands) was used at this study, because of similar particle size compared to melibiose batches used. Microcrystalline cellulose (Avicel PH-200; EMC Biopolymers Ireland) was used as a plastically deforming reference and calcium dihydrogen phosphate (Emcompress; Edward Mendell CO Inc. New York USA), as a fragmenting reference material.

5.3 Densities

Bulk density of powders was measured by filling a 250 ml cylinder with a known weight of material and reading the volume of the powder. Tapped density was then measured for the samples according to Ph. Eur. with a standard apparatus (Erweka SVM 1UZ, Erweka Apparatebau, Hemseustaum Germany). The volume of powders after 1250 taps was used to calculate the tapped density value. The measurements were done in triplicate. Samples were stored in room humidity and temperature prior to density measurements.

True density of powders was measured with a heliumpycnometry (Multivolume pycnometer 1305, One Micromeretics Dr. Norcross USA). Three samples of each material were measured in triplicate. Sample cup was filled with 2/3 of material and sample was accurately weighed. Helium flow through every fresh sample was used to remove moisture from samples. The flow pressure of helium was 5 Pa and it was sustained for 5 minutes. Pressure in the system was allowed to stabilize for 30 s and the pressure values were used to calculate the true volume of the sample. Average values of the true density was calculated from mass of the sample and from the true volume, and used for this study.

5.4 Flowability of materials

Flow properties of materials were studied with an experimental “Flowpro” apparatus (University of Helsinki, Finland, Department of Pharmaceutical Technology). All materials were stored in conditioned space (RH 40 - 50%, 25 °C) for several days before testing. Testing was done in same conditions. Metallic cylinder with a volume of 5 ml was filled with powder and placed to the apparatus. The apparatus shakes the cylinder at a constant rate and measures the mass of the powder flowing through 2 mm orifice on the bottom of the cylinder. Minimum of 5 measurements for each of the materials were performed. The Hausner ratio and the Carr index values calculated from

the bulk and tapped densities of the materials were also used to estimate the flow properties of the powders.

5.5 Scanning electron microscopy

A scanning electron microscopy (SEM) (Zeiss DSM 962, Oberkochen, Germany) was used to investigate the particle size, shape and morphology of the melibiose batches. Rough estimates of a median particle size and particle size range were also made from SEM images. Samples were prepared so that the powder was sprinkled on top of carbon tape and loose particles were removed with pressurized air. Samples were then coated with conductive platinum coating in vacuum evaporation coater. SEM micrographs were obtained at an acceleration voltage of 10 kV and magnification from 100x to 500x was used.

5.6 Thermal analysis

Dehydration rates as a function of temperature and melting points were measured with a differential scanning calorimeter DSC823e (Mettler-Toledo Inc., Switzerland) with a cooling unit (Labplant RP 100MT). Samples were weighed with an analytical scale and sealed to 50 µl aluminum pans with manually pierced holes on lids. Samples were first stabilized in 25 °C for 3 minutes and then heated from 25 °C to 250 °C with heating rate of 10 °C/min. Nitrogen was used as a protective gas at flow rate of 50 ml/min. Melibiose from both manufacturers were measured as received. Measurements were also done to samples stabilized at least one week in RH of 40% and 75% as well as to dried samples stored in desiccators with phosphorous pentoxide and silica gel as drying substances. Measurement for the stabilized samples was done to study the effect of the RH on the behaviour of substances during heating.

Moisture content as well as dehydration temperatures and behaviour were studied using thermogravimetry (TG) analysis (TGA 850 (Mettler-Toledo Inc., Switzerland).

Measurements were done to samples as received as well as to samples stabilized at least one week in RH of 10%, 20% and 75%. Sample of 3-10 mg of material was transferred to open aluminum oxide pan. The pan and material were weighed and then heated from 25 °C to 190 °C in the TG apparatus using nitrogen flow of 50 ml/min as protective gas. Heating rate was 10 °C/minute. Temperature was kept constant for 3 minutes after it had reached 190 °C. Weight loss of the sample material was recorded as a function of time and temperature.

5.7 Fourier transform infrared and near-infrared spectroscopy

Fourier transform infrared spectrometer (FT-IR) (Vertex 70, Bruker optics, Germany) with ATR reflector with MIRacle single bounce diamond crystal 45 degree face angle and digital high pressure clamp was used to determine IR spectra for both melibiose batches. The ATR reflector was used to minimize the effect of preparation of the sample on measured spectra. Background and samples were measured 20 times with resolution of 4 cm^{-1} between 4000 cm^{-1} to 650cm^{-1}. Samples were measured as received from the manufacturers.

Near–infrared (NIR) spectra were measured using a Fourier transform (FT)-NIR spectrometer (Bühler NIRVIS, Uzwil, Switzerland) with fiber-optic probe. Diffuse reflectance spectra of materials were measured over the range of 4008–9996 cm^{-1} (1000–2500 nm). All measurements were performed three times to confirm repeatability. All samples were poured into a glass vial with clear flat bottom so that it was covered completely with the material. All measurements were done in same vial washed and dried between each material. Sample distance to the probe was constant in every measurement.

5.8 Raman spectroscopy

Raman spectrometer (Raman RXN Systems, RXN1-PhAT-785-D, Kaiser Optical Systems inc., USA) was equipped with external laser source (Kaiser Optical Systems Inc., USA) and operated at wavelength of 785 nm, and had a cooled charge-coupled device (CCD). Temperature of CCD was stabilized in -60 °C. Holograms 4.1 software (Kaiser Optical Systems Inc., USA) was used in measurements. Exposure time was 6 seconds and accumulation of spectra was 3. Samples measured included bulk sample (as received), samples dried in a desiccators containing phosphorous pentoxide and silica gel and samples stabilized in the RH of 75%. Each sample was measured in open glass vials in triplicate.

5.9 X-ray powder diffraction

Crystal properties of materials was studied with a X-ray powder diffractometer (XRPD) (Bruker D8 Advance x-ray powder diffractometer, Bruker Axs Inc., WI, USA). CuKα – radiation (λ = 1,54 Å) was used to obtain the spectra. The angular range was 5–40º (2theta), and the measurement rate 0.1º per second. Diffractograms were compared to the diffractograms for melibiose obtained from the Cambridge Structural Database of The Cambridge crystallographic data centre (CCDC). Diffractograms for melibioses from different manufacturers were also compared. Samples were measured as received. To study the effect of moisture content, samples stabilized in RH of 20%, 45%, and 75% were measured and compared. XRPD measurements were also done during heating. Measurement points were 25 °C, 130 °C, 170 °C and 185 °C to study the effect of heating and dehydration on measured spectra. Heating rate was 0.2 °C/min and sample was stabilized 300 seconds in each temperature points.

5.10 Compression studies

The compressibility and compactibility of the two melibiose batches and reference materials were studied with two different methods. Eccentric tableting machine was used for compression in two different pressure levels and the crushing strength of the resulting tablets was measured. Materials stabilized in different relative humidities were compressed to study the effect of moisture content on the compactibility profiles of the materials.

The Heckel analysis was performed with a compaction simulator to study the volume reduction mechanisms of melibiose, α-lactose monohydrate and microcrystalline cellulose. Differences in the densification behaviour were determined comparing the shape of the Heckel plots, the yield pressure values and strain rate sensitivity index values.

5.10.1 Compactibility

The compactibility of the two batches of melibiose and the reference materials; calcium dihydrogen phosphate, α-lactose monohydrate (Pharmatose 80M) and microcrystalline cellulose, was studied with instrumented eccentric tableting machine (Korsch EK0; Erweka Apparatebau, Germany). Flat faced punches with diameter of 9 mm were used. Machine was calibrated so that the upper punch was adjusted to lowest position. Position of the lower punch was then adjusted with 3.000 mm calibration disk between the punches to keep the upper punch and the tablet height constant during tableting. The die and the upper punch were prelubricated with 5% magnesium stearate acetone suspension to prevent sticking problem. This was done because internal lubrication was not used. The die was filled manually with powder and flywheel of the tableting machine was twisted backwards as long as the lower punch started to rise again. This was to make sure that the machine would have enough time to speed up to constant velocity after starting the motor. Machine speed of 36 rpm was used for this study.

Powders were sieved with analytical sieves to obtain particle size fraction between 125 and 355 micrometers. Vibrating sieving apparatus (Fritsch laborgerätebau 03,562 Germany) was used and amplitude used was 6 during 5 minutes sieving time for each material. The particle size distribution of material is known to affect the tensile strength, and the same sieve fraction of each material was used to get comparable data.

Weight of the sample materials was adjusted so that similar compression pressure values at two stages were reached for each material (Appendix 2). Mean of upper and lower punch forces was used to calculate the compression pressure in MPa. The lower values of compression pressures were between 50-100 MPa and the upper values between 150-200 MPa. Relative compactibility of materials is examined by comparing compression pressure to the tensile strength of resulting tablets. Statistical analysis between tensile strengths of tablets was done with t-test. At 95% confidence interval, P value lower or equal to 0.05 was considered the limit of significance between tensile strengths.

Different samples of materials were stored for several days in RH of 10%, 40% and 75% to study the effect of humidity on the tabletability. Tableting was performed in conditioned space. The RH were stabilized so that the RH of the room was 20% for tableting of 10% samples, 40% for tableting of 40% samples and 75% for tableting of 75% samples. Water activity (a_w) was measured for each material (n=1) with a water activity meter (Aqualab Series 3, Decagon Devices Inc. US).

Dimensions of tablets were measured with digital micrometer (Sony U30-F Digital indicator, Sony magnescale Inc., Japan). Tablet crushing strength was measured with a single column material test machines (Lloyd LRX, Lloyd instruments UK).

5.10.2 Heckel analysis and strain rate sensitivity

Heckel analysis was done to evaluate deformation mechanism of materials. A compression simulator (Puuman Oy Kuopio Finland) was used for the Heckel analysis. Flat tableting punches of 10 mm were used for the compression of powders. Machine set-up were made so that the lower punch was kept stationary when the upper punch

moved down according to the standard “saw tooth” displacement/time profile and the minimum distance between the upper and the lower punch was 3 mm during compression cycle. The powder sample weights were determined from the true density values so that each material would give a 3 mm thick compact at zero porosity to allow comparison between materials. For both melibiose batches the target weight was 365 mg, for lactose monohydrate 360 mg and for microcrystalline cellulose 384.1 mg. “At pressure” methods were used and therefore the punch displacements and punch forces were measured during the whole compression cycle.

Sieve fraction of 125-355 micrometers of the materials was obtained as described earlier and used for this study. At the first study, melibiose batches and α-lactose monohydrate were used. The powder samples were stored for several days in desiccators containing saturated salt solution to get RH of 20% and 40%. Another compression study was also done two months after the first study. At the second study same equipment was used and all machine parameters and sample weights were kept same as in the first study, but the samples were not humidity stabilized. At the second study microcrystalline cellulose was also used as a plastically deforming reference material. The tableting in both studies was performed at space without humidity and temperature control but samples were kept conditioned until weighing and compression were performed.

Each powder sample was individually weighed with accuracy of +- 0.2 mg with analytical scale and manually poured to a die. The die were prelubricated with 5% solution of magnesium stearate in acetone using cotton stick to prevent sticking problem and to minimize the effect of die wall friction to the measurements. Each tablet was weighed after compression.

Two punch velocities, 3.7 mm/s and 300 mm/s, were used and three compacts for each material was compacted with both velocities. The lower punch velocity was higher than 0.033 mm/s used by Robers and Rowe (1985), because software did not allow lower velocities to be used.

Thickness of the compacts was calculated from the displacement data of the upper punch and lower punch so that the distance of the punches was the thickness of the compacts. Relative tablet density at each pressure point was calculated using the weight of the tablet after compression and from the thickness of the tablet at each pressure point. Elastic deformation of machine parts was not calculated and taken into account in this study. This mostly likely causes some degree of systematic error to determined values.

Average of the upper and the lower punch forces were used to calculate pressure values. Heckel plots were calculated according to Equation 13 (Heckel 1961a). The linear parts of the Heckel plots were determined by taking a linear regression from the data between correct pressure values (for 3.7 mm/s 100-300 MPa and for 300 mm/s 40-180 MPa). The linearity were determined from the correlation coefficients (>0.9900). The yield pressure values were calculated from the K values of the linear part of the Heckel plots using Equation 14. The SRS -index values were calculated from the yield pressure values according to equation 15 (Roberts and Rowe 1985). Three tablets were compressed for each material and mean values of P_y were used to calculate the SRS – index.

6. RESULTS AND DISCUSSION

6.1 Densities

The bulk, tapped and true density values are shown in the Table 2. According to the Handbook of Pharmaceutical Excipients (2000) Pharmatose 80M has a bulk density of 0.75 g/ml and tapped density of 0.92 g/ml. The true density for α-lactose monohydrate is 1.545 g/ml. The true density value obtained with our method is slightly lower than mentioned in this literature. The density values for melibiose are not mentioned in literature.

Table 2. Density values and calculated Hausner ratio and Carr –index values for materials.

Material	Bulk density (g/ml)	Tapped density (g/ml)	True density (g/ml) (standard deviation)	Hausner ratio	Carr index
Melibiose (Senn)	0.62	0.72	1.5526 (0.004)	1.2	13.9
Melibiose (Sigma)	0.49	0.59	1.5515 (0.003)	1.2	16.9
α-lactose monohydrate (Pharmatose 80M)	0.74	0.83	1.5291 (0.003)	1.1	10.8
Microcrystalline cellulose (Avicel PH-200)	0.38	0.48	1.5501 (0.006)	1.3	20.8

6.2 Flowability of powders

The Hausner ratio (Eq. 1) and the Carr index (Eq. 2), calculated from the ratio of the poured and the tapped density were used to estimate flowability of powders. Pharmatose 80M has smallest Carr index value and Hausner ratio values so it can be considered to have best flow properties (Table 2). Melibiose (Senn) can also be assumed to have good flowability as its Carr index is 13.9.

Values of the flowability of materials measured with the Flowpro apparatus are shown in Table 3. According to these results melibiose (Senn) has superior flowing properties compared to other materials. These good flow properties of the melibiose (Senn) are probably due to spherical particle shape, larger particle size and uniform particle size distribution (Fig. 6). Poor flow properties of Sigma melibiose are caused by smaller particle size (Fig. 7 and 8). Melibiose (Senn) would be the material of choice over melibiose (Sigma) for direct compression when flowability is taken into account.

Table 3. Flow properties of materials measured with Flowpro apparatus. RSD = relative standard deviation, RH = relative humidity during measurements, n = number of parallel measurements, Pharmatose = α-lactose monohydrate.

Material	Flow rate (mg/s)	RSD%	Flow Rate (mm^3/s)	RSD%	Mass (g)	RH%	n
Melibiose (Senn)	38.452	7.8	80.604	8.6	2.387	50.4	5
Melibiose (Sigma)	18.957	5.6	46.586	5.5	2.035	50.2	6
Pharmatose 80M	24.605	3.5	35.137	2.4	3.501	54.1	5
Pharmatose 200M	6.386	6.7	15.305	7.5	2.088	49.8	6

The calculated and measured values for flowability have some inconsistencies. The Hausner ratio and the Carr index indicate that Pharmatose 80M would have superior flowing than melibiose (Senn). The experimentally determined values on the other hand show that melibiose (Senn) has superior flowing properties (Table 3).

6.3 Scanning electron microscopy

Figure 6 shows SEM images of different batches of the melibiose and α-lactose monohydrates. The melibiose (Senn) (a) has smaller primary particles aggregated to uniform sized spherical aggregates. The particle size of melibiose (Sigma) (b) is smaller and it has more heterogeneous particle size distribution as some bigger particles can be seen. For comparative reasons the SEM images of Pharmatose 80M (c) and Pharmatose 200M are shown. In SEM images of melibioses, smaller particles cannot be seen (Fig. 7). This might be due to the sample preparation. Pressurized air was used to remove loose particles from carbon tape and it is possible that this was enough to remove the smallest particles from the samples.

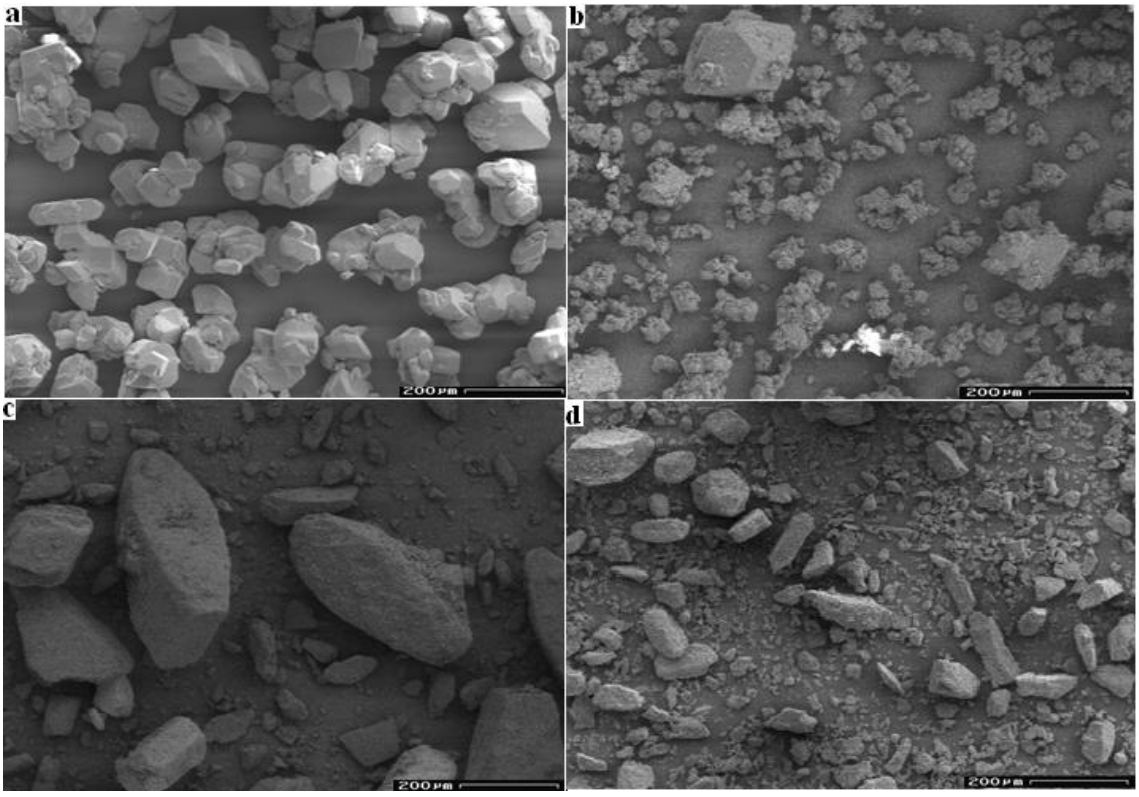

Figure 6: SEM images of melibiose (Senn) (a), melibiose (Sigma) (b), Pharmatose 80M (c), Pharmatose 200M (d) in 100x magnification.

In larger magnifications some similarities between the melibiose batches (Fig. 7) can be seen. Both qualities are aggregated but the primary particle size of melibiose (Senn) (a) is significally larger than the primary particle size of melibiose (Sigma) (b).

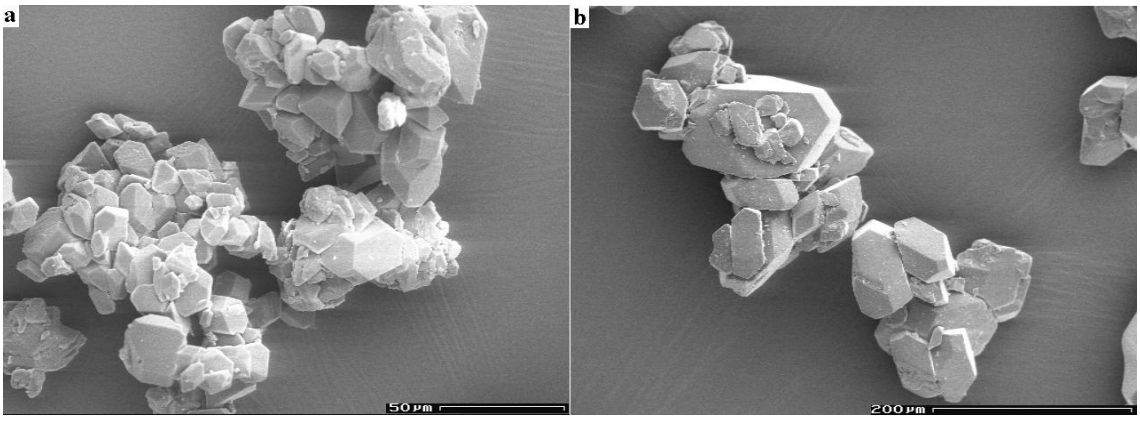

Figure 7: SEM images of Melibiose (Sigma) (a) in 500x magnification and melibiose (Senn) (b) in 200x magnification.

Estimates of particle median can be made from SEM images. For melibiose (Senn) agglomerate size is between 100 and 200 µm primary particles being between 20 and 200 µm (Fig. 7). Agglomerate size of melibiose (Sigma) is approximately between 20 to 100 µm, but some larger agglomerates can also be seen in the images (Fig. 7). Primary particle size for melibiose (Sigma) is approximated to be between 5 and 30 µm. Pharmatose 80M particles are 70 – 90% between 100 and 250 µm (DMV-Fonterra). Sieve experiment to determine the particle size distribution was not done due to defiency of material. The sieve analysis was also evaluated to be too inaccurate for powders and most likely to be impaired because of the agglomerated nature of melibioses. For further studies some other methods as laser diffraction and specific surface area measurements should be done to determine the particle size distribution and micromeritics.

6.4 Thermal analysis

DSC curves for both melibiose batches show an endothermic reaction at temperatures around 100 °C (Fig. 8). This endotherm is most likely evaporation of water. Melting onset temperature for melibiose (Senn) is from 182 °C to 184 °C (Table 4.) which is slightly higher than the melting point from 179 °C to 181 °C for α-melibiose monohydrate mentioned in the literature (Flecther and Diehl 1952). Melibiose (Sigma) has slightly higher melting onset temperature being from 185 °C to 187 °C (Table 4).

Melibiose (Senn) also shows a small endothermic “shoulder” at 170 °C that cannot be seen in DSC curve of melibiose (Sigma). This “shoulder” recurred in parallel measurements and is most likely some residual water evaporating at higher temperatures but presence of impurities cannot be excluded. This is most likely the cause for lower melting point of melibiose (Senn) compared to melibiose (Sigma)

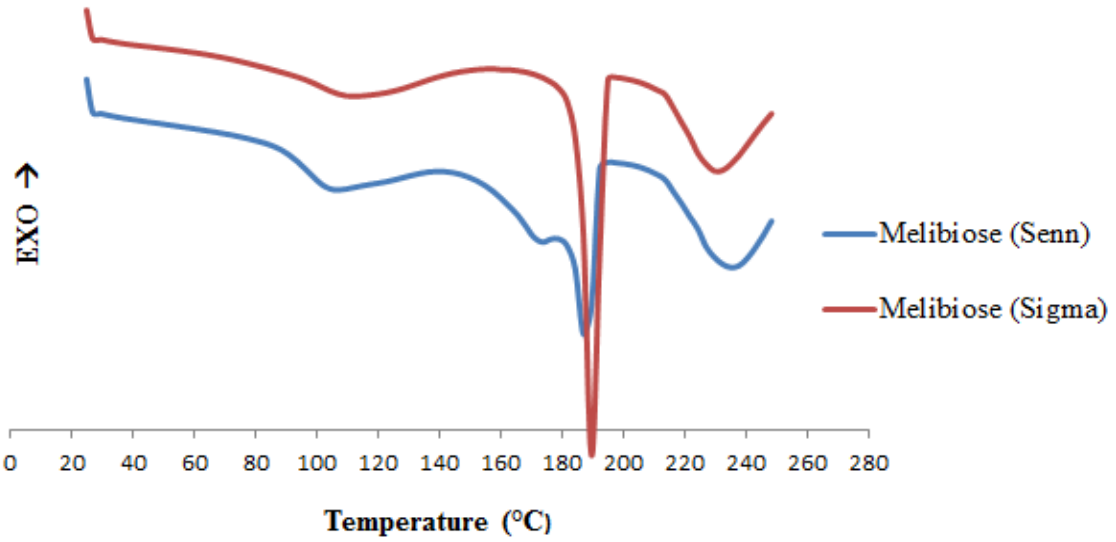

Figure 8: DSC curves for both melibiose batches stabilized in RH of 40%.

Thermogravimetry curve for melibiose (Senn) shows that loss of weight occurs in two steps (Fig. 11). First step is between 32 °C – 170 °C and loss of mass is 2.6% (Table 4). Second step is between 170 °C – 190 °C and the loss of weight in the second step is 1.2%. Overerall loss of mass is 3.8%. Second step of loss of mass at after 170 °C confirms that the “shoulder” seen in DSC curves for melibiose (Senn) is caused by evaporation of water. TG curve for melibiose (Sigma) differs from the one of melibiose (Senn). For melibiose (sigma) the loss of weight is a one single step and the loss of mass is 2.7%.

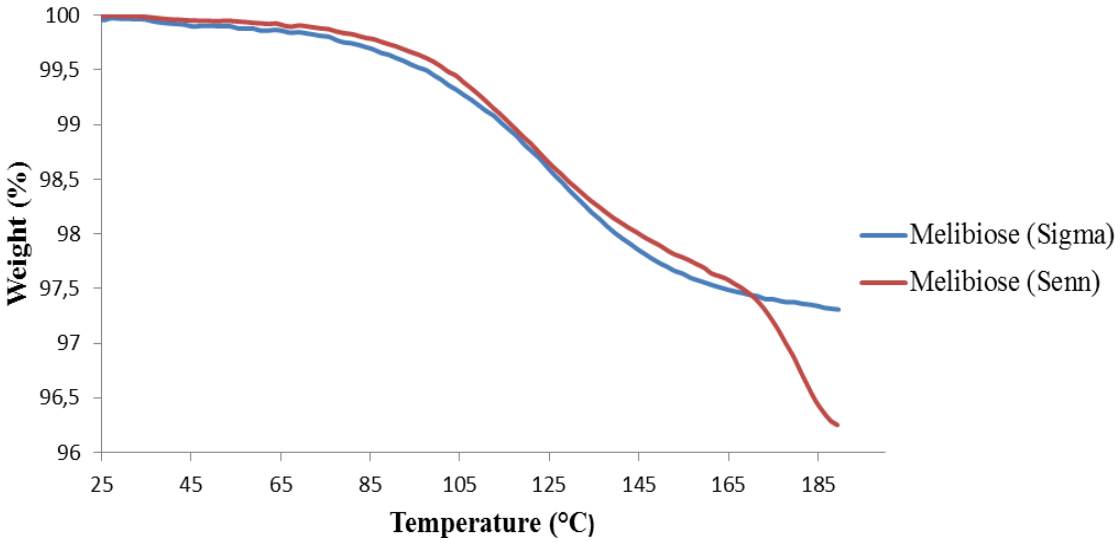

Figure 9. TG curve for both melibiose batches stabilized in a RH of 20%.

DSC measurements were done to samples dried in a desiccator containing phosphorous pentoxide and silica gel to determine if the “shoulder” of melibiose (Senn) is actually water leaving the particles. The RH in phosphorous pentoxide and silica gel desiccator was found to be between 5 – 10% and not 0% as expected. It can be concluded that dried samples used in thermal analysis and spectroscopic methods are not completely dry.

However in Figure 10, from the DSC curve for the dried sample, can be seen that the “shoulder” disappears confirming that the “shoulder” is caused by the residual water. It can also be concluded that the phosphorous pentoxide and silica gel drying was effective in removing tightly bound water from materials even though small endotherm indicating evaporation of water, is observed after 100 °C. The curve of melibiose (Senn) still has different shape than melibiose (Sigma) (Fig. 11). It is possible that crystal structure of melibiose (Senn) is more unstable after evaporation of tightly bound water which lowers the melting point. One conclusion made from Figure 9 is that further increase in RH do not have and significant effect on thermal behaviour of melibiose (Senn).

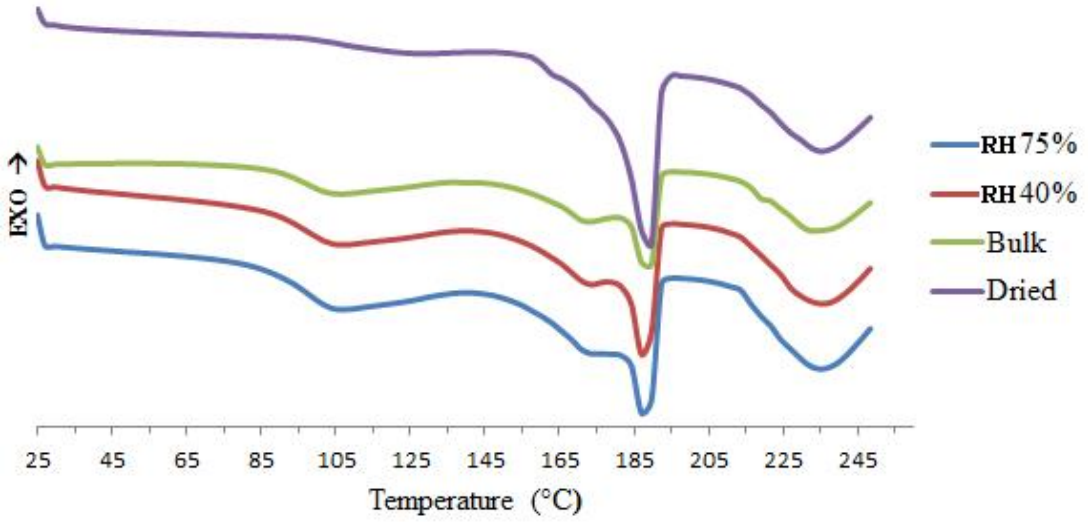

Figure 10: DSC curves for melibiose (Senn) stabilized in different RH, measured as received and dried in desiccator containing phosphorous pentoxide and silica gel.

For melibiose (Sigma) effect of drying or moisturizing do not have an effect on DSC curves (Fig 11). From the absence of dehydration endotherm around 100 °C can be concluded that the phosphorous pentoxide and silica gel drying has been effective in removing water from melibiose (Sigma).

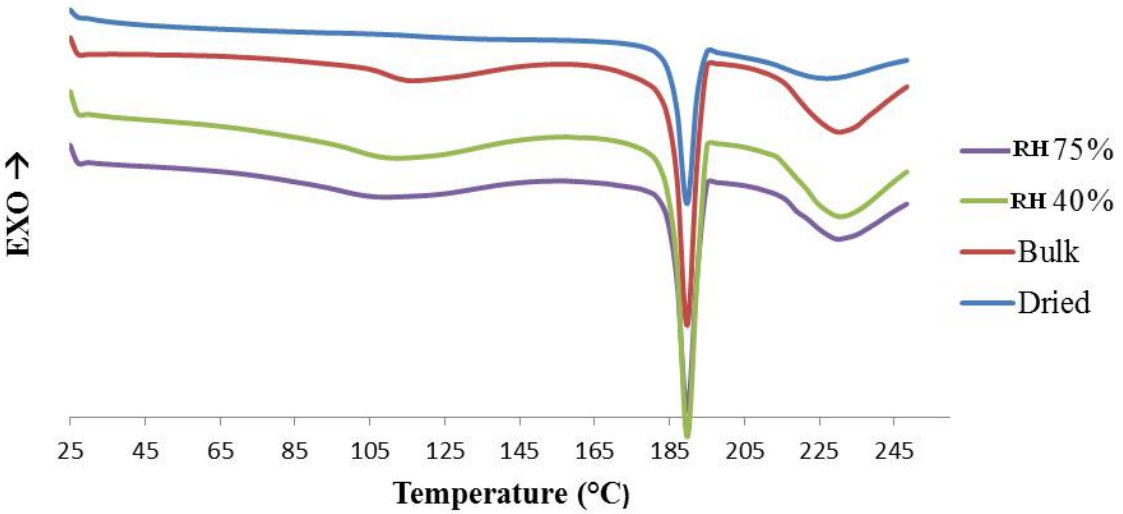

Figure 11: DSC curves for melibiose (Sigma) stabilized in different RH, measured as received and dried in phosphorous pentoxide.

It can be concluded that RH increase lowers melting peak temperatures of both melibioses (Table 4). Melting onset temperatures on the other hand behave differently. For melibiose (Senn) the increase in RH upraise the melting onset temperature. For melibiose (Sigma) the effect of increasing RH lowers the melting onset temperature. Melting enthalpy values are higher for melibiose (Senn). Most likely the "shoulder" phenomena cause error in determination of correct values for melting onset temperatures and melting enthalpy of melibiose (Senn).

In Table 4, the total mass loss and onset temperature of mass loss measured with TG are reported for samples stabilized in different humidity levels. Total loss of mass increases slightly with the increasing humidity level as expected. Levels of total loss of mass are higher for melibiose (Senn), which might indicate higher degree of hygroscopicity or larger degree of porosity and tortuosity of the pores.

Table 4. Thermal analysis data of melibioses. (n=1)

Material	RH	Melting onset temperature (°C)	Melting peak temperature (°C)	Integral normalized enthalpy of melting (Jg^{-1})	Onset temperature of steps (°C)	Overall loss of mass (%)
Melibiose (Senn)	Dried	182.1	188.5	-109	-	0.4
	Bulk	182.5	188.4	-124	1^{th} 32 2^{th} 170	5.0
	40%	183.4	188.0	-109	-	-
	75%	183.6	187.8	-88	1^{th} 30 2^{th} 170	5.1
Melibiose (Sigma)	Dried	186.8	190.1	-73	31	0.5
	Bulk	186.2	190.5	-83	32	4.5
	40%	185.7	189.8	-109	-	-
	75%	185.2	189.4	-53	35	4.9

Numbers of variables, most importantly the mass of the sample, heating rate and particle size distribution, are known to effect DSC and TG measurements (van Dooren 1982; Agbada and York 1994). In this study the heating rate was kept constant and variation of the sample mass was relatively small. Particle size distribution on the other

hand differs significantly between melibiose (Senn) and melibiose (Sigma). Particle size of melibiose (Senn) is significantly larger (Fig. 7) and this can cause the observed “shoulder” in DSC curves and the two steps in TG curves. It is possible that it takes more time for tightly bound water inside crystals to evaporate from larger crystals. More DSC and TG measurements with same particle size fraction of materials and different heating rates are needed to confirm this. Some estimates of purity of materials can be made from DSC measurements. Impurities in material are known to affect for example melting point. However in because pure reference material was not available quantification of impurities could not be done. Lower melting point of melibiose (Senn) is most likely caused by the residual water which is confirmed with TG measurements and DSC curve for dried sample.

Interestingly, when it was exposed to RH over 40%, melibiose (Senn) got lumby formation of a solid “cake”, which however disintegrated back to powder when pressed gently. Melibiose (Sigma) did not show any “cake formation” until RH increased to 75%.

6.5 Spectroscopic methods

6.5.1 X-ray powder diffraction

XRPD spectra for both melibiose batches stabilized in RH of 20% are shown in Figure 12. For comparative reasons XRPD spectra for α-melibiose monohydrate obtained from the CCDC database is shown. From the spectra it can be made a conclusion that materials are in a crystalline form. Major differences cannot be seen in the spectra. Major peaks in the measured spectra and in the spectra of α-melibiose monohydrate are at the same positions. Spectra for other polymorphs of melibiose cannot be found from databases and this causes limitations to this study. Measured spectra cannot be compared to spectra of β-melibiose dihydrate or α-melibiose anhydrate for example so it cannot be confirmed that melibioses used in this study are pure α-melibiose monohydrate. Other polymorphs and hydrate forms of melibiose in melibiose batches

cannot be quantified. Residue impurities from manufacturing process, raffinose, glucose and galactose could also cause differences in spectra.

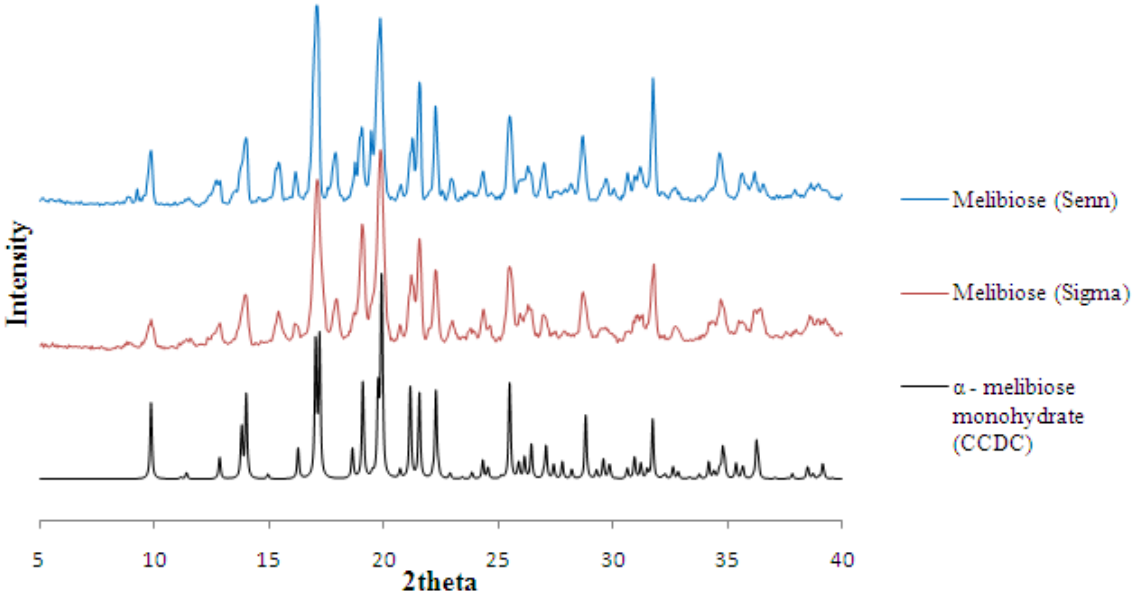

Figure 12. XRPD spectra for melibioses stabilized in RH 40% and α-melibiose monohydrate from CCDC database (Gress et al. 1978). Normalized intensities are used.

XRPD spectra for melibiose Senn stabilized in different relative humidities are shown in Figure 13. Overall there are no significant differences between spectra of different samples. Intensities of peaks in the 2theta scale increases as moisture content increases at 17°, 22°, 29° and 34.5°.

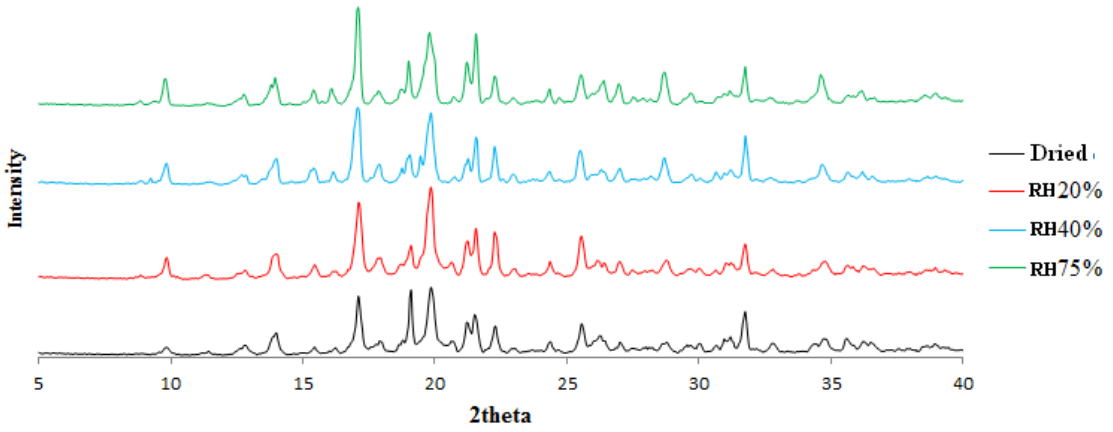

Figure 13. XRPD spectra for melibiose (Senn) stabilized in different relative humidities.

With melibiose (Sigma) moisture seems to have more effect on XRPD spectra. The patterns of dried sample, and sample stabilized in RH of 20%, appears to be almost identical (Fig. 14). But, as mentioned earlier, the phosphorous pentoxide and silica gel system might have not be able to dry materials completely. Between samples stabilized in relative humidities of 20% and 40% a chance happens as a shift of peaks can be observed at points 18° - 20° and 26°- 27° in the 2theta scale. Intensities of peaks in the 2theta scale increases as moisture content increases at same locations as observed for melibiose (Senn) at 17°, 22°, 29° and 34.5°.

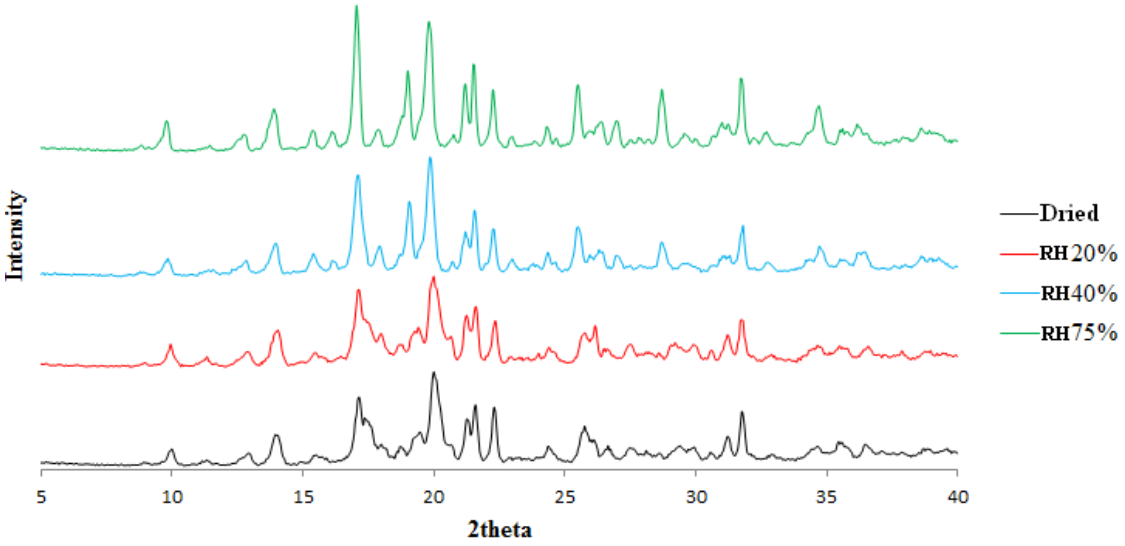

Figure 14.XRPD spectra for melibiose (Sigma) stabilized in different relative humidities.

Changes in XRPD spectra were measured at different temperatures. Reference point was at 25 °C. Spectra after dehydration was measured at temperature of 130°C to determine if evaporation of water present in crystal structure causes change in crystalline structure. Diffraction was also measured at 170 °C where melibiose (Senn) has endothermic “shoulder” in the DSC curves. Diffraction at 185 °C was measured to confirm melting of the material.

Changes can be observed in the spectra for melibiose (Senn) dried with phosphorous pentoxide measured at different temperatures (Fig. 15). Most of the changes take place between temperatures of 25 °C and 130 °C. The differences can be seen between 17° and 22° in the 2theta scale. Lack of crystalline peaks in diffraction pattern measured at temperature point 185 °C confirms total melting of the material

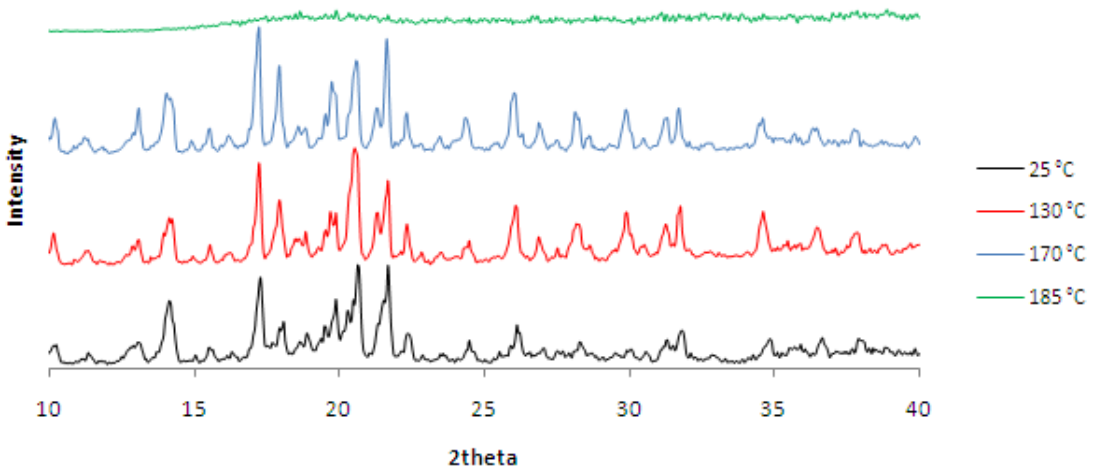

Figure 15. X-ray diffraction for melibiose (Senn) dried in a desiccator containig phosphorous pentoxide and silica gel. Spectras measured during heatingin different temperatures.

Stabilization of samples in RH of 40% caused a change in spectra of melibiose (Senn) (Fig. 16). Most of the changes take place between 25 °C and 130 °C as it can also be observed with dried samples. Evaporation of water after 100 °C seems to change the crystal structure of melibiose. Surprisingly no significant change can be observed in the spectra between 130 °C and 170 °C where the second step of dehydration was observed

with thermal analytic methods. Decrease of intensity at temperature point 170 ºC might be caused by increase in amorphous nature or melting and this can in fact be caused by the water evaporating at this point. It is possible that when the tightly bound water leaves the crystal structure it collapses. Water can also decrease the melting point of the material.

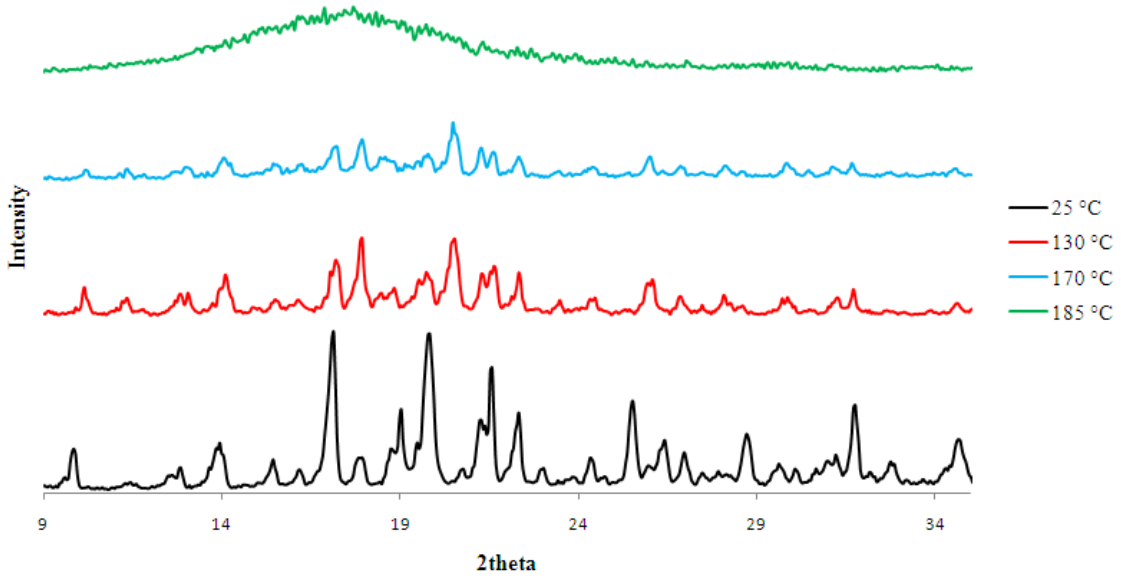

Figure 16. X-ray diffraction for melibiose (Senn) stabilized in RH of 40% at different tempererature points during heating.

XRPD spectra for dried melibiose (Sigma) at different temperature points are shown in Figure 17. No significant change can be observed between diffraction at different temperature point. Diffrences between diffraction patterns measured at temperatures of 25 °C and 130 °C are minimal contrary to melibiose (Senn), where some differences could be found (Fig. 15). It is possible that this can be caused by the fact that in melibiose (Senn) amount of water is larger after drying than in melibiose (Sigma), as can be seen in DSC curves (Fig. 10 and 11).

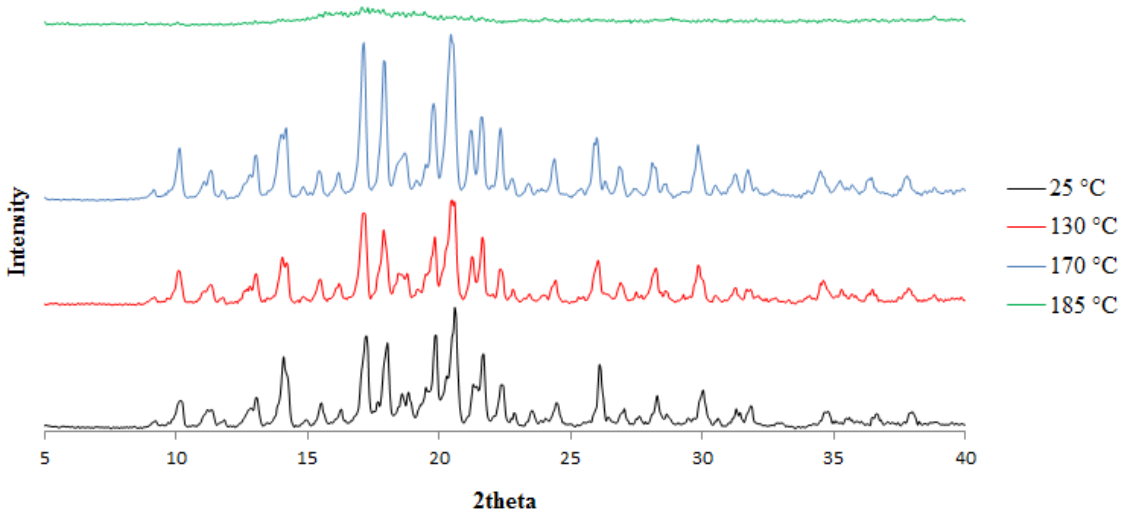

Figure 17. XRPD spectra for melibiose (Sigma) dried in a desiccator containing phosphorous pentoxide and silica gel. Spectras measured during heating at different temperature points.

Crystal structure of melibiose (Sigma) changes after dehydration as can be seen in Figure 18. Same degree of decrease of intensity, seen in Figure 16 for melibiose (Senn), cannot be observed in the spectra. Difference between the spectras measured in 25 °C and 130 °C can be clearly seen. Most likely evaporation of water changes the crystal structure as the peaks shift between 17° and 22° in the 2theta scale.

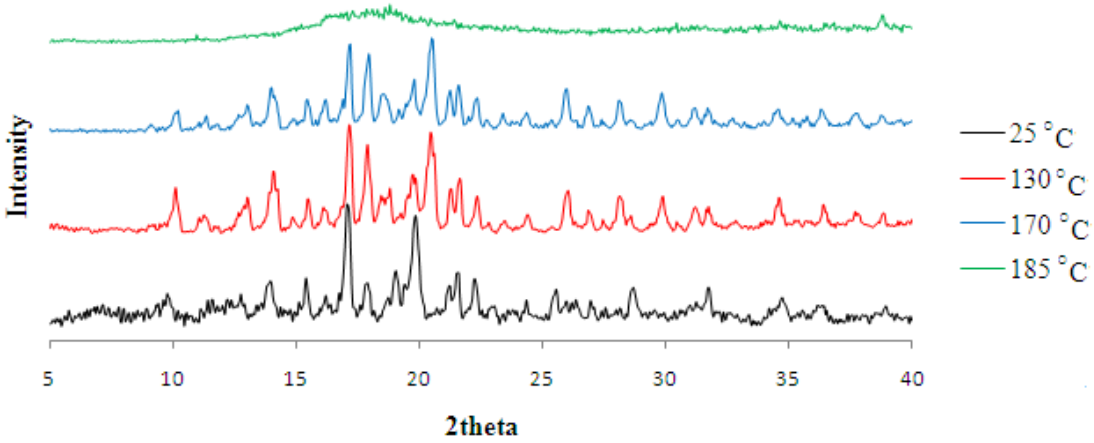

Figure 18. XRPD spectra form melibiose (Sigma) stabilized in RH of 40% at different temperatures during heating.

XRPD measurements confirmed that both materials are in crystalline form. Confirmation about the polymorphic form or presence of impurities cannot be made because lack of reference spectra for other polymorphic form of melibiose or possible impurities. There seems to be difference between materials from the two manufacturers but the effect of sample preparation and the significant particle size difference cannot be excluded. Difference in the moisture content seems to have slight effect on measured diffraction patterns and dehydration causes change in the structure of melibiose (Senn). Dehydration changed crystal structure of both melibiose batches, as could be observed when samples stored in elevated moisture atmosphere were measured. Heating however had more effect on diffraction patterns of melibiose (Senn) when dried samples are measured

6.5.2 Fourier transform infrared and near-infrared spectroscopy

FT-IR spectra measured for both melibiose batches appear to be similar (Fig. 19). As FT-IR gives information about molecular structure of substances, it is expected that there should not be any significant difference between two qualities.

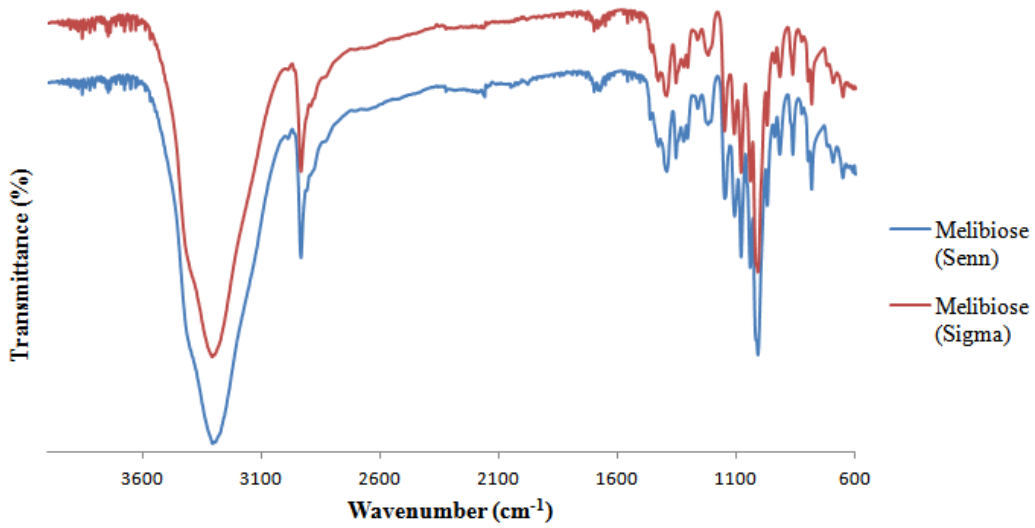

Figure 19. FT-IR spectra for melibiose (Senn) and melibiose (Sigma) measured as received.

FT-IR can also give information on how the molecule is packed in the solid state and how the groups of the molecule are orientated respect to other molecules and therefore it can be used to detect different polymorphs and amorphous forms (Steele 2004). Therefore it can be made careful estimates that two batches of melibiose are most likely same polymorphic form and that not a large degree of impurities or other substances can be found from either melibiose qualities.

For comparative reasons the infrared spectra of α-melibiose monohydrate and β-melibiose dihydrate from the study of Fletcher and Diehl (1952) and spectra measured with FT-IR in this study, are shown in figure 20. Spectra for melibiose batches measured as received, are similar that the one of α-melibiose monohydrate. Spectra of β-melibiose dehydrate differs significantly from the spectra of α-melibiose monohydrate at wavenumber area of 1300 – 700 cm^{-1}.

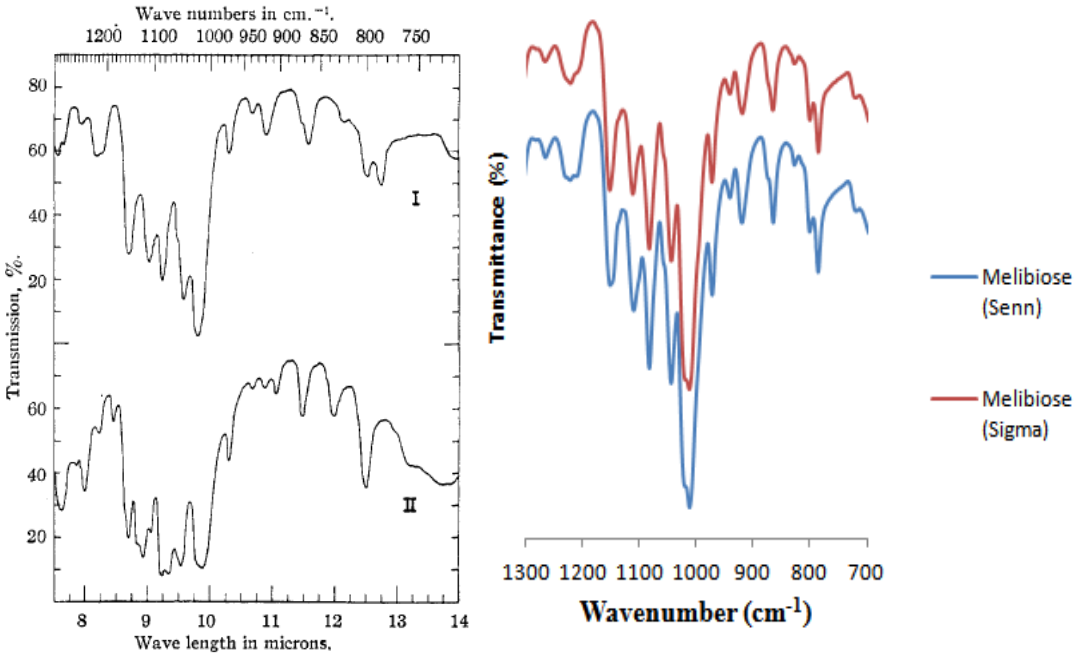

Figure 20: Infrared spectra of melibiose. On the left α- melibiose monohydrate (I) and β-melibiose dihydrate (II). (Fletcher and Diehl 1952). On the right melibiose (Senn) and melibiose (Sigma) as received.

NIR spectra for melibiose (Senn) and melibiose (Sigma), measured as received, are shown in the Figure 21. Difference in the shape of spectra can be observed between wavelengths of 2200 to 2500 nm.

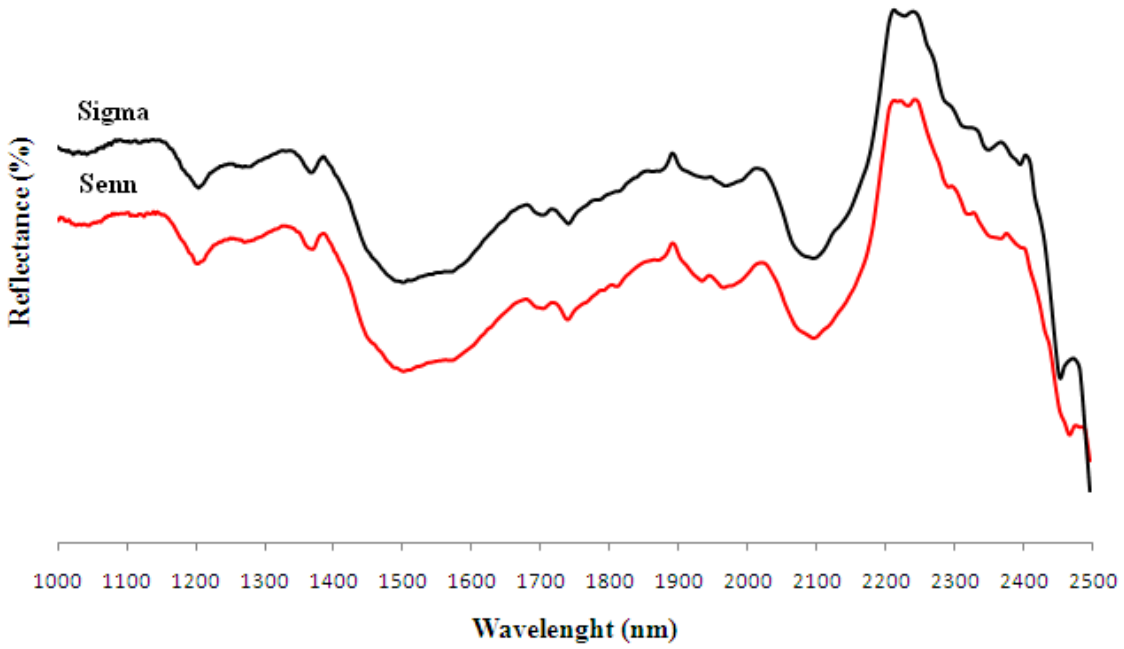

Figure 21. NIR spectra for melibiose (Senn) and melibiose (Sigma) stabilized in RH of 20%.

NIR spectra were also measured from samples stabilized several weeks in different relative humidities as well as from sample dried in desiccators containing phosphorous pentoxide and silica gel. All spectra measured from all samples are almost identical for both melibiose (Senn) (Fig. 22) and melibiose (Sigma) (Fig. 23). Drying causes change in the shape of the spectra in wavelength of 2200 nm. NIR was quite insensitive for water content of the material, or the differences in moisture content of the samples were too small to be observed. However NIR should be sensitive to changes in moisture content of materials as it is even used to determine the moisture content.

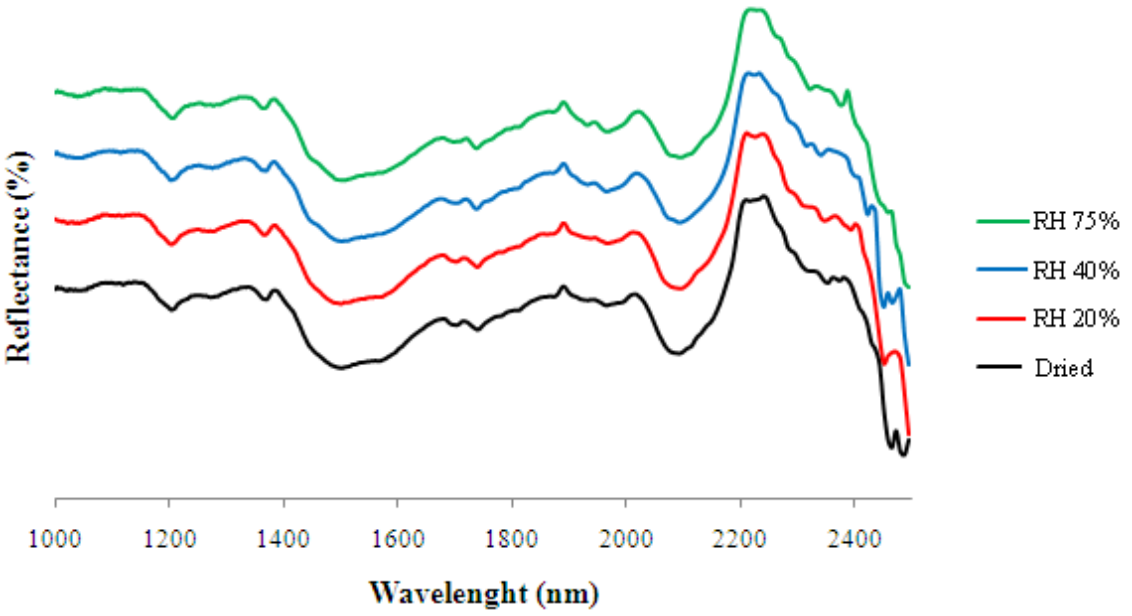

Figure 22. NIR spectra for melibiose (Senn) stabilized in different RH levels and dried in a desiccator containing phosphorous pentoxide and silica gel.

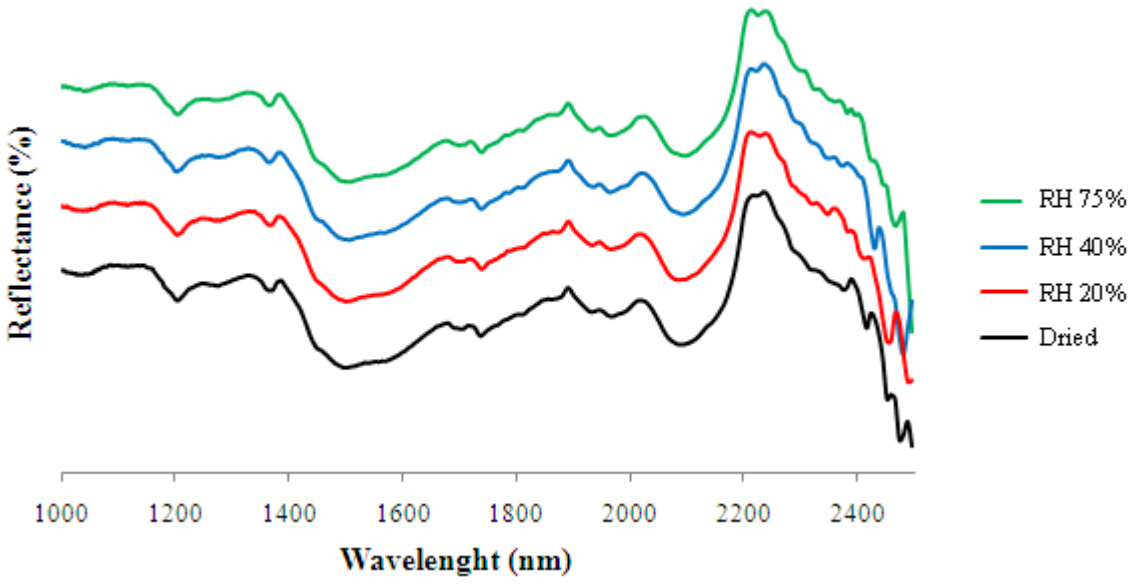

Figure 23. NIR spectra for melibiose (Sigma), stabilized in different RH levels and dried in a desiccator containing phosphorous pentoxide and silica gel.

Overall FT-IR and NIR did not show any differences between the two melibiose batches.

6.5.3 Raman spectroscopy

Raman spectra for melibiose (Senn) and melibiose (Sigma), measured as received, are shown in Figure 24. Excluding the slight intensity difference in the peaks, the spectra appear similar. There is however change in the spectra between 800 and 900 cm^{-1} in Raman shift in a form of extra peak in the spectra of melibiose (Senn) in a point of 813 cm^{-1}.

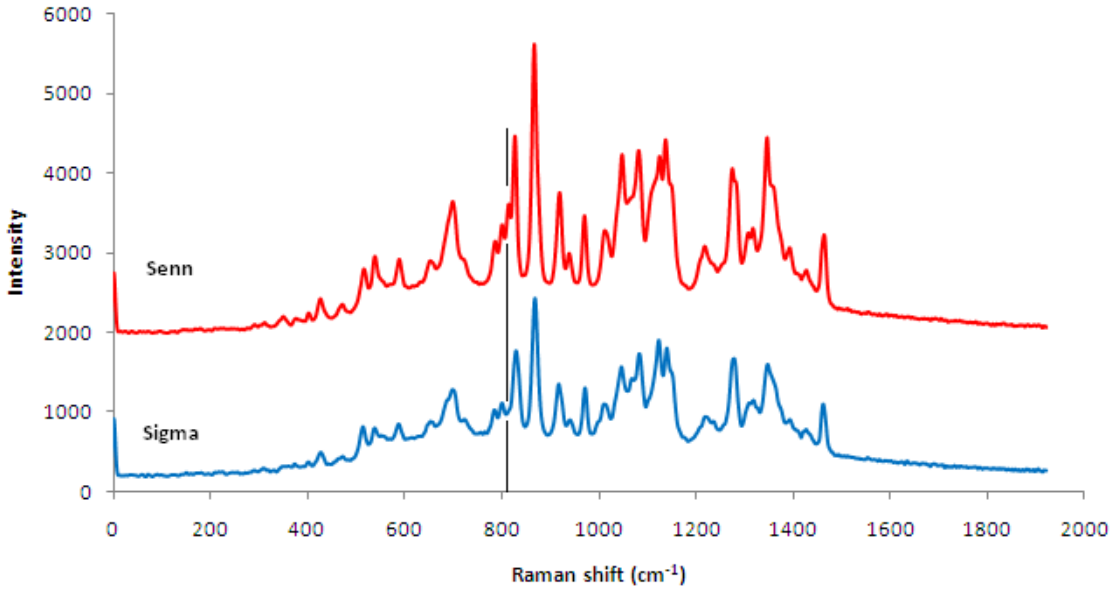

Figure 24. Raman spectra for melibiose (Sigma) and melibiose (Senn), measured as received.

When samples were dried in phosphorous pentoxide spectra for both melibioses became close to identical (Fig. 25). The positions of the peaks are at the same points and the extra peak of melibiose (Senn) disappears. This is interesting because water itself does not cause changes in Raman spectra. Most likely difference in moisture content causes change in some crystal properties of materials. Raman can be used to determine hydration state of material (Hausman et al. 2005). Water loss or uptake on the crystal lattice causes change in vibrational states of the molecule causes differences in spectra.

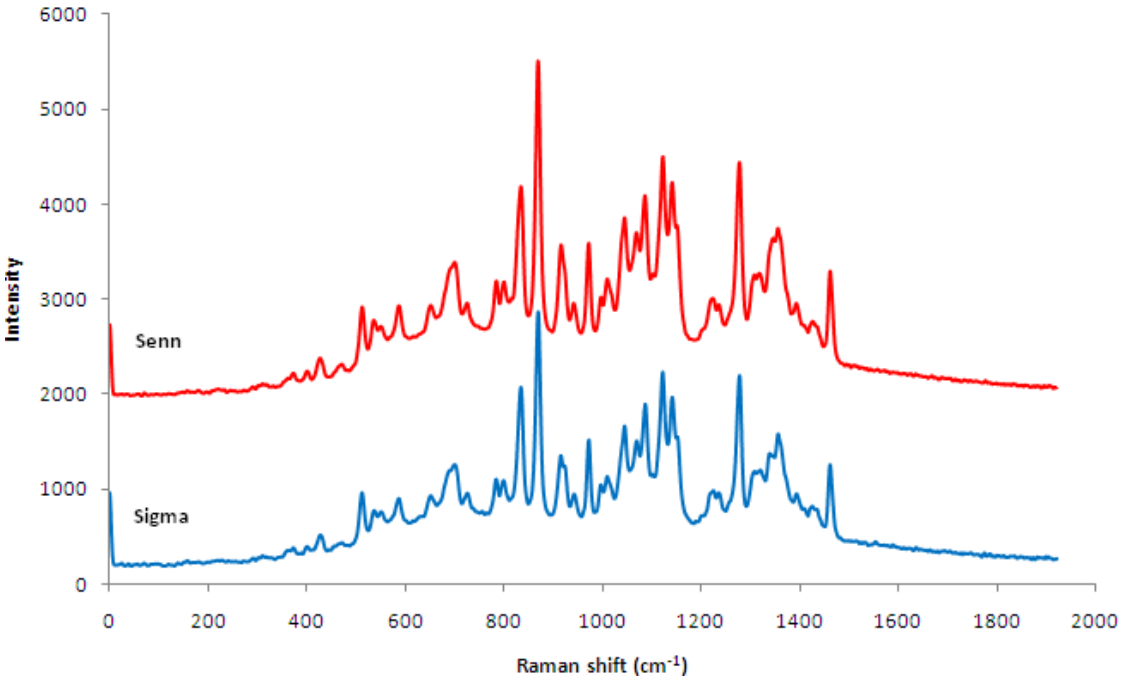

Figure 25. Raman spectra for melibiose (Senn) and melibiose (Sigma). Samples dried in a desiccator containing phosphorous pentoxide and silica gel.

The effect of moisture content can be seen clearly in Figure 26. The Raman spectrum of dried sample differs significantly from the spectra of samples exposed to moisture (bulk sample measured as received). Further increase in moisture content (sample stabilized in the RH of 75%) does not cause change in the spectra but the intensity of peaks increases.

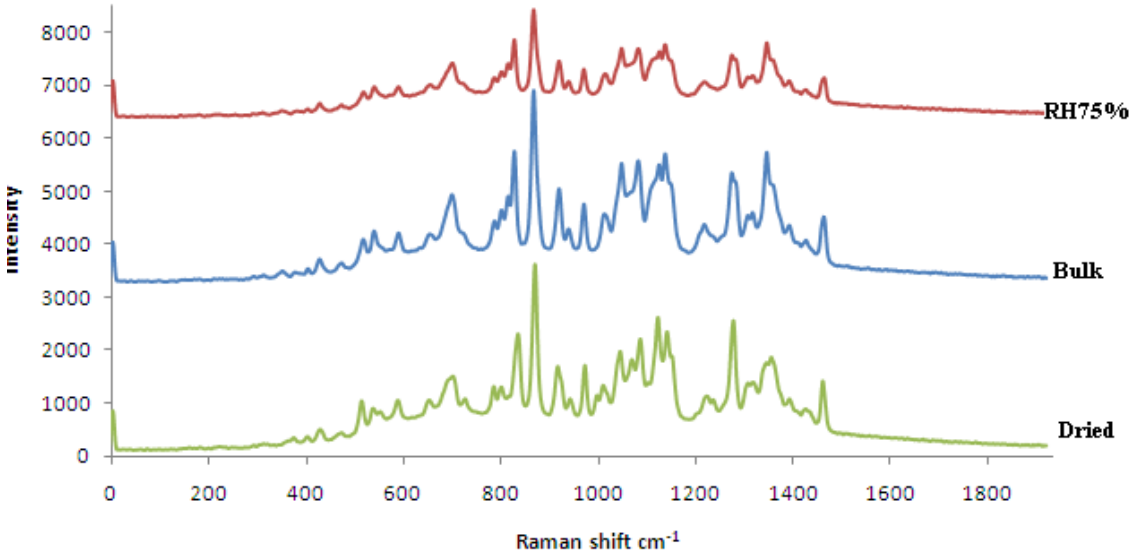

Figure 26. Raman spectra for melibiose (Senn) measured for sample stabilized in RH of 75%, for sample measured as received and for sample dried in a desiccator containing phosphorous pentoxide and silica gel.

Interestingly, exposure to elevated moisture atmospheres also causes change in the measured spectra of melibiose (Sigma) but the change can be observed at higher RH levels (75%) (Fig. 27)

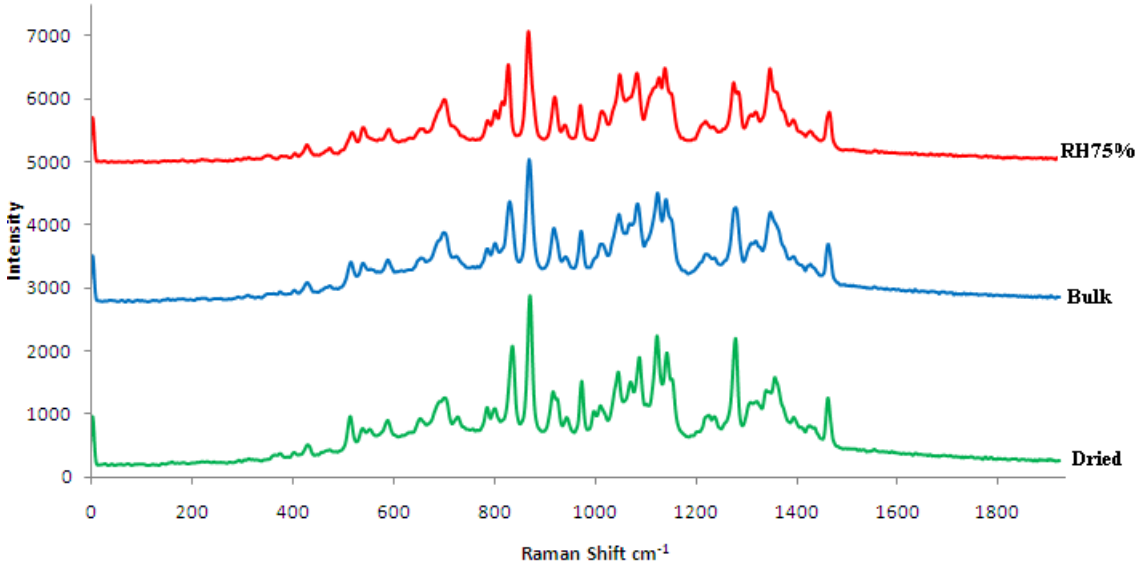

Figure 27. Raman spectra for melibiose (Sigma) measured for sample stabilized in RH of 75%, for sample measured as received and for sample dried in a desiccator containing phosphorous pentoxide and silica gel.

Interestingly Raman spectra of both melibiose batches became close to identical when samples were stabilized in RH of 75% (Fig. 28).

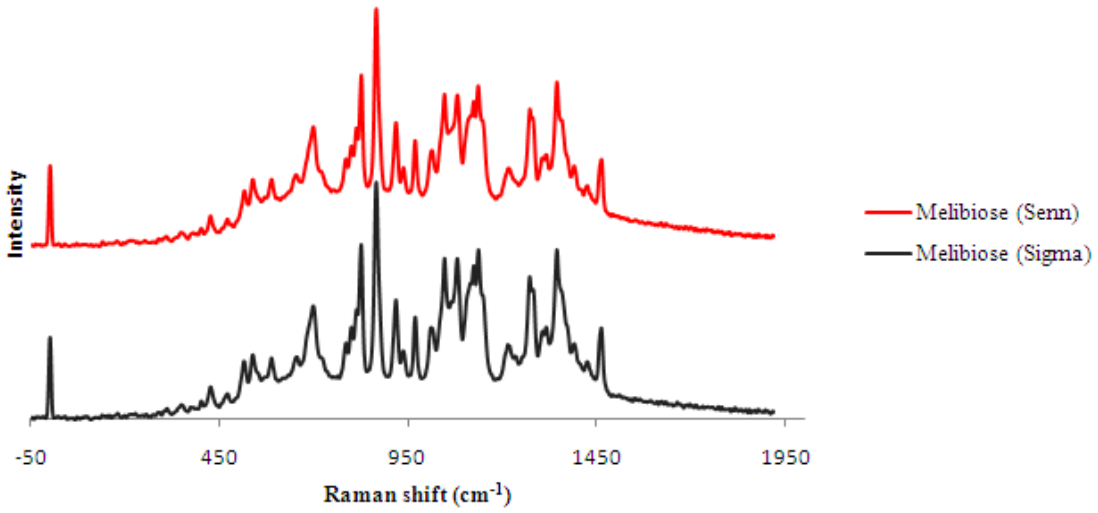

Figure 28. Raman spectra for melibiose (Senn) and melibiose (Sigma), stabilized at RH of 75%.

6.6 Compression studies

6.6.1 Compactibility

Compactibility of materials is examined by comparing compression pressure to the tensile strength of resulting tablets. Compactibility of materials stabilized in RH of 10% is represented in Figure 29. Microcrystalline cellulose (Avicel PH-200) had superior compactibility compared to other materials ($P < 0.05$). Microcrystalline cellulose produced tablets with manyfold higher tensile strength with lower compression pressures than other materials (Appendix 2). It seems that both melibiose batches produced slightly stronger tablets at same pressure levels than lactose (Pharmatose 80M) and calcium dihydrogen phosphate (Emcompress), but the difference is not statistically significant ($P > 0.05$). The difference can be seen more clearly in higher compression

pressure levels. Melibiose (Senn) seems to have better compactibility than melibiose (Sigma), but not the difference is not significant (P = 0.1).

Both batches of melibiose caused sticking problems during compression even with prelubricated punches and die when the relative humidity increased from 20% to 40%. Sticking can have significant effect on crushing strength measurements (Fell and Newton 1970). Therefore, tablets with high degree of flaws caused by sticking were excluded from the measurements.

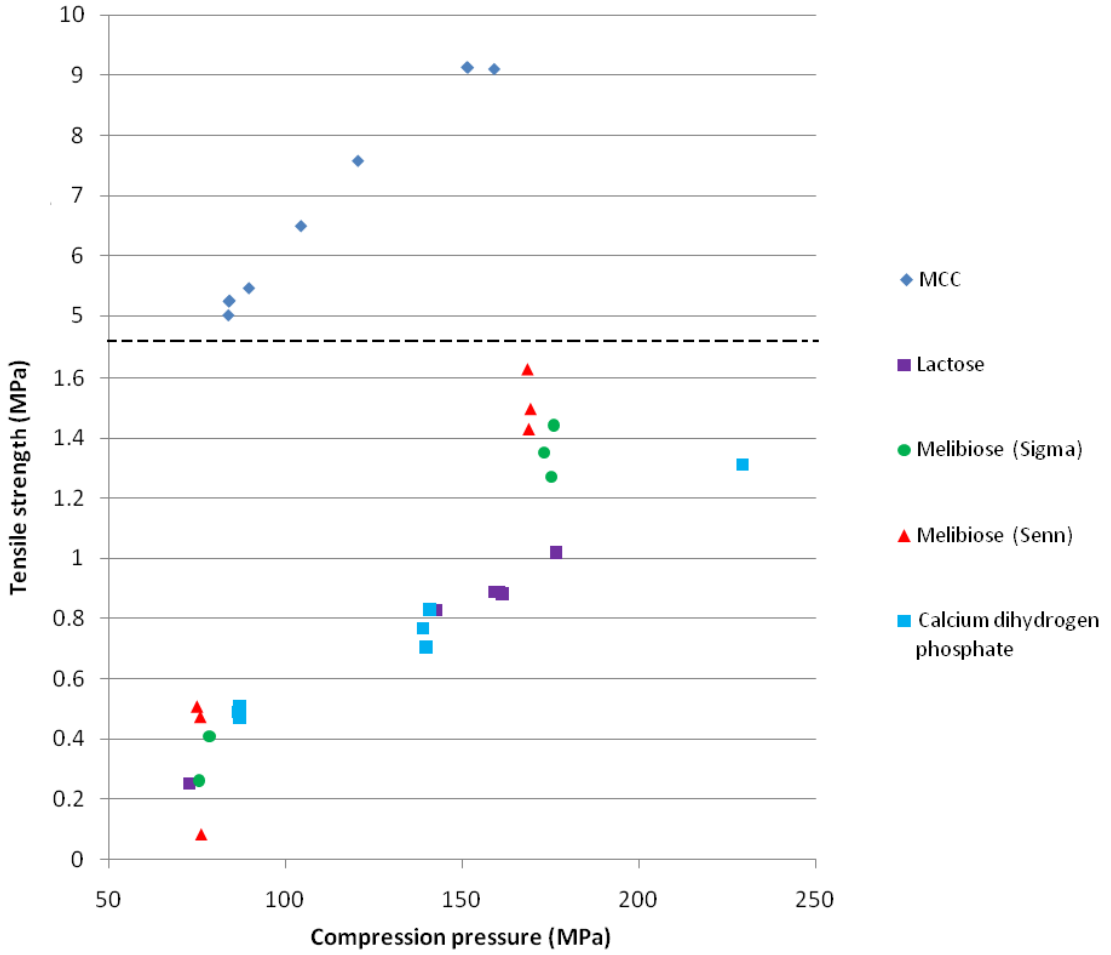

Figure 29. The effect of compression pressure on the tensile strength of resulting tablet at two different pressure levels. Samples stabilized in RH of 10%. Each symbol represents individual tablet. MCC=microcrystalline cellulose.

Materials stabilized in RH of 40% were compressed to study the effect of moisture content to compactibility of materials. Increase in moisture content is known to increase crushing strength of tablet (Jaffe and Foss 1959). Tensile strength of tablets increased when moisture content was increased (Fig. 30 and 31).

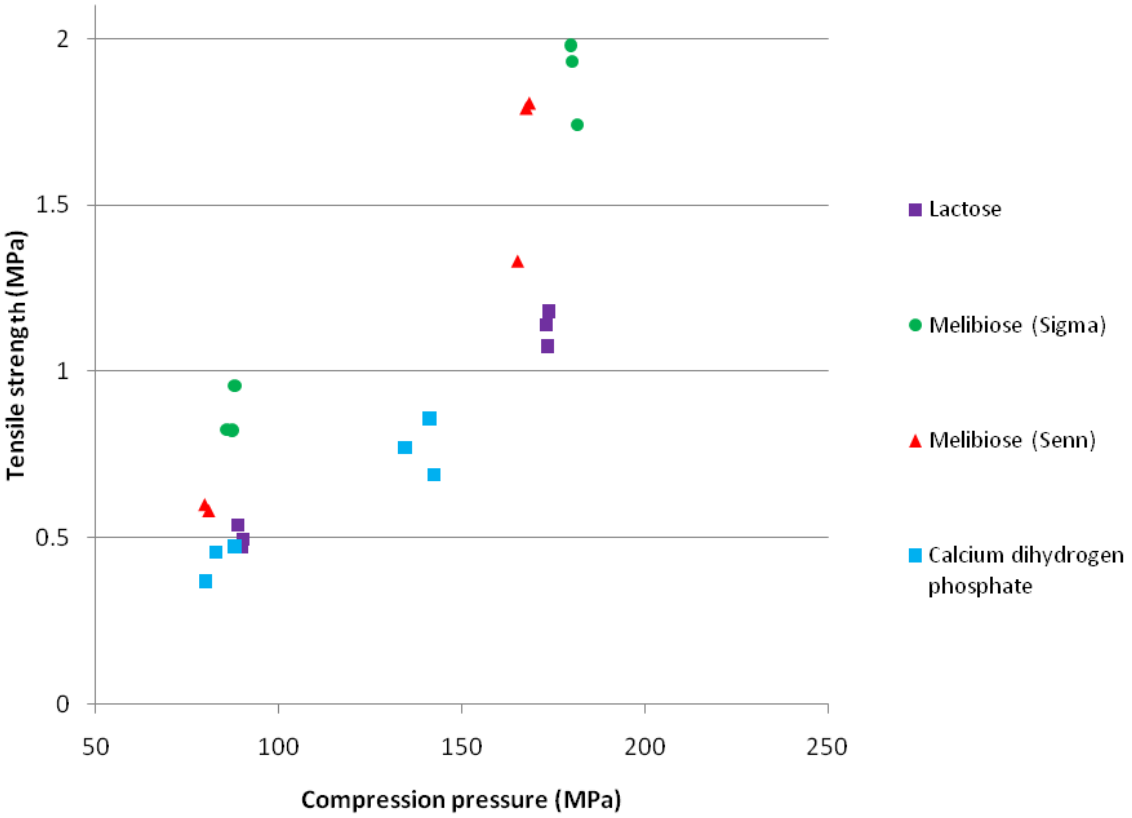

Figure 30. The effect of compression pressure on the tensile strength of resulting tablet at two different pressure levels. The materials are stabilized in RH of 40%. Each symbol represent individual tablet.

Increase in moisture content increases the tensile strength of tablets for each material. The increase in the tensile strength of tablets was significant for melibiose (Sigma) at higher pressure levels ($P < 0.05$). Melibiose (Sigma) produced tablets with higher tensile strength than melibiose (Senn) and α-lactose monohydrate ($P < 0.05$) (Fig. 31).

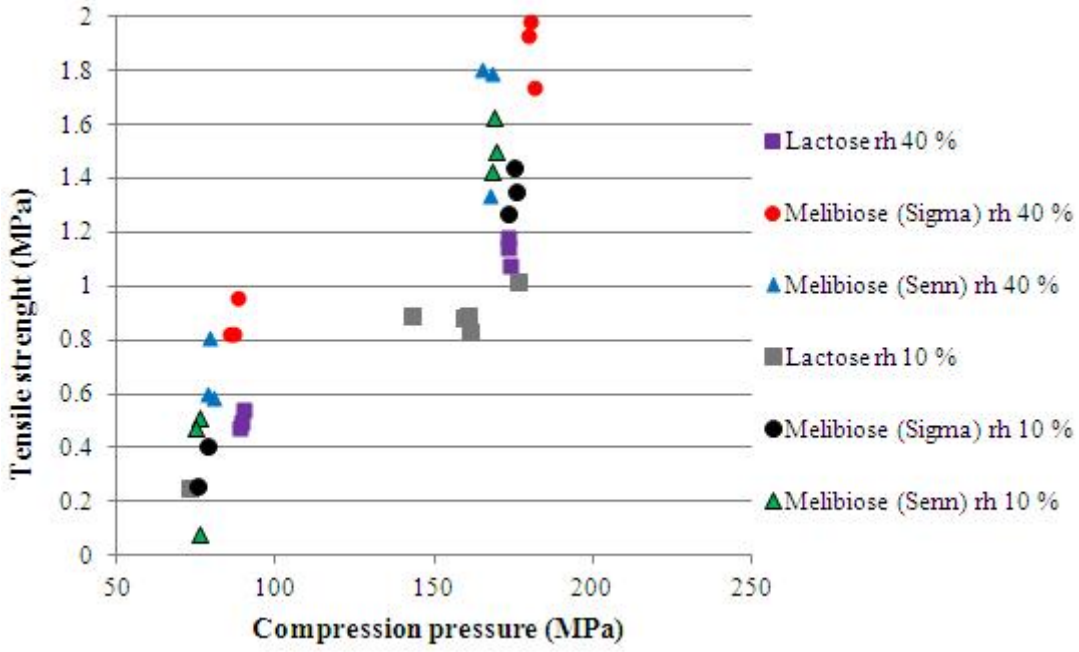

Figure 31. The effect of compression pressure and moisture content of material on the tensile strength of resulting tablet at two different pressure levels. The materials are stabilized in RH of 10% or 40%. Each symbol represent individual tablet.

According to these results melibiose might have slightly better compactibility than α-lactose monohydrate. The difference however, is not statistically significant because of the large variations in the tensile strengths. Further, the compression pressures were not equal for all materials. More studies are needed to make any further conclusions. Materials should be compacted using different pressure levels so that the compression pressure versus tensile strength could be drawn to a curve. Statistical analysis could be conducted to determine the statistical difference between the compactibility of materials.

6.6.2 Heckel analysis and strain rate sensitivity

Shape of Heckel plots for lactose monohydrate and different batches of melibiose are quite similar in both punch velocities (Fig. 32). The shape of Heckel plot can give good indication of deformation mechanism of materials (Roberts and Rowe 1985). According

to shape of the Heckel plots shown in the Figure 32, it can be assumed that lactose and melibiose have similar mechanism of deformation during compression.

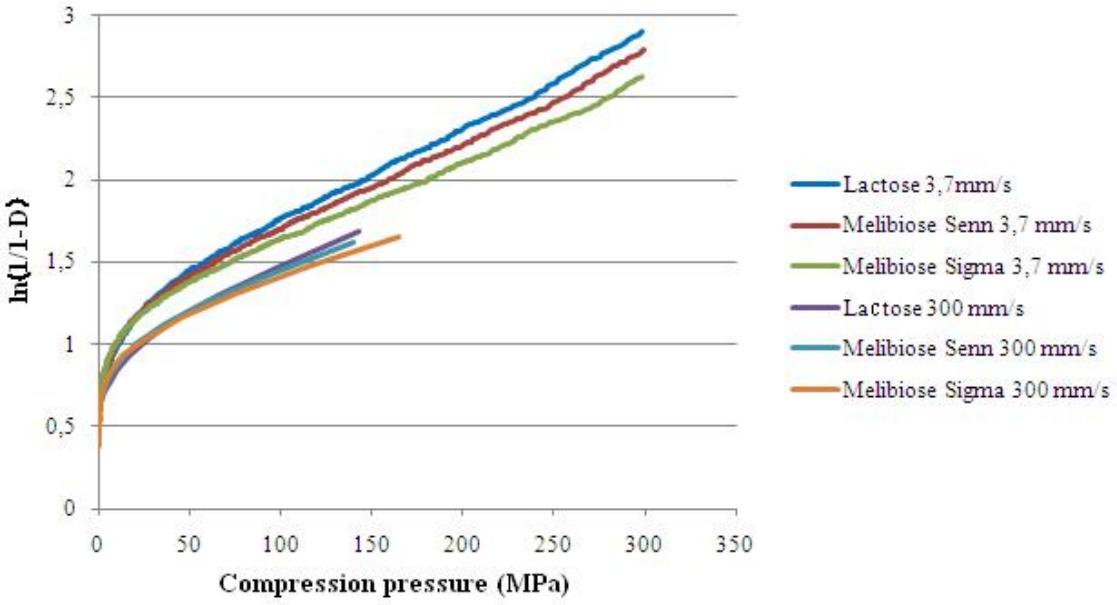

Figure 32. Heckel plots for materials at compression velocities of 3.7 mm/s and 300 mm/s. Materials stabilized in RH of 20%.

Calculated mean yield pressure and SRS-index values are shown in Table 5. Mean yield pressure values (P_y) for melibioses and lactose monohydrate are relatively close to each other, but being a bit higher for melibioses. This indicates that they are slightly more fragmenting. SRS index -values on the other hand indicates that melibioses are more plastic than lactose monohydrate but the difference is relatively small. In the original study Roberts and Rowe obtained SRS-index of 16.2% for lactose which is higher than determined in this study. Although the 1^{th} derivates were not used to determine the linearity of the Heckel plots, the materials used in this study show good linearity as correlation coefficients are over 0.995. Only three tablets of each material were compacted. The deviation in the values of P_y for lactose monohydrate influenced the calculated SRS index –values, so one compression cycle had to be excluded from both studies. More compression data is needed to confirm the results. Also the compaction simulator must be adjusted so that similar compression pressures and tablet thicknesses

can be reached in both compression velocities. Correcting the effect of machine part deformation to punch displacement values would also be important.

Differences in mean P_y values show that both melibiose batches becomes more plastic when RH rises from 20% to 40% as can be predicted absorbed water acting as plasticizer (Garr and Rubenstein 1992). Strain rate sensitivity increases as well when moisture content of the material increases, indicating an increase in plasticity.

Table 5. Calculated yield pressure (P_y) and SRS -values for materials from the first study. R^2= Correlation coefficient for linear regression of the linear part of the Heckel plots

Material	**P_y (MPa)**	**Standard deviation**	**R^2**	**SRS-index (%)**	**n**
Melibiose Senn RH 20% 3.7mm/s	192	4	0.998	7.0	3
Melibiose Senn RH 20% 300mm/s	207	3	0.995		3
Melibiose Sigma RH 20% 3.7mm/s	207	3	0.998	11.8	3
Melibiose Sigma RH 20% 300mm/s	235	9	0.998		3
Melibiose Senn RH 40% 3.7mm/s	169	3	0.998	13.6	3
Melibiose Senn RH 40% 300mm/s	195	9	0.995		3
Melibiose Sigma RH 40% 3.7mm/s	176	2	0.998	18.3	3
Melibiose Sigma RH 40% 300mm/s	216	7	0.996		3
Lactose RH 20% 3.7mm/s	177	2	0.995	5.1	2
Lactose RH 20% 300mm/s	187	7	0.997		3

Compacts with density close to true density were compressed when slower punch velocity was used. However, when higher punch velocity was used, the machine was unable to compress powders close to true density, and considerably thicker compacts were obtained. Maximum compression pressures were also much higher when slower punch velocity was used. This phenomenon is most likely due the fact that with higher

punch velocities particles of the materials have less time to rearrange and cause more resistance to deformation (Roberts and Rowe 1985). Usually this causes higher compression pressures when higher punch velocities are used because higher compression pressures must be used to obtain same density with higher punch velocities.

It is possible that some degree of deformation of machine parts affected the measurement especially at higher punch velocities, but quantification of this deformation could not be precisely done. Lower punch displacement was taken into account as the distance between punches was used to calculate the thickness of compacts. Maximum compression pressure is known to have effect on calculated yield pressure values (Rees and Tsardaka 1994). Yield pressure values are known to increase as the maximum compression pressure increases. Therefore SRS–index values obtained might not be comparable with those determined with different machine and set-up.

Maximum compression pressures in the second compression study were significantly lower than in the first study. In the second study materials were not humidity controlled prior to the test. But the effect of differences between moisture content of the materials do not explain the differences in measured values of compression pressures. It is most likely that this is caused by some change in the set-up of compression simulator after the first study..

Shapes of Heckel plots obtained (Fig. 33 and 34) in the second study also show similarity between lactose and melibiose grades. Shape of Heckel plot of microcrystalline cellulose differed significantly from the ones of lactose and melibioses. Slope of the plot of microcrystalline cellulose is higher and the initial curvature that can be seen in Heckel plots of melibioses and lactose cannot be observed. This indicates higher degree of plasticity. Including the microcrystalline cellulose to the study is important because it deforms plastically and the P_y -and SRS –values can be used as reference to other materials.

Due to low bulk density of microcrystalline cellulose the whole amount of sample did not fit to the die when poured. The material was therefore compressed manually with a round tool to fit all material in the die. This procedure most likely caused some weight variation. Absence of initial curvature of in Heckel plot of microcrystalline cellulose

can also be cause of the “precompression” although plastic materials usually show less initial curvature in the Heckel plot (Denny 2002). The impact of the material handling before compression to overall shape of the Heckel plots in higher compression pressure levels was estimated to be insignificant.

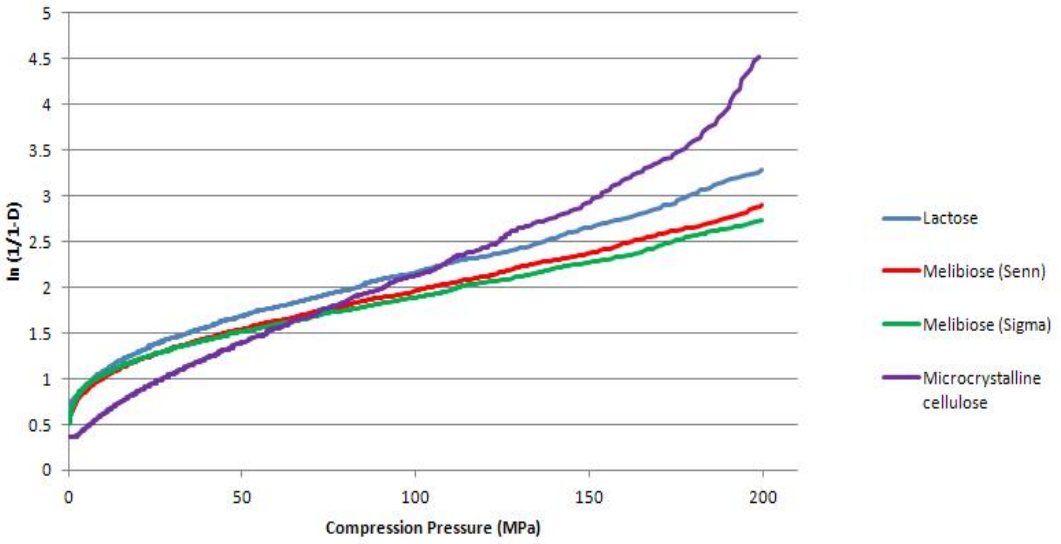

Figure 33. Heckel plots for materials at compression velocity of 3.7 mm/s. MCC = Microcrystalline cellulose.

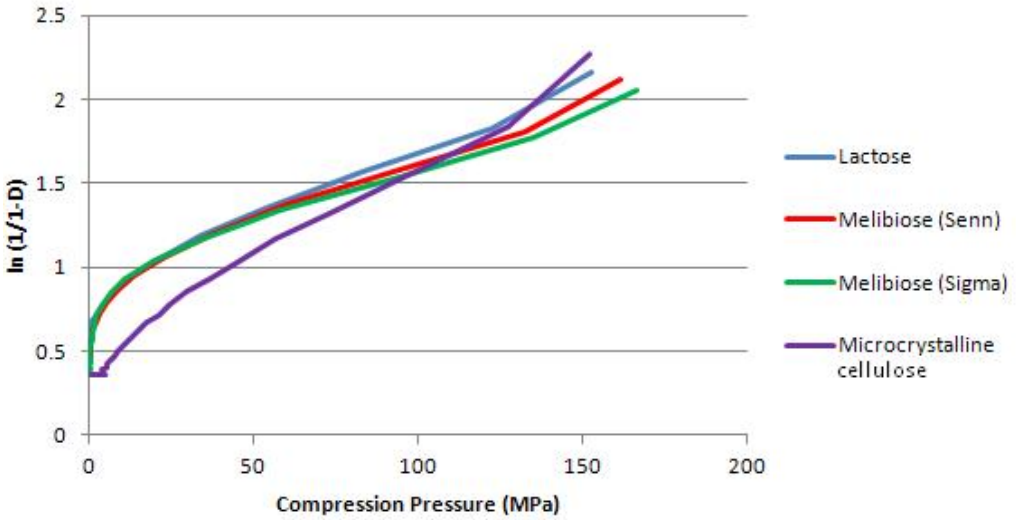

Figure 34. Heckel plots for materials at compression velocity of 300 mm/s. MCC = Microcrystalline cellulose.

Calculated P_y and SRS-values for materials are shown in Table 6. Values differ from the first study. P_y are significantly lower and SRS-values higher than in the first study. In the second study however, P_y and SRS index –values, indicate that both melibiose batches are slightly more fragmenting than α-lactose monohydrate. Microcystalline cellulose seems to be significantly more plastic than other materials as the P_y –values are lower and the SRS index –values higher than the ones of other materials. The SRS index value for microcrystalline cellulose is 38.9 which is precisely the same than represented in Table 1. (Roberts and Rowe 1985).

Table 6. Calculated yield pressure (P_Y) and SRS values for materials from the second study. MCC=Microcrystalline cellulose, R^2= Correlation coefficient for linear regression of the linear part of the Heckel plots.

Material	**P_y (MPa)**	**Standart deviation**	**R^2**	**SRS-index (%)**	**n**
Melibiose (Senn) 3.7 mm/s	116	0.8	0.998	26.6	3
Melibiose (Senn) 300 mm/s	158	1.4	0.999		3
Melibiose (Sigma) 3.7 mm/s	127	0.9	0.998	25.4	3
Melibiose (Sigma) 300 mm/s	170	1.7	0.999		3
Lactose monohydrate 3.7 mm/s	101	2.9	0.996	27.6	2
Lactose monohydrate 300 mm/s	140	1.1	0.998		3
MCC 3.7 mm/s	62	5.3	0.988	38.9	3
MCC 300 mm/s	101	0.6	0.998		3

7. SUMMARY AND CONCLUSIONS

The aim of this research was to conduct a range of pre-formulation studies to determine the fundamental physicochemical and mechanical properties of melibiose. According to the different methods used the two melibiose batches have differences in physico-chemical properties as well as in compactibility and compressibility.

Particle size of the two melibiose batches is significantly different, but particle morphology is similar between materials. Particles of both melibioses are aggregated. Aggregates of melibiose (Senn) are larger and more spherical, and its size distribution is more uniform and due to this it has better flowing properties. The flow properties, measured with Flowpro apparatus, of melibiose (Senn) are superior to the flow properties of crystalline α-lactose monohydrate with similar particle size. Therefore, it would be preferable material for direct compression over melibiose (Sigma).

In thermal analysis a difference was found between behaviour of melibiose (Senn) and melibiose (Sigma) during heating. Melting point for both materials are slightly higher than the one mentioned in literature for α-melibiose monohydrate. In DSC measurements melibiose (Senn) shows an additional endothermic reaction at 170 °C. This endotherm is confirmed to be caused by evaporation of residual water by TG measurements. Drying of melibiose (Senn) changes its DSC behaviour so that the endotherm disappears also confirming that the residual water is causing the difference between materials. Lower melting point of melibiose (Senn) compared to melibiose (Sigma), is most likely caused by the residual water. This is most likely caused by the larger aggregate size of melibiose (Senn).

Infrared methods are unable to detect major differences between two batches of melibiose. In Raman measurements differences between materials can be seen depending on the moisture content. Raman spectrometry shows differences between both qualities stabilized in different relative humidities. For melibiose (Senn) the difference in Raman patterns can be observed when dried sample is compared to sample stored in room humidity. Melibiose (Sigma) shows the difference when moisture content is increased. Raman spectra for both melibioses become close to identical when

materials are dried as well as stabilized in RH of 75%. It is possible that differences in Raman spectra indicate that there might be differences in crystal structure of the two melibiose baches. Moisture content seems to cause the difference and it is also possible that water is more easily bound to melibiose (Senn) which causes changes in Raman spectra in lower relative humidities compared to melibiose (Sigma).

In XRPD measurements differences in spectra can be found between the two melibiose batches. Both melibiose Batches are most likely α-melibiose monohydrate, but amounts of impurities could be identified or quantified because of the lack of reference spectra for other forms of melibiose. Increase in moisture content has more effect on measured spectra of melibiose (Sigma). During heating both melibiose batches show differences in XRPD spectra. Evaporation of water most likely changes the crystal structure of materials. Effect of heating is more dramatic for melibiose (Senn), which is assumed to be due to effect of the residual water evaporating at higher temperatures.

Tableting studies showed that melibiose has potential to be used as a replacer of lactose in tablet formulations, because of the similar or even better compactibility. Melibiose produces tablets with higher tensile strength in similar compression pressures than lactose. Increase in moisture content increases compression pressures and resulting tensile strength of tablets for both melibiose batches, but it seems to have more effect on compactibility of melibiose (Sigma). Although the results of the study seem promising, some limitations must be taken into account. Only a small number of tablets were compressed and large variation in the results prevent the detecting of statistical difference between compactibility of materials.

According to the P_y and SRS –values, calculated from the data of Heckel analysis, melibiose is concluded to be fragmenting, even at higher degree than α-lactose monohydrate. The overall deformation behaviour of melibiose is similar than the one of α-lactose monohydrate. More studies are however needed to confirm these results because of the small number of compression cycles were used to calculate the P_y and SRS –values and because of the high variations in the results. The elasticity of the materials needs more investigation as well as the effect of aggregated nature of melibiose batches to the values obtained from Heckel analysis. Two compression studies was done with same machinery and parameters but large variations in resulting

values was observed, most likely caused by faults in tableting simulator. Due to limitations of the tableting simulator used, the results from this study might not be comparable with the results obtained with different machinery or set-up.

For further studies melibiose could be used in a tablet formulation and the tableting properties of this formulation should be compared to similar formulation containing lactose. The “cake formation” of melibiose (Senn), when exposed to higher humidity levels can be considered as a problem for tablet manufacturing. Sticking problem of both melibiose batches must be also taken into account when designing manufacturing processes. Further tableting studies are needed to investigate the effect of internal lubrication, for example magnesium stearate, in the sticking problem of melibiose. Other polymorphic forms or amorphous of melibiose could be used in tableting studies and the materials could be processed to get more favorable quality of material for tableting. Before melibiose can be used as a pharmaceutical excipient, physicochemical properties like solubility, hygroscopocity and particle size distribution of melibiose still need further studies. Also chemical purity analysis of melibiose should be conducted with suitable analytical method. Further, disadvantage of melibiose as an excipient in tablet formulations can be risen from the facts that it still is rare and expensive. Melibiose also loses advantage over lactose as it is also able to react in Maillard reaction and may cause symptoms similar to lactose intolerance to patients.

As conclusion, there still is some exploring to do and hurdles to win, but the potential for using melibiose as a tablet excipient exists.

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APPENDIX 1

Mechanical constants/parameters and techniques used in characterization mechanical properties of powdered materials (Jain 1999).

Property	**Mechanical parameters or constants**	**Technique or method**	**Information derived**
Plasticity or ductility	Yield strength	Density pressure profiles (Heckel, Shapiro, Walker and Kawakita equations)	Minimun pressure to form coherent compact
	Yield pressure	Identation hardness	Local plasticity of materials
		Compression cycles	Capping potential, plastic/elastic deformation
	Network of plastic deformation	Force displacement curves	Precompression possibilities
			Work of die wall friction
			Simulated final scale compaction
Brittleness	Brittle fracture index	Hiestand tableting indices	Laminating tendencies
	Critical stress	Notched beam fracture, douple torsion	Fracture toughness
	Intensity factor	Radial edge cracked tablet or disc	
Elasticity	Young's modulus	Beam bending, indentation testing	
	Brinell hardness number	compression testing	
	Elastic recovery (%)	Force displacement curves	
		Work done on the lower punch in a second compression	Work of elastic deformation
	Strain index	Hiestand tableting index	
Viscoelasticity	Stress relaxation		Plastic flow, die stricking potential,
	Viscoelastic slope		laminating tendency
	Strain rate sensitivity	Heckel analysis	Effect of scaling-up to high speed tablet presses
		Compaction simulator	
	Creep compliance		
	Elastic and viscous moduli		
Compactibility	Tablet strength	Compression force versus crushing strength profile	Laminating tendencies
	Deformation hardness	Leuenberger equation, identation hardness	Laminating tendencies
	Bonding index	Hiestand tableting indices	Capping and sticking potential
Compressibility	Compressibility, γ	Leuenberger equation	
		Heckel equation	

APPENDIX 2

Table showing avarages of sample masses used in compression studies, compression pressure, thickness of resulting tablet and tensile strength of resulting tablet. 1 = Higher pressure level, 2 = lower pressure level, RH = Relative humidity, StDev = Standard deviation, AW = Water activity, n = number of tablets compressed, Avicel PH-200 = Microcrystalline cellulose, Pharmatose 80M = α-lactose monohydrate, Emcompress = Calcium dihydrogen phosphate.

Material		Sample mass (mg)		Thickess of tablet (mm)		Compression pressure (MPa)		Tensile strength (MPa)		AW (%)	n
RH 10%			StDev		StDev		StDev		StDev		
Avicel PH-200	1	295.5	8.0	3.57	0.060	146	17.1	8.7	0.77	0.143	3
	2	262.9	6.7	3.50	0.045	89	8.8	5.5	0.58	23.1 °C	3
Pharmatose 80M	1	294.4	3.6	3.42	0.022	165	8.1	0.9	0.07	0.127	3
	2	262.4	2.5	3.37	0.000	165	49.3	0.1	0.15	24.7 °C	3
Melibiose (Sigma)	1	293.5	0.6	3.44	0.017	175	1.5	1.4	0.09	0.142	3
	2	270.3	0.6	3.38	0.119	77	2.1	0.3	0.10	24.7 °C	3
Melibiose (Senn)	1	293.6	0.1	3.41	0.016	169	0.4	1.5	0.10	0.145	3
	2	258.2	0.6	3.33	0.055	76	0.6	0.4	0.24	24.9 °C	3
Emcompress	1	383.5	9.9	3.40	0.024	140	0.9	1.1	0.71	0.161	3
	2	348.6	0.5	3.33	0.043	87	0.2	0.5	0.02	24.9 °C	3
RH 40%											
Avicel PH-200	1	310.3	0.1	3.50	0.010	158	15.3	9.8	0.03	0.422	3
	2	270.4	0.5	3.40	0.001	85	0.5	5.8	0.18	25.1 °C	3
Pharmatose 80M	1	294.8	0.1	3.40	0.004	173	0.3	1.1	0.05	0.177	3
	2	264.5	0.0	3.27	0.012	90	0.6	0.5	0.03	23.8 °C	3
Melibiose (Sigma)	1	293.9	0.7	3.40	0.037	180	0.9	1.9	0.13	0.256	3
	2	258.8	0.3	3.24	0.008	87	1.1	0.9	0.08	25.2 °C	3
Melibiose (Senn)	1	293.6	0.4	3.37	0.027	167	1.7	1.6	0.27	0.229	3
	2	258.6	0.4	3.26	0.026	80	1.1	0.7	0.12	24.9 °C	3
Emcompress	1	379.2	1.0	3.35	0.008	139	4.2	0.8	0.08	0.264	3
	2	349.2	0.2	3.24	0.020	84	4.1	0.4	0.06	24.1 °C	3