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Influence of water-cellulose binding energy on stability of acetylsalicylic acid

Mina Heidarian [a], Albert Mihranyan [a], Maria Strømme [b,*], Ragnar Ek [a,**]

[a] *Department of Pharmacy, BMC, Uppsala University, Box 580, 75123 Uppsala, Sweden*
[b] *Department of Engineering Science, The Ångström Laboratory, Uppsala University, Box 534, 75121 Uppsala, Sweden*

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Abstract

The aim of the present study was to investigate how the energies of water binding in cellulose tabletting excipients influence the availability of moisture to induce hydrolysis of acetylsalisylic acid (ASA). Cellulose powders of varying degree of order, denoted as low-crystallinity cellulose (LCC) and high-crystallinity cellulose (HCC), were produced by treating ordinary microcrystalline cellulose (MCC) in $ZnCl_2$ solutions of varying concentrations. Microcrystalline cellulose (MCC) and lactose monohydrate were used as reference excipients. The samples were then studied by X-ray diffraction, scanning electron microscopy, and differential scanning calorimetry (DSC). Different ratios of each excipient mixed with ASA were stored at 40% RH and 50 °C for 35 days to investigate the hydrolytic stability of the mixtures. Stability studies indicated that as concentration of HCC and MCC in binary mixtures with ASA was raised from 1 to 50% (w/w), ASA became increasingly unstable with respect to hydrolysis. Although LCC contained more moisture than the other celluloses, no such trend was observed in the LCC and lactose samples. DSC analysis revealed that each water molecule on the average was bound by more than three hydrogen bonds in the LCC and lactose structures and therefore remained predominantly unavailable to induce hydrolysis. The current study elucidates the necessity of evaluating the energy of water bindings in a pharmaceutical excipient when predicting the excipient's performance in mixtures comprising moisture-sensitive drugs.

Keywords: Microcrystalline cellulose; Lactose; Crystallinity; Moisture content; Water interactions; Stability of moisture-sensitive drug

1. Introduction

Hydrolysis is the main stability threat for acetylsalicylic acid (ASA), Fig. 1, as it is for many other esters in pharmaceutical formulations (Khan and Olagbemiro, 1985; Shija et al., 1992; Grit et al., 1993). Many excipients used in the production of solid dosage forms contain some moisture, which may act both as a reagent and reaction medium (Waterman et al., 2002). Water may be associated with the excipient in various ways including water of crystallization, tightly bound water of sorption (also known as non-freezing water), intermediately bound water of sorption, and free/bulk water (also known as freezing water) (Kontny and Conners, 2002). The energy needed to remove water differs substantially among the types, which, in turn, influences moisture's availability to induce hydrolysis.

Thermal analysis, particularly differential scanning calorimetry (DSC), has been successfully used to extract information about the energies of water binding to hydrophilic polymers (Nakamura et al., 1981; Hatakeyama et al., 2000; Agraval et al., 2003). DSC measurements of pure water are not always easy to interpret due to the high surface tension of water, and the fact that multiple peaks are observed (Hatakeyama and Hatakeyama, 1998). In contrast, transition temperatures and enthalpies of sorbed water on polymers are easily measured by DSC. It has previously been shown that thermal analysis is able to separate three energetically distinct states of water in cellulose, viz., the tightly bound, the intermediately bound, and the bulk water (Stamm and Hansen, 1937; Zografi et al., 1984; Blair et al., 1990).

Microcrystalline cellulose (MCC) and lactose monohydrate are commonly used excipients in formulation of solid dosage forms. One of the limitations of using MCC lies in its hygroscopicity, which may induce instability of moisture-sensitive drugs such as ASA (Ahlneck and Lundgren, 1985). Whilst no hydrolytic degradation of ASA is observed in mixtures

* Corresponding author. Tel.: +46 18 471 7231; fax: +46 18 50 0131.
** Corresponding author. Tel.: +46 18 471 4112; fax: +46 18 471 4223.
E-mail addresses: Maria.Stromme@angstrom.uu.se (M. Strømme), Ragnar.Ek@farmaci.uu.se (R. Ek).

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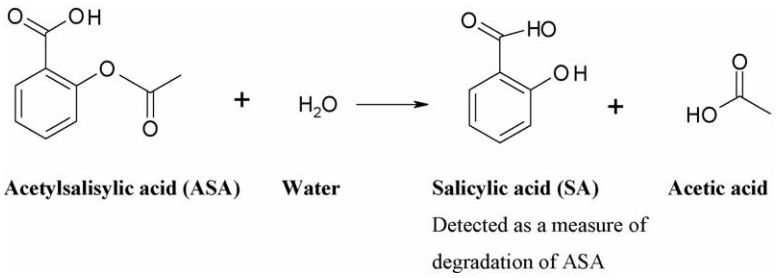


Fig. 1. Hydrolysis of acetylsalicylic acid (ASA).

with lactose, the limitations of its use lie in its inferior tablettability as well as some regulatory and ethical aspects linked to lactose intolerance, lactose being a potential bovine spongiform encephalopathy infection (the so-called "mad-cow disease") carrier, and lactose being a non-vegetarian dairy product.

As water in drug formulations has significant impact on drug stability, the moisture content of excipients is subject of regulation. According to the European Pharmacopoeia (European Pharmacopoeia, 2005a), the moisture content of MCC should not exceed 7.0% (w/w), whereas that of lactose monohydrate is set between 4.5 and 5.5% (w/w) (European Pharmacopoeia, 2005b). It should, however, be noted that moisture content per se says little about an excipient's propensity to promote hydrolysis. For instance, it has been shown that while the cellulose powders of lower degree of crystallinity contain more moisture than their counterparts with higher degree of order (Mihranyan et al., 2004), cellulose powders of low-degree of crystallinity exhibit lower rates of degradation of ASA than those with higher degree of crystallinity (Ahlneck and Alderborn, 1988; Mihranyan et al., 2006).

The aim of the present study was to investigate how the energies of water binding in cellulose powders of different degree of order and of different moisture content influence the availability of moisture to induce ASA hydrolysis. The low-hygroscopic excipient lactose is used as a model low-hygroscopic material for comparison in the present study.

2. Materials and methods

2.1. Materials

Microcrystalline cellulose, MCC (Avicel PH 102: FMC, Ireland), acetylsalicylic acid, ASA (Sigma–Aldrich Chemie Gmbh, Germany), and lactose monohydrate (α-Lactosemonohydrat, Pharmatose 200M, Sweden) were used as supplied. Low-crystallinity cellulose (LCC) was produced by swelling 50 g MCC in 1 kg 70% (w/w) $ZnCl_2$ (Merck KGaA, Germany) solution for 1 h. Additional water was added to a final volume of 2 l. The cellulose was then filtered and washed with 95% ethanol. The filtrate was washed with de-ionized water until the conductivity of the wash water was below 14 μS/cm (the upper limit for conductivity for MCC is 75 μS/cm, European Pharmacopoeia, 2005a) and subsequently dried in an oven at 45 °C for 2 days. The resulting powder was milled in a mortar grinder (KM1, Retch, Germany) and sieved. High-crystallinity cellulose (HCC) was produced by swelling MCC in 1 kg 48% (w/w) $ZnCl_2$ and washing in de-ionized water as described above for LCC, followed by spray-drying (Minor type 53, Niro Atomizer A.S., Denmark) and sieving. The LCC and HCC sieve fraction <106 μm was used in this study.

High purity indium, tin (Acros Organics, New Jersey, USA), gallium (Sigma–Aldrich GmbH) and zinc (TA Instruments, Delaware, USA) were used in the calibration of the differential scanning calorimeter instrument.

2.2. Methods

2.2.1. X-ray diffraction

The cellulose materials were studied using an X-ray diffractometer with Bragg-Brentano focusing geometry (D5000, Siemens, Germany) and Cu Kα radiation (λ = 1.54 Å). The samples were scanned at room temperature (25 °C) from 12° to 28° (2θ), with 0.1° steps (step-time 20 s). The ratio between the total peak height and the baseline level of the amorphous background was used to extract the crystallinity index as

$$\mathrm{CrI} = \frac{I_{002} - I_{\mathrm{am}}}{I_{002}} \times 100 \quad (1)$$

where I_{002} is the maximum intensity of the peak at 2θ of about 21–22° and I_{am} is the diffraction peak intensity at 2θ about 18° (Segal et al., 1959).

2.2.2. Moisture content

The cellulose samples were equilibrated at 40% RH over saturated K_2CO_3 solutions at room temperature throughout the study. The moisture content of the samples was measured using a Halogen Moisture Analyser (HR 73, Mettler Toledo, Switzerland) at 130 °C.

2.2.3. Scanning electron microscopy (SEM)

LCC, MCC, and HCC particles were studied by SEM (Hitachi 3500, Japan) to analyze the differences in particle morphology at 100× magnification.

2.2.4. Thermal analysis

A Seiko DSC 220 differential scanning calorimeter (SSC/5200h, Seiko, Japan) was used for thermal analysis of

MCC, LCC, HCC, and lactose. The instrument was calibrated for melting point and heat of fusion (T_m (°C), ΔH_m (J/g)) of indium (156.60 °C, 28.59 J/g), tin (232.00 °C, 60.62 J/g), gallium (29.80 °C, 80.17 J/g), and zinc (419.60 °C, 111.40 J/g). Each sample was heated from 25 to 50 °C above the melting temperature with appropriate heating rate and was purged by nitrogen gas. The gas flow rate was 80 ml/min.

The samples were carefully weighed (5–20 mg) in aluminum pans with cover (TA Instruments, Delaware, USA) and placed in the DSC equipment. The experiments were performed in a N_2 atmosphere. Empty pans were used as references. The temperature was first lowered to −15 °C at 5 °C/min and then held constant for 5 min followed by scanning from −15 to ∼170 °C at 1.5, 3, and 10 °C/min for the cellulose samples. Lactose was heated to 260 °C at similar heating rates. The temperature was then lowered to 19 °C by 20 °C/min. The temperature associated with the maximum point of the endothermic curve was then documented, and the area under curve was extracted as a measure of the total amount of energy (J/g) required for releasing the moisture present in the samples.

2.2.5. Stability studies

LCC, MCC, HCC, and lactose were blended with ASA to make four mixture series. In each series, the amount of the excipient was 1, 5, 10, 25 and 50% (w/w), respectively. A Turbula mixer (Willy A. Bachofen AG, Switzerland) was used. A total amount of 4 g of each sample was prepared, and the samples were mixed for 15 min. The concentration of salicylic acid indicated the extent of ASA hydrolysis as shown by Fig. 1. The mixtures were stored at constant relative humidity (40%) over saturated K_2CO_3 solution at 50 °C for 35 days. The concentration of salicylic acid was measured weekly by removing 50 mg samples from each mixture and blending them with 25 ml 95% ethanol. The ethanol mix was shaken vigorously and centrifuged (model 5403, Eppendorf, Germany) at 5000 rpm and 20 °C for 5 min. The supernatant was then analyzed by a UV-spectrophotometer (Hitachi U1100, Japan) at λ = 303 nm.

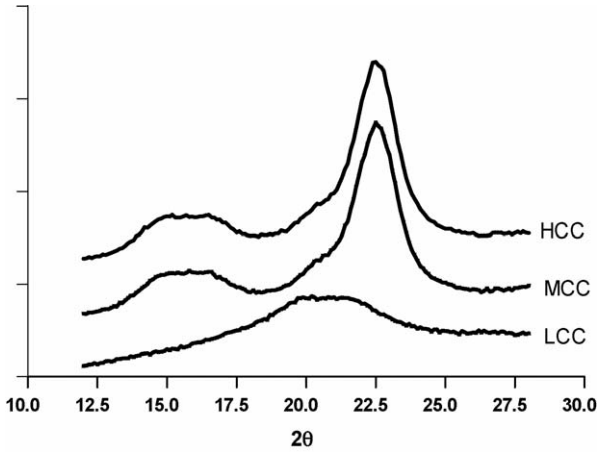


Fig. 2. Powder X-ray diffraction patterns of LCC, HCC and MCC.

3. Results

3.1. Solid-state characteristics

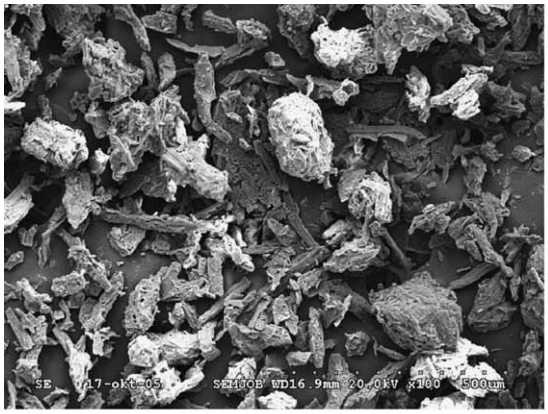
Fig. 2 depicts X-ray diffraction patterns of the cellulose powders. The peaks for MCC and HCC were very similar, though the HCC peak was somewhat sharper and had higher intensity. HCC and MCC diffraction patterns showed typical cellulose I structure, with a sharp peak at 22.5°, and a wide peak between 14° and 17.5°. The LCC diffraction pattern had a markedly different appearance with a single, smeared out peak of maximum intensity at around 20.5°. The CrI values, extracted from the XRD analysis, are listed in Table 1. In the same table, the moisture contents of the samples are also displayed. The moisture content of the cellulose samples varied between 5.27 and 9.27% (w/w) with the highest value pertaining to LCC. The lowest water content among all samples was observed for lactose, viz. 4.70% (w/w).

In Fig. 3, the SEM pictures of the samples show the differences in morphology between the cellulose samples. Treating MCC in 48% (w/w) $ZnCl_2$ solution, to produce HCC, did not result in appreciable changes in the morphology of the particles.

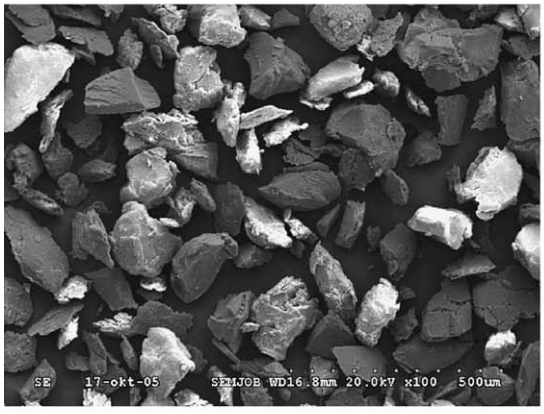
Table 1
Solid-state characteristics of the samples and DSC thermal analysis data

	Temperature[a] (°C/min)			I_{cr}[b] (%)	Moisture content[c] (%) (w/w)	$-\Delta H$[d] (J/g$_{sample}$) (1.5 °C/min)	$-\Delta H$[e] (J/g$_{water}$) (1.5 °C/min)	N_{Hbonds}[c,f]
	1.5	3.0	10.0					
HCC	36.7 (4.1)	49.4 (3.7)	81.1 (2.5)	82.1 (1.7)	5.27 (0.24)	121.1 (17.4)	2298 (393.8)	1.98 (0.34)
MCC	35.2 (3.0)	54.2 (2.9)	87.8 (4.9)	81.7 (4.0)	5.47 (0.08)	133.6 (23.3)	2442 (443.7)	2.10 (0.38)
LCC	49.9 (1.3)	61.0 (2.9)	90.6 (8.1)	30.5 (11.3)	9.27 (0.24)	376.1 (35.9)	4057 (439.6)	3.49 (0.38)
Lactose	136.3 (0.8)	144.0 (1.2)	150.6 (1.4)	–	4.70 (0.36)	177.1 (11.0)	3768 (377.7)	3.24 (0.32)

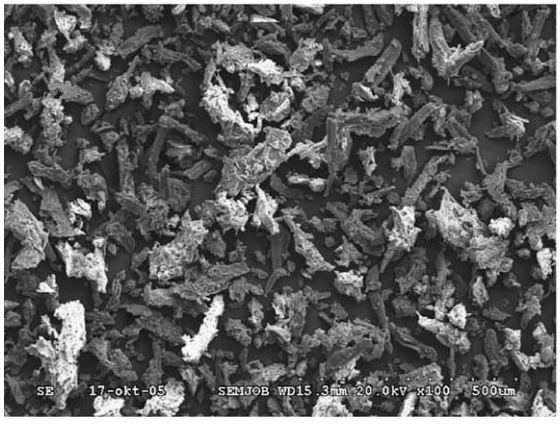
The values in parentheses are standard deviation over three to six measurements.
[a] The maximum temperature of the endothermic peaks in the DSC measurements. For lactose, the temperature is extracted for the peak pertaining to evaporation of crystal water.
[b] Crystallinity index.
[c] Equilibrated at 40% RH.
[d] Heat absorbed normalized per sample weight.
[e] Heat absorbed normalized per sample moisture content.
[f] Average number of H-bonds holding the water molecule in the excipient structure.



MCC



LCC



HCC

Fig. 3. SEM pictures of: (a) MCC; (b) LCC; (c) HCC particles with 100× magnification. The white-dots scaling bar has a length of 500 μm.

This was not the case for LCC as the swelling in 70% (w/w) $ZnCl_2$ solution caused profound changes in the appearance of particles both in terms of their shape and particle size distribution, compared to MCC. The LCC particles also appeared more dense.

3.2. Stability studies

The results of the stability studies are illustrated in Fig. 4. Each data point represents the average value of three measurements. The standard deviation in each displayed data point, which was less than 4%, is too small to be visualized by error bars in the figure. In mixtures with the highest drug/excipient ratio, there were no significant differences among the samples. However, when the drug concentration was decreased below 90% (w/w), the extent of hydrolysis elevated as the MCC and HCC concentration increased, whereas the trend was opposite for mixtures containing LCC and lactose. The drug degradation was progressively inhib-

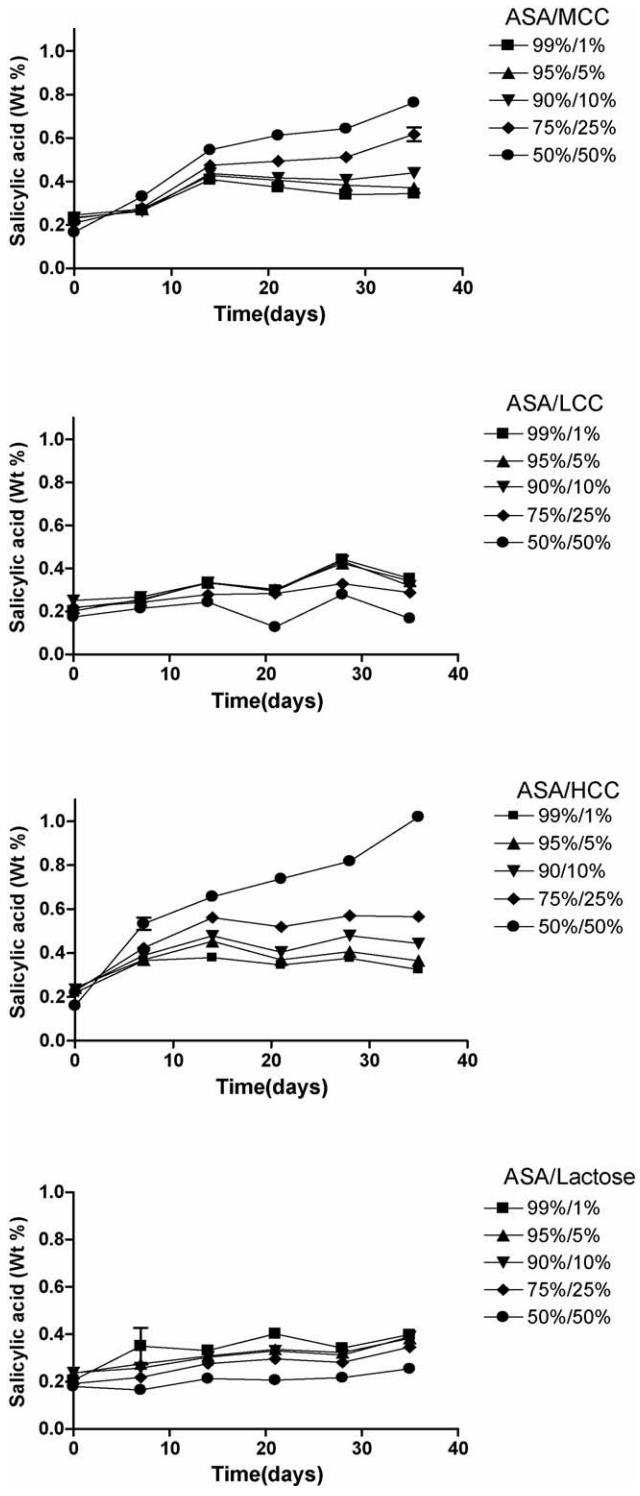


Fig. 4. Salicylic acid concentration vs. time in mixtures of ASA and MCC, LCC, HCC, as well as lactose at the five indicated drug/excipient ratios stored over saturated K_2CO_3 solution at 50 °C. Lines are drawn as guides to the eye.

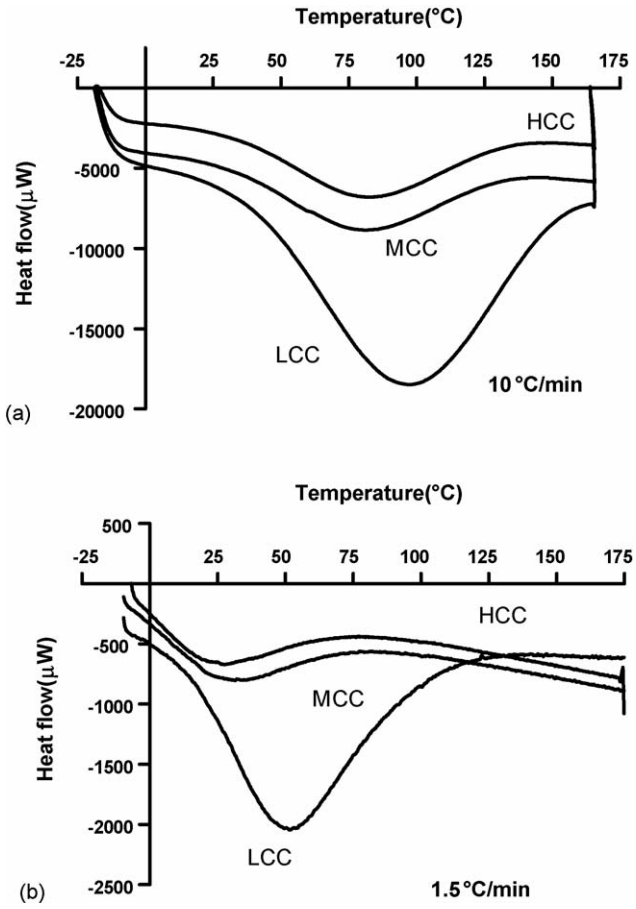


Fig. 5. Heat flow vs. temperature for HCC, MCC, and LCC equilibrated over saturated K_2CO_3 solution (40% RH) registered by DSC at two different heating rates: (a) 10 °C/min and (b) 1.5 °C/min.

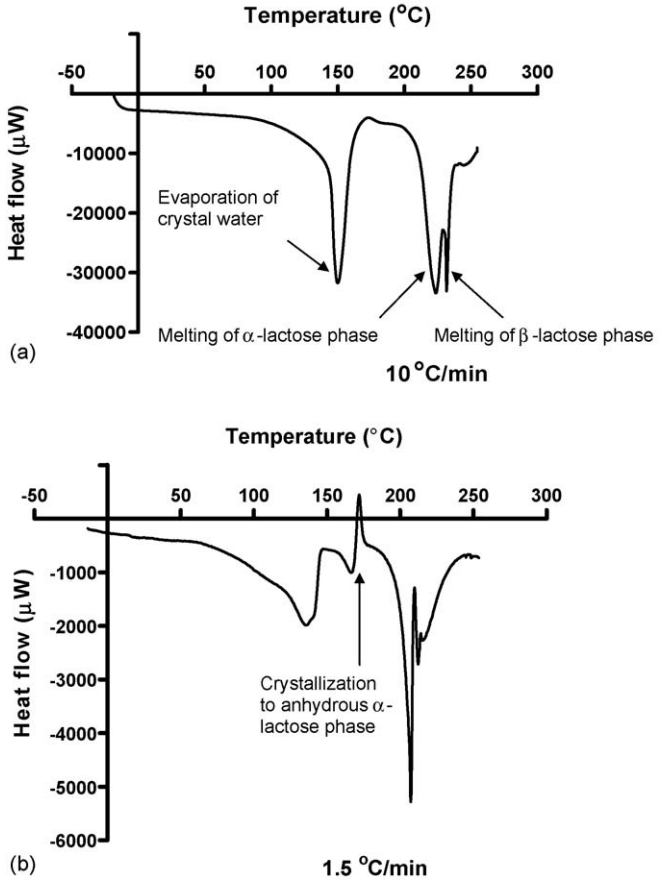


Fig. 6. Heat flow vs. temperature for lactose monohydrate equilibrated over saturated K_2CO_3 solution (40% RH) registered by DSC at two different heating rates: (a) 10 °C/min and (b) 1.5 °C/min.

ited in these mixtures as the excipient concentration was increased.

In mixtures containing 50% (w/w) excipient, the difference in drug degradation among the samples was most pronounced. The samples containing HCC and MCC showed a substantially higher degradation of ASA than did the LCC and lactose mixtures. The degradation was highest in the samples containing HCC followed by the MCC mixtures, whereas in mixtures of LCC and in those of lactose ASA remained virtually intact.

3.3. Thermal analysis

Fig. 5 shows the DSC analysis results for the cellulose samples for the highest (panel a) and the lowest (panel b) temperature sweep rates employed. The DSC graph for each cellulose sample was dominated by a peak pertaining to the melting and evaporation of water from the cellulose structure. The areas under the endothermic curves for HCC and MCC were small in comparison to that under the LCC curve, and the evaporation peaks of HCC and MCC reached maximum faster, i.e., at lower temperatures, than did the LCC peak.

Fig. 6 shows the corresponding DSC analysis plots of the lactose sample. The endothermic events of phase transition of different lactose phases were clearly visible in both of the plots. However, at the lowest temperature sweep rate (Fig. 6b) the DSC spectrum was more resolved with respect to the exothermic peak located at 173.5 °C. The numerical results from the DSC analyses are summarized in Table 1, showing the location of the water evaporation peaks for the different samples at different temperature sweep rates as well as the energy required to dehydrate the samples at the lowest sweep rate. From the table it is obvious that the water evaporation peaks were shifted to higher temperatures with increasing sweep rate, while the mutual order amongst the samples remained unchanged. The table also shows that the energy required to evaporate water from the LCC and lactose structures was significantly larger than the same for HCC and MCC, even when normalized with respect to the sample moisture content.

4. Discussion

The moisture content of the presently investigated celluloses was found to increase with decreasing crystallinity, Table 1. This result can be explained by the fact that lowering the degree of cellulose order created a higher number of available OH-groups to which water molecules could bind.

The CrI of the LCC in the present study of ~30.5% was found to be significantly lower than the earlier reported value of 45%, whereas the moisture content at 40% RH was found to be larger,

∼9.3% (w/w), compared to the earlier obtained value of ∼7.0% (w/w) (Mihranyan et al., 2004). The obtained lower value of crystallinity in this work can be explained by the fact that the cellulose was more dispersed and consequently more available for swelling. A similar swelling of pure cellulose in a dissolving pulp sheet form resulted in a crystallinity value (measured with NMR) of 25% (Ek et al., 1994).

In the SEM pictures, Fig. 3, the LCC particles appeared denser than the MCC and HCC particles. This densification of MCC by swelling in 70% (w/w) $ZnCl_2$ is supported by earlier pore volume estimations from nitrogen adsorption measurements (Mihranyan et al., 2004). The fact that the LCC particles may be denser than the other cellulose particles under study, could contribute to the location of the evaporation peak (Table 1) being at higher temperatures than the corresponding peaks for MCC and HCC due to longer equilibration times in LCC. However, the fact that the difference in peak location remains, and even becomes more pronounced as the temperature sweep rate is decreased down to 1.5 °C/min, shows that the difference between LCC and the other investigated celluloses is not predominantly due to equilibration kinetics.

The three major endothermic processes in lactose, Fig. 6, can be attributed to evaporation of crystal water, melting of anhydrous α- and β-lactose, respectively (Sebhatu et al., 1994). The exothermic process, which took place after the endothermic event of dehydration, can be associated with re-crystallization of the hygroscopic instable lactose $L\alpha_H$ phase to the stable anhydrous lactose $L\alpha_S$ phase (Garnier et al., 2002).

From Fig. 4, it is evident that the structure of the cellulose had significant effect on the hydrolysis degradation of ASA in mixtures with cellulose. As degradation increased with increasing the cellulose content for HCC and MCC, the low-crystallinity cellulose (featured with highest moisture content in the series) obviously had a degradation protecting effect on ASA; the higher the LCC content, the lower was degradation. Lactose had a similar influence on ASA degradation as did LCC. From Fig. 3 and Table 1, it is evident that the energy of water-excipients interactions, as normalized per gram water content, was significantly higher for LCC and lactose than for HCC and MCC. The increased bond strength of water in LCC as compared to HCC and MCC can be explained by differences in the local nanostructure environment. The local nanostructure of LCC, hosting the sorbed water, on the average provides each water molecule with more hydrogen bonds than do MCC and HCC. Considering that the energy, $E_{\text{H-bond}}$, of dissociation per H-bond is about 5 kcal/mole (20950 J/mole) (More, 1970), the average number, $N_{\text{H-bond}}$, of H-bonds attaching each water molecule in the excipient structure presented in Table 1, was calculated according to

$$N_{\text{H-bonds}} = \frac{\Delta H(\text{J/g}_{\text{water}}) \times M_{\text{H}_2\text{O}}(\text{g/mole})}{E_{\text{H-bonds}}(\text{J/mole})} \quad (2)$$

Here, ΔH denotes the heat absorbed during water removal normalized per sample moisture content, Table 1, and $M_{\text{H}_2\text{O}}$ is the molar mass of water (18 g/mole). The content of the brackets denotes the relevant units for the different quantities. The number of H-bonds attaching the water molecule to the HCC and MCC structures at 40% RH was found to be of the order of 2, while the average number of H-bonds holding each water molecule in the LCC and lactose structures was found to be ∼3.5 and 3.2, respectively, Table 1. These differences are suggested to be pivotal to explaining the lower rates of ASA degradation in LCC despite the latter's relatively high moisture content.

5. Conclusion

Stability tests of the model moisture degradable drug ASA were performed at various concentrations in three different structures of microcrystalline cellulose as well as in lactose. The results showed that lactose as well as LCC, featured with the highest moisture content in the series, inhibited ASA degradation, whereas HCC and MCC, featured with lower moisture content and higher degree of crystallinity, promoted ASA hydrolysis. DSC analysis showed that each water molecule was bound to the LCC structure on average by ∼3.5 bonds, whereas the corresponding number of H-bonds per water molecule for HCC and MCC was ∼2. The current study elucidates the necessity of evaluating the energy of water bindings in a pharmaceutical excipient when predicting the excipient's performance in a mixture comprising moisture-sensitive drugs. The presented results support earlier studies showing that the low-crystallinity cellulose, containing more moisture than commercially available MCC, may be a viable alternative in pharmaceutical formulations comprising moisture-sensitive drugs.

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