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Spray-dried nanofibrillar cellulose microparticles for sustained drug release

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ABSTRACT

Nanofibrillar cellulose (also referred to as cellulose nanofibers, nanocellulose, microfibrillated or nanofibrillated cellulose) has gained a lot of attention in recent years in different research areas including biomedical applications. In this study we have evaluated the applicability of nanofibrillar cellulose (NFC) as a material for the formation of matrix systems for sustained drug delivery. For that purpose, drug loaded NFC microparticles were produced by a spray drying method. The microparticles were characterized in terms of size and morphology, total drug loading, and physical state of the encapsulated drug. Drug release from the microparticles was assessed by dissolution tests, and suitable mathematical models were used to explain the drug releasing kinetics. The particles had spherical shapes with diameters of around 5 μm; the encapsulated drug was mainly in amorphous form. The controlled drug release was achieved. The drug releasing curves were fitted to a mathematical model describing the drug releasing kinetics from a spherical matrix. Different drugs had different release kinetics, which was a consequence of several factors, including different solubilities of the drugs in the chosen medium and different affinities of the drugs to the NFC. It can be concluded that NFC microparticles can sustain drug release by forming a tight fiber network and thus limit drug diffusion from the system.

1. Introduction

Controlled drug delivery (CDD) technology represents a widely studied area in the field of pharmaceutical sciences. The main aim when formulating CDD systems is to increase the effectiveness of drug therapy. The benefit could be in a form of increased therapeutic activity, reduced toxicity, avoidance of the first pass metabolism, elimination of a specific drug administration route (e.g. injections), or reduction in a dosing frequency. A convenient administration route and reduction in the dosing frequency leads to better patient compliance. Therefore, there is a great interest in the development of systems that would be capable of delivering drugs over a long time period and, thus, reduce the number of drug doses required during the treatment. Desirable drug delivery rates and extents from these kinds of systems are in the most cases achieved by the application of suitable polymers. This incites constant research in the area of designing new and improved polymers.

In recent years, many methods have been introduced to disintegrate cellulose fibers originated from the wood to the level of elementary fibrils. These methods involve mechanical fibrillation using various devices, such as high pressure homogenizer (Paakko et al., 2007; Stelte and Sanadi, 2009), microfluidizer (Henriksson et al., 2007; Zimmermann et al., 2004), or grinder (Iwamoto et al., 2005). The mechanical treatment could be combined with processes of refining (Stelte and Sanadi, 2009), cryocrushing (Chakraborty et al., 2005), enzymatic treatment (Henriksson et al., 2007; Paakko et al., 2007) or ultrasonication (Zhao et al., 2007), in order to increase the yield and to obtain more uniform fibers with smaller diameters. Elementary fibrils and fibril bundles obtained in these ways have typically diameters in the range of 5–40 nm and lengths up to several micrometers (Zimmermann et al., 2010), referred to as nanofibrillar cellulose (NFC). In the literature, names like cellulose nanofibers, nanofibrillated cellulose, microfibrillated cellulose or nanocellulose are also used.

NFC has gained increasing attention in recent years due to its interesting properties such as high strength and stiffness combined with low weight, biodegradability and renewability. Therefore, different forms of the material have been produced such as spray dried particles, hydrogels, aerogels and films. Furthermore, a wide variety of composites containing mixtures of NFC with different polymers have been introduced (Siro and Plackett, 2010). Since the manufacturing methods of the different forms of NFC have been significantly developed, it is now possible to produce applications with tailored properties.

Various types of nanoparticles have been assembled on the surfaces of cellulose fibers to allow functionalities, such as antibacterial or catalytic activity and permanent colors. It has been showed (Valo et al., 2011) that NFC can be successfully used for

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the stabilization of drug nanoparticles in a suspension, as well as in a freeze dried NFC matrix. In this way, the nanoparticles could be stored for more than ten months without major changes in their morphology. Loading of silver entities, referred to as silver nanoclusters, within NFC films, which exhibit antimicrobial properties, has been reported (Diez et al., 2011). We have shown (Kolakovic et al., 2011) that NFC microparticles produced by a spray drying method can be used as fillers in tablet production by direct compression, as well as in wet granulation.

Here, we introduce the ability of NFC to form porous microparticles in the spray drying process, and simultaneously to encapsulate drugs inside the NFC carrier. The particles produced in this way should have a matrix system structure, and be able to release the drug over a long time period. The mechanism of the sustained drug release would rely on the tight fiber network formed around the drug entities, presenting a release rate controlling structure.

2. Material and methods

2.1. Materials

The NFC used for the microparticles production was obtained from UPM-Kymmene Corporation (Helsinki, Finland) in form of a 1.66% water suspension. The material has been produced by mechanical grinding of bleached birch pulp. Indomethacin, purchased from Hawkins, Inc. (Minneapolis, USA), nadolol, atenolol, metoprolol tartrate, verapamil hydrochloride from Sigma–Aldrich (Munich, Germany) and ibuprofen from Orion Pharma (Espoo, Finland) were used as the model drug compounds. All other chemicals were of analytical grade obtained from standard sources and used without further purification.

2.2. Methods

2.2.1. Preparation of suspension for spray drying

All the suspensions were prepared in the same manner. First, the drug was dissolved in a suitable solvent. Atenolol, nadolol, metoprolol tartrate, and verapamil hydrochloride were dissolved in water and ibuprofen and indomethacin in 50 mM aqueous NH_4OH. The solution was then mixed with NFC suspension so that the total concentration of the both, dissolved and suspended material, was 0.5%. The ratios, in which the NFC and drug were mixed, were 20/80, 30/70 and 40/60 (Table 1). Since the used NFC was in a form of 1.66% water dispersion, the concentration of feeding suspension and the ratios were calculated using the content of dry fibers in the NFC suspension. The prepared suspensions were sonicated for 15 min using a high intensity ultrasound processor (Sonics, Newtown, USA) equipped with a 13 mm probe, and then mixed with a mechanical stirrer for 15 min at a speed of 1800 rpm. Batches containing 30% and 40% of ibuprofen and atenolol were not produced, since the preliminary particles with an aimed ratio of 20/80 did not have the desirable properties.

2.2.2. Spray drying

The suspensions were dried using a Mini spray dryer Büchi B-191 (Flawil, Switzerland). The spray dryer was equipped with a two-fluid nozzle and operated in a co-current mode (the feeding suspension and the drying air flow in the same direction). The drying was performed using the following parameters: inlet temperature 220 °C, outlet temperature in a range from 120–127 °C, spray flow 700 l/h, air pressure 7 bar, aspirator setting 95% and pump setting 18% (corresponds to approx. 7 ml/min). The feeding suspension was mixed continuously during the drying process using a magnetic stirrer to prevent sedimentation of the suspended cellulose fibers. The spray dried microparticles were collected from the dryers' collection vessel and stored in closed vials at room temperature.

2.2.3. Electron microscopy analyses

Micrographs of the spray dried indomethacin microparticles were obtained using Scanning electron microscope (SEM) Zeiss DSM 962 (Carl Zeiss, Hamburg, Germany). The samples were fixed onto a two-sided carbon tape and sputtered with platinum for 25 s with an Agar sputter device (Agar Scientific Ltd., Stansted, UK). The images of metoprolol tartrate and verapamil hydrochloride microparticles were obtained using FEI Quanta™ FEG scanning electron microscope (FEI, Hillsboro, USA). The samples were prepared in the same way as the indomethacin samples. The micrographs were used for morphological characterization and particle size determination.

Transmission electron microscopy (TEM) images of the microparticles were obtained using FEI Tecnai F12 (FEI, Hillsboro, USA). Microparticles dispersions were dried on Formvar film-coated copper grids with a mesh size of 300 (Agar Scientific, Stansted, UK). Microparticles were characterized by TEM after the dissolution test.

2.2.4. Thermal analyses

Thermal analysis of the spray dried particles and the physical mixtures of NFC and tested drugs were carried out using a differential scanning calorimeter Mettler Toledo DSC 823e (Mettler Toledo, Giessen, Germany). The physical mixtures were prepared by mixing spray dried NFC powder and drug at a ratio that corresponded to the ratio in the drug loaded particles. The samples were placed in sealed aluminum pans with pierced lids and heated at a scanning rate of 10 °C/min between 25 and 200 °C in nitrogen atmosphere. The data was analyzed with STARe software (Mettler-Toledo, Giessen, Germany).

2.2.5. Determination of drug loading

1-N-allyl-3-methylimidazolium chloride (AMIMCl) has been reported to be a good solvent for native cellulose (Zhang et al., 2005). Therefore, the total drug content in the spray dried particles was assayed by dissolving the microparticles in AMIMCl. AMIMCl has a melting point of 22 °C and it is a highly viscous liquid at room temperature. To provide easy handling, AMIMCl was used in solid state by storing it in a freezer prior to use. The test was performed by mixing known amounts of the microparticles with AMIMCl. The concentration of microparticles in AMIMCl was 1%. The mixture was stirred continuously overnight at 60 °C. Solutions were then diluted with DMSO and analyzed by a suitable HPLC method (Agilent Technologies, 1100 Series, Santa Clara, USA). The DMSO is not a solvent for cellulose, but it is a swelling agent. Therefore, the addition of DMSO kept the fibers in a dissolved form. The addition of other solvents, such as water or ethanol, would have caused precipitation of NFC and entrapment of certain amount of drug in the network of precipitated fibers and, thus, caused incorrect values of total drug loading.

During the spray drying process, a certain portion of drug was not loaded into the particles, but spray dried separately or located on the particles surface. In order to quantify this amount, 40 mg of the microparticles were placed on a hydrophilic polypropylene membrane with a pore size of 0.2 μm and washed with 400 ml of the medium using a vacuum filtration system. In the case of metoprolol tartrate, verapamil hydrochloride, nadolol and atenolol, deionized water, and for indomethacin and ibuprofen phosphate buffer at pH 7.4 was used as the medium. The medium was chosen based on the drug solubility. Concentration of the drug in the filtrate was measured by a suitable HPLC method and was used to quantify the unbound drug fraction. The details on conditions used for the HPLC analyses are given in Table 2. The unbound fraction

Table 1
Content of NFC/indomethacin, NFC/metoprolol tartrate, NFC/verapamil hydrochloride, NFC/nadolol, NFC/ibuprofen and NFC/atenolol suspensions used for particles production. The numbers are mass ratios of drug and NFC fibers used in the initial feeding suspensions. Labels for the different formulations are also given in the table.

API/NFC (%/%)	Indomethacin	Metoprolol tartrate	Verapamil hydrochloride	Nadolol	Ibuprofen	Atenolol
20/80	INDO20	METO20	VERA20	NADO20	IBU20	ATE20
30/70	INDO30	METO30	VERA30	NADO30	–[a]	–[a]
40/60	INDO40	METO40	VERA40	NADO40	–[a]	–[a]

[a] Batches containing ibuprofen and atenolol and NFC in ratio 30/70 and 40/60 were not produced

was calculated as a percentage of drug mass compared to the total mass of drug used (100% represents the total amount of the drug used in the production). These values have been further used for calculation of the amount of drug remained in the particles after the washing process. The remaining amount was calculated as the difference in the amount of drug before the washing process and washed unbound fraction. The final loading was then calculated as a percentage of remained drug mass compared to the total mass of the particles (100% represents the total mass of the particles).

2.2.6. Drug release studies

After the unbound fraction was removed, the samples were transferred to 50 ml glass bottles with 10 ml of medium. Deionized water was used as the medium for metoprolol tartrate, verapamil hydrochloride, nadolol and atenolol, and phosphate buffer at pH 7.4 for indomethacin and ibuprofen. The bottles were placed into a shaking water bath (Julabo SW 23, Seelbach, Germany) equipped with a tray for Erlenmeyer flasks. Shaking frequency was set at 110 min^{-1}. At various time points the total volume of dissolution medium was replaced by fresh medium. This was done by filtering the particles from the dissolution medium and resuspending them in the same volume of fresh medium. The amount of the released drug was quantified by a suitable HPLC method (Table 2). The results of the dissolution tests were fitted to a mathematical model developed by Baker and Lonsdale (1974) for spherical particles, in order to analyze the release kinetics.

3. Results and discussion

3.1. Microparticle production

NFC has strong affinity to water, which makes it difficult to dry. The residence time in the spray-drying chamber is short and, therefore, a high temperature is necessary for complete drying. If a lower temperature and/or higher feeding rates were to be used, the wet material would adhere to the chamber. The use of a high temperature, when spray drying the aqueous suspension of NFC containing an API, increases the risk of degradation of the API during the drying process. High temperature is also a limitation for the choice of the API, if the melting point is low (Table 3).

Generally, particles reach a maximum temperature during spraying, which is 15–20 °C below the outlet temperature of the co-current dryer (Broadhead et al., 1992). During the particle production, the outlet temperatures were in the range of 120–127 °C, which was sufficient for successful drying. However in the case of ibuprofen, the outlet temperature was above the melting point of ibuprofen (75–78 °C) (Lerdkanchanaporn et al., 2001), and the drug melted inside of the drying chamber and adhered to the chamber walls. Hence, a very small portion of the drug was incorporated into the microparticles. The second limitation step of the spraying process is the API's solubility to the spraying fluid (Table 3). In the present work, the spray-drier was limited to aqueous solvents. Therefore in the case of atenolol, nadolol, metoprolol tartrate and verapamil hydrochloride, water was used as the solvent, whereas indomethacin and ibuprofen were dissolved in 50 mM aqueous NH_4OH solution. NH_4OH was chosen since indomethacin and ibuprofen have pH dependent solubility, and high pH values of aqueous medium were needed to dissolve the drug prior to spray drying. Furthermore, NH_4OH was a convenient solvent, since NH_3 was removed as gas during the drying process due to the high temperature in the drying chamber, leaving the product almost completely free of the base. In all cases, the concentrations of model drug and NFC together in the feeding suspension was kept at 0.5%, since higher amounts caused clogging of the spray nozzle due to the high viscosity. The employed method resulted in the production of white powders with the exception of indomethacin, which resulted in a yellow powder. The color was a consequence of yellow

Table 2
Experimental parameters of HPLC analysis used for drugs' quantification.

Drug	Column	Eluents	Eluents flow (ml/min)	Retention time (min)	Absorption wavelength (nm)
Indomethacin	Phenomenex Gemini-NX 3 μm C18 100 mm × 4.6 mm	ACN/0.2% PA[a] 35%/65%	1	4.5	320
Metoprolol tartrate	Phenomenex Prodigy 3 μm ODS 100 mm × 4.6 mm	ACN/0.05% TFA[b] 25%/75%	1	3.4	225
Verapamil hydrochloride	Phenomenex Prodigy 3 μm ODS 100 mm × 4.6 mm	ACN/0.05% TFA 50%/50%	1	1.7	230
Nadolol	Agilent Zorbax Eclipse XDB C8 5 μm 150 mm × 4.6 mm	ACN/0.03% TFA 20%/80%	1	2.8	270
Ibuprofen	Agilent Zorbax Eclipse XDB C8 5 μm 150 mm × 4.6 mm	ACN/0.1% TFA 56%/44%	1.5	3.5	264
Atenolol	Phenomenex Prodigy 3 μm ODS 100 mm × 4.6 mm	ACN/0.05% TFA 10%/90%	1	4.1	225

[a] PA denotes phosphoric acid (all dilutions were made in water).
[b] TFA denotes trifluoroacetic acid (all dilutions were made in water).

Table 3
The values of melting points (T_m), water solubility and stability in water solution of the tested drugs.

API	Solubility in water	T_m (°C)	Stability in water solution (37 °C)	Reference
Indomethacin	6 μg/ml MQ 13 μg/ml pH = 5 302 μg/ml pH = 7.4	158 (α)[a] 165 (γ)[a]	pH 7.4 up to 4 days	Borka (1974), Jain (2008), and Varughese et al. (2010)[a]
Ibuprofen	<1 mg/ml 6 mg/ml pH = 7.4[c]	75–78[b]	–	Lerdkanchanaporn et al. (2001) and Levis et al. (2003)
Nadolol	6.77 mg/ml	127[d]	Up to 30 days	Domańska et al. (2010)[d]
Atenolol	26 mg/ml[e]	146–148[e]	–	Thomas and Rubino (1996)[e]
Metoprolol tartrate	Freely	120–122[f]	Up to 60 days	Glaessl et al. (2009) and Varshosaz et al. (2006)[f]
Verapamil hydrochloride	Freely	142–145[g]	Up to 7 days	Passerini et al. (2003) and Rustichelli et al. (1999)[g]

amorphous indomethacin. The intensity of powder color was increased with the increasing amount of indomethacin. The yield was in the range of 19–35%, since certain amount of the material adhered to the drying chamber (Table 4). As mentioned above NFC has high affinity to water which makes its drying difficult. This property was pronounced during the spray drying process since the certain portion of droplets was not completely dry before they reach the wall of the drying chamber or the cyclone wall. This caused sticking/adhesion of the wet material to the chamber and cyclone wall and thus affected the yield of the production process.

3.2. Microparticle characterization

SEM images of all the produced particles (Figs. 1 and 2) show irregular, roughly spherical particles with diameters around 5 μm. The shape of the drug loaded NFC particles produced in this case had similar shape and size as the unloaded NFC particles produced by the spray drying method (Kolakovic et al., 2011). However, in this case, the most interesting characteristics observed were related to the spray-dried microparticle surfaces, where higher magnifications revealed the fibrous structure of the particle surfaces. In the case of indomethacin loaded powders, certain amount of indomethacin was present as free drug that had dried separately. This can be seen in Fig. 2 as needle-shaped drug crystals. This phenomenon was not seen when verapamil hydrochloride and metoprolol tartrate were used (Fig. 1). This is possibly a consequence of indomethacin precipitation that occurred before the spraying process, when the drug solution was added to the NFC suspension, but may also be a consequence of different drying kinetics of the drug and NFC.The physical state of the drugs inside the dry particles was assessed by thermal analysis. The DSC thermal profiles of the produced powders were compared to the corresponding drug-NFC physical mixtures (Figs. 3 and 4). The DSC curves of INDO20, INDO30 and INDO40 (Fig. 3) showed broad endothermic peaks in the temperature range of 60–120 °C, which is related to the loss of water retained in the samples after the spray drying process. For physical mixtures, sharp peaks were observed at the temperature that corresponds to the melting of crystalline γ-indomethacin (Varughese et al., 2010). For spray dried particles, the peak was shifted to 150 °C, and the peak area was significantly decreased. The shifted positions of the peaks indicates that in the produced powders, indomethacin might exist in its α-form, since the γ-form is known to have a melting temperature between 160 and 164 °C and α-form between 153 and 155 °C (Borka, 1974; Varughese et al., 2010). INDO40 sample consisted of two separate peaks for both the α- and γ-forms. The reduced peak area indicated that the crystallinity was decreased during spray drying. Furthermore, the change in indomethacin color from white to yellow indicated the presence of the amorphous form. The production conditions, which involved the use of 50 mM NH_4OH as the solvent and high temperature during the drying, caused the drug to exist partly in an amorphous form. Similar results were obtained in the case of metoprolol tartrate (Fig. 4) and verapamil hydrochloride (data not shown). Sharp melting peak (Glaessl et al., 2009; Varshosaz et al., 2006) seen in the physical mixtures disappeared

Table 4
The yield, total loading, unbound fraction calculated as the amount of drug washed in 24 h compared to the total loaded amount of drug, and final drug loading of spray dried particles as mass percents.

Sample	Yield (%)	Total loading (%)	Unbound fraction (%)[a]	Final loading (%)[b]
INDO20	29.5	19.5	73.2	6.1
INDO30	20.2	28.8	70.1	12.2
INDO40	19.2	40.3	74.1	15.1
METO20	31.3	17.1	81.2	4.5
METO30	30.9	23.9	85.1	5.1
METO40	29.2	34.2	87.5	7.7
VERA20	33.7	17.2	81.8	4.5
VERA30	28.4	27.3	79.0	5.4
VERA40	29.3	38.1	83.3	8.2
NADO20	33.5	20[c]	87.4	3.6[d]
NADO30	35.2	30[c]	95.6	1.8[d]
NADO40	25.7	40[c]	96.8	2.4[d]
IBU20	23.2	11.7	84.5	1.7
ATE20	31.6	20[c]	95.7	1.1[d]

[a] Values calculated as a percentage of drug mass compared to the total mass of drug used (100% represents the total amount of the drug used in the production).
[b] Values calculated as a percentage of drug mass compared to the total mass of the microparticles (100% represents the total mass of the microparticles).
[c] Theoretical loading used as total loading.
[d] Final loading calculated from theoretical total loading.

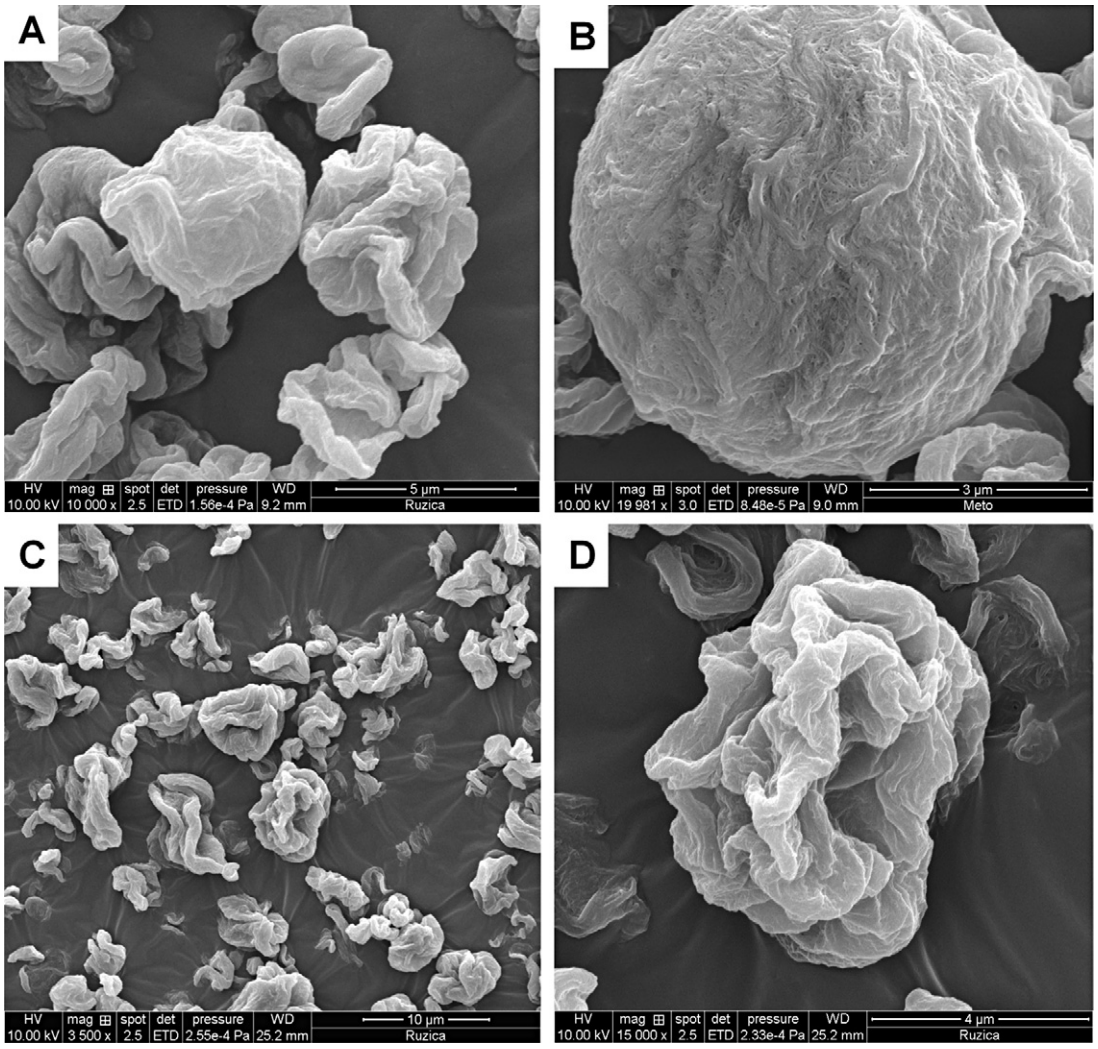

Fig. 1. SEM images of the spray dried NFC microparticles: (A) particles containing 20% of metoprolol imaged with lower magnification and (B) higher magnification, and (C) particles containing 20% of verapamil imaged with lower magnification and (D) higher magnification.

in the spray dried products, indicating that the drugs are also in the amorphous state or disordered crystalline phase as a molecular dispersion in the polymer matrix after the production process. These results are in accordance with literature data – spray drying processes produce amorphous materials (Chen et al., 2007; Corrigan, 1995; Patterson et al., 2007).

3.3. Drug loading

The total drug loading in the dry microparticles includes all the drug material present in the powder product, i.e. the tightly bound encapsulated drug, the weakly bound drug adsorbed inside the cavities on the particle surface, and the non-encapsulated drug that

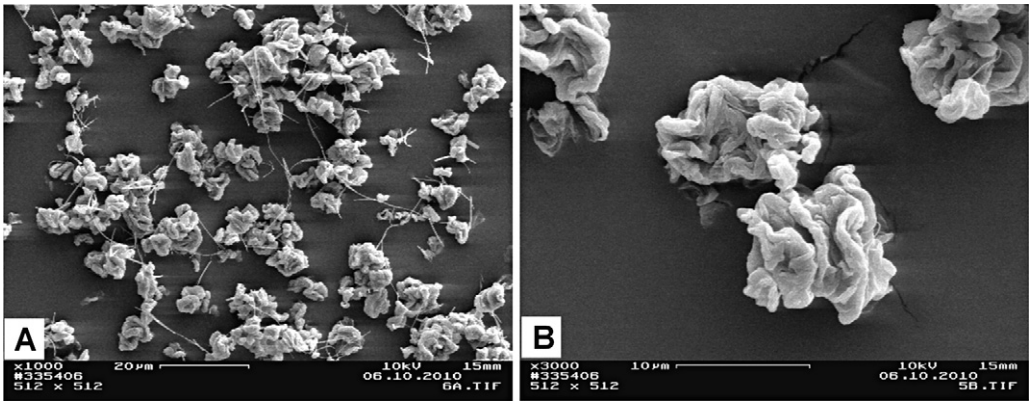

Fig. 2. SEM images of spray dried NFC particles containing 20% of indomethacin: (A) lower magnification and (B) higher magnification.

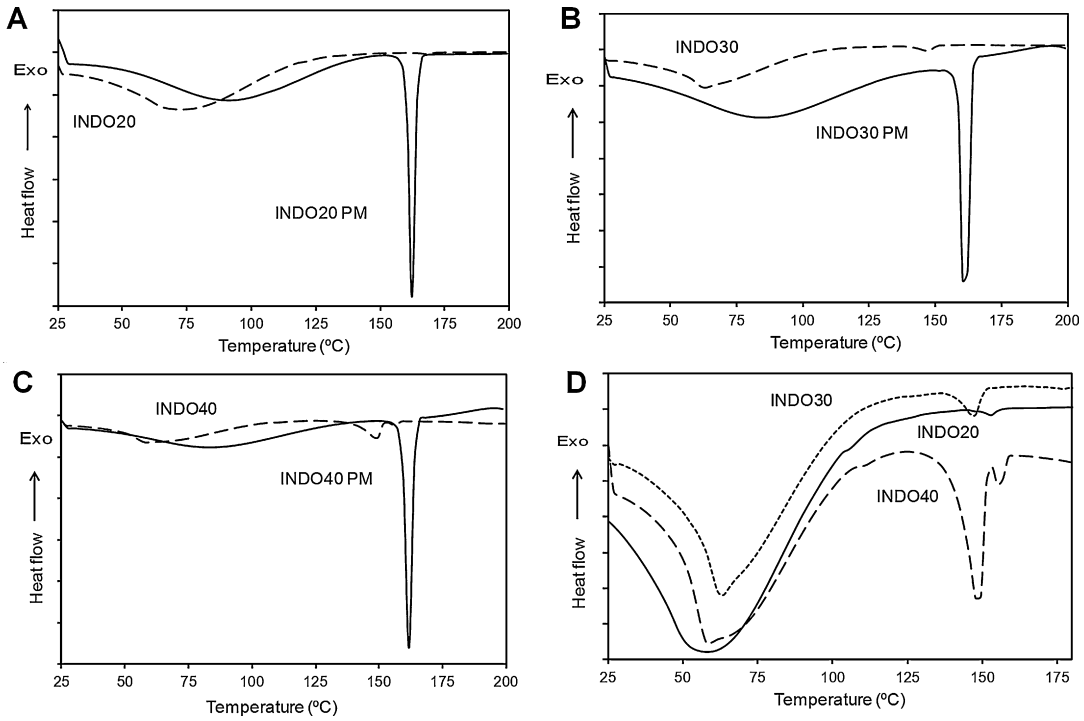

Fig. 3. DSC curves of the NFC microparticles containing indomethacin (A) INDO20, (B) INDO30, (C) INDO40 compared to the corresponding physical mixtures (PM) and (D) comparison of curves INDO20, INDO30, and INDO40.

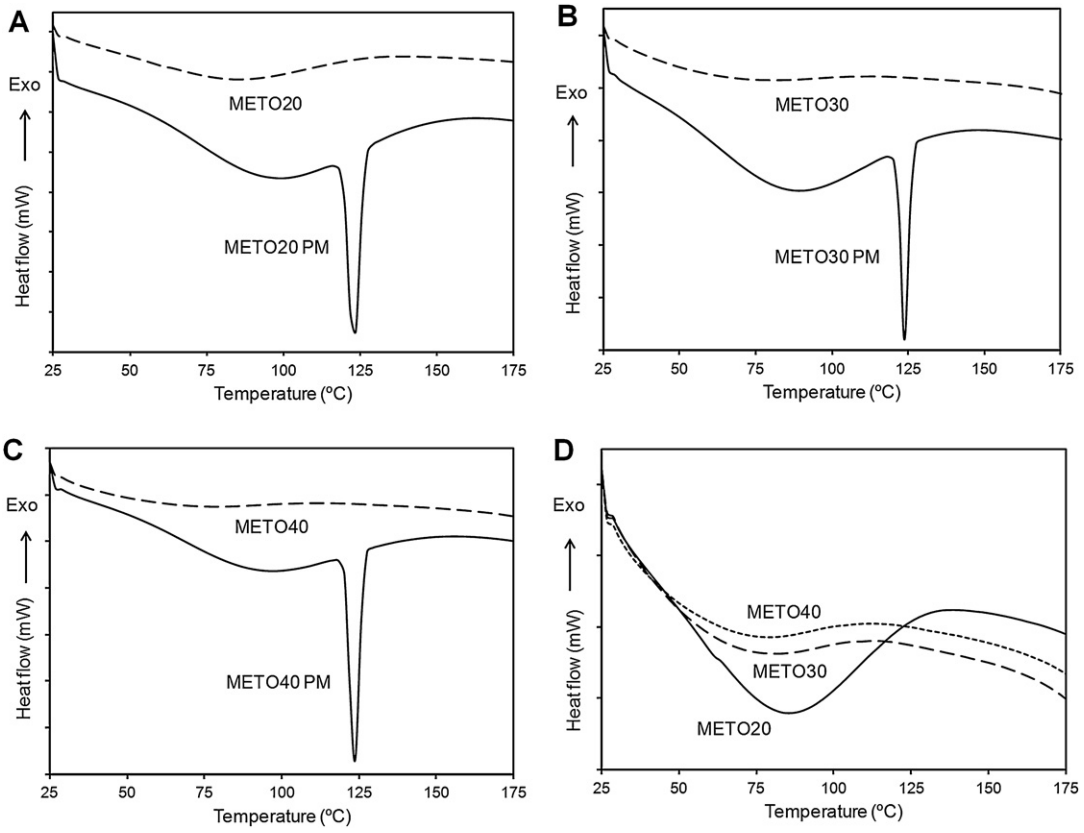

Fig. 4. DSC curves of the NFC microparticles containing metoprolol (A) METO20, (B) METO30, (C) METO40 compared to the corresponding physical mixtures (PM) and (D) and comparison of curves METO20, METO30, and METO40.

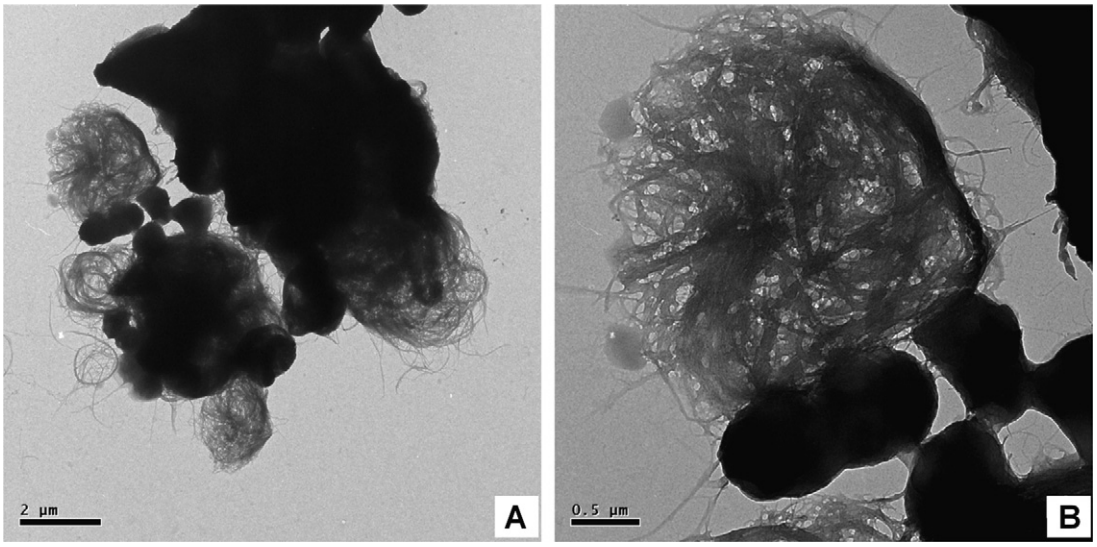

Fig. 5. TEM images of the NFC microparticles containing indomethacin (INDO20) after the unbound fraction was released imaged with lower (A) and higher (B) magnification.

had dried separately. The unbound fraction consists of the drug that had either spray dried separately or loosely bound drug that was released within the first 30 min in the dissolution medium.

Final loading presents the amount of drug that had been either bound to the NFC by hydrogen bonding or physically entrapped inside of the particles. Details of the drug loadings for each batch are given in Table 4. Indomethacin microparticles had the highest final loading, in the range from 6.1% to 15.1%, while in the case of metoprolol tartrate and verapamil hydrochloride particles these values were between 4.5% and 8.2%. Powders containing those three drugs were chosen for further studies. Particles containing ibuprofen had the lowest final loading of 1.7% due to the combination of high inlet temperature and low melting point of ibuprofen (Table 3), that caused high portion of the drug to melt and adhere to the walls. Drug solubility plays an important role in precipitation during drying, but the main difference in the final loadings was most probably due to the different affinity of the drugs to the cellulose fibers. It has been shown that drugs may bind directly to the surface of cellulose crystals, and that the ionic interactions, as well as the choice of dispersion medium, have important roles in the binding process (Jackson et al., 2011). Further studies on pH dependent drug binding to the NFC are needed for better understanding of this phenomenon.

The unbound fraction was relatively high (Table 4) and presents the fraction of drug that was spray-dried separately as well as the weakly bound fraction that was easily accessible to water. Fig. 5 shows particle appearance after the unbound drug fraction was released. The shown particles were loaded with indomethacin (INDO20) and the unbound fraction in this case was 73.2% of the total loaded drug amount (Table 4). The figure shows that certain portions of the particles appear to have a highly porous fibrous structure after they have released the weakly bound drug fraction, while there still are darker regions with presumably tightly bound drug material.

3.4. Drug release studies

The drug release studies were performed from the particles after the unbound drug fraction was removed. The releasing curves for all the tested batches are shown in Fig. 6. The curves are characterized by a burst phase within the first 10–14 days.

After this burst phase in release, the dissolution profiles are characterized by an extremely slow release rate that is a consequence of releasing the tightly bound fraction of the drug. Dissolution curves were fitted to a mathematical model describing the drug releasing kinetics from a spherical matrix developed by Baker and Lonsdale (1974). The dissolution rate is given by the equation:

$$\frac{3}{2}\left[1-\left(1-\frac{M_t}{M_\infty}\right)^{2/3}\right]-\frac{M_t}{M_\infty}=\frac{3D_{\mathrm{m}}C_{\mathrm{ms}}}{r_0^2C_0}t \quad (1)$$

where M_t is the released drug amount at time t and M_∞ is the amount of drug released at an infinitive time, D_{m} is the effective diffusion coefficient of the drug inside the particle, C_{ms} is the drug solubility in the matrix, r_0 is the radius of the spherical matrix and C_0 is the initial concentration of drug in the matrix. Eq. (1) can be modified as following:

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

where the release constant k, corresponds to the slope of the curves obtained when the left side of the equation is plotted against time (Fig. 7). As the diffusion coefficients of the small molecular weight drugs and the NFC microparticle sizes obtained here were quite similar, it can be assumed that the most important factors affecting k and, thus, the dissolution rate, are loading (C_0) and solubility of the drug (C_{ms}). The diffusion coefficient could be indirectly affected by binding of the drug molecules to the fibrous matrix, which would reduce the effective diffusion coefficient.

In the case of metoprolol tartrate, the release profiles in Fig. 7 consist of two distinctive parts. The first two week part shows faster drug release than the latter testing period. This is probably due to the release of a drug fraction located close to the particle surface or a loosely bound fraction. After this amount has been released, a pronounced reduction in the drug release rate could be seen. During both periods, the drug was released by diffusion through the matrix system, as is indicated by the linear profiles when analyzed with Eq. (2). As the solubility of the drug and the loading did not change during dissolution, the difference must come from different binding characteristics. This indicates that some metoprolol tartrate fractions have been encapsulated differently. The drug released in the latter part could be more physically bound inside the matrix, and the faster released fraction would correspond to a fraction bound by hydrogen bonding near the particle surfaces.

The two-phase phenomenon was not visible in particles containing verapamil hydrochloride or indomethacin. Constant slope in Fig. 7 is seen throughout the dissolution process. The difference

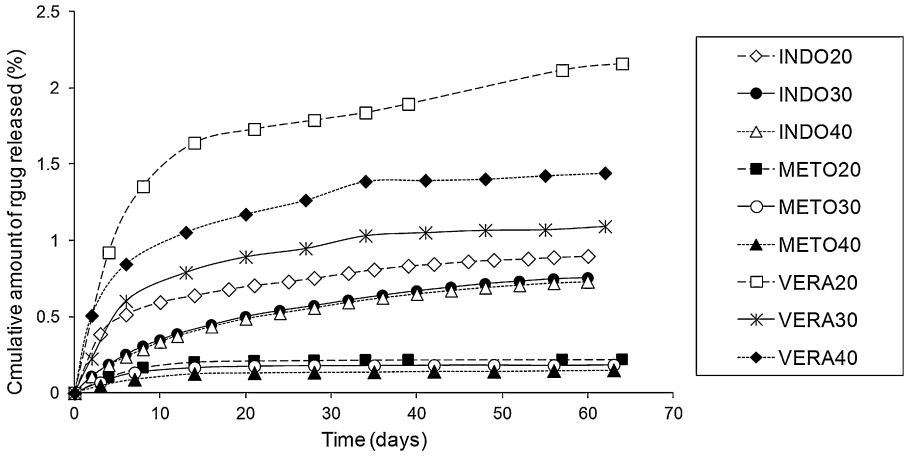

Fig. 6. Drug release curves of indomethacin, metoprolol tartrate and verapamil hydrochloride loaded in the NFC microparticles.

in the shape of the dissolution curves could be a consequence of different solubility of the drugs into the dissolution medium and/or binding to the cellulose fibers. Indomethacin has lower solubility, which causes slower release of the loosely bound fraction. Metoprolol tartrate, on the other hand, had much lower overall released amounts than the other two drugs, which might indicate that it was bound more strongly to the fibrous structure of the NFC particles.

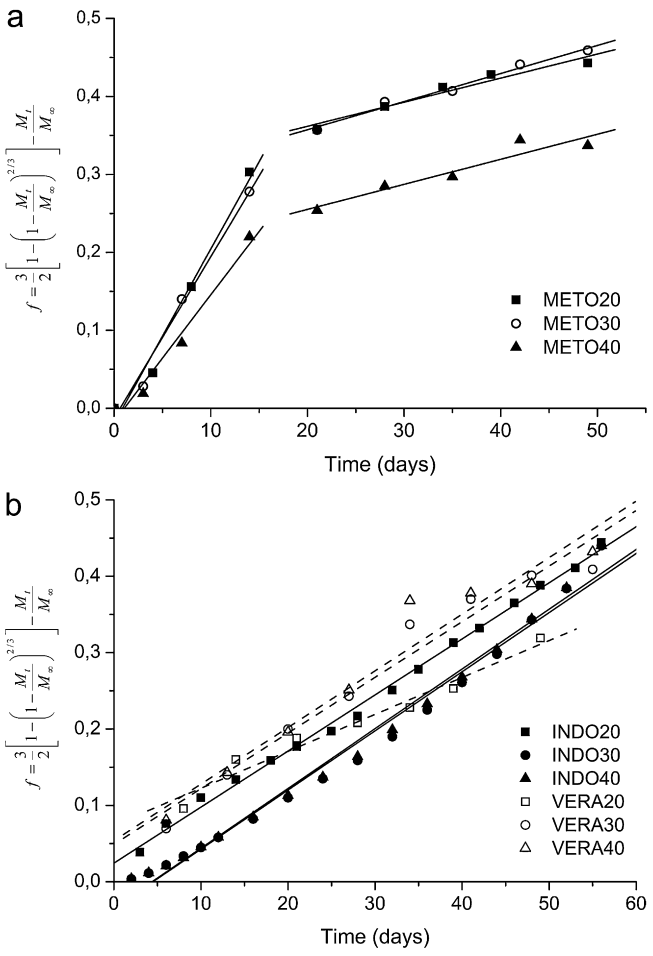

Fig. 7. Plots of a function of dissolved fraction of the drug as per Eq. (2) against the dissolution time for (A) metoprolol and (B) indomethacin and verapamil. Linear regions indicate diffusion controlled release and the lines are best fits to the data points.

Table 5
Values of coefficients of determination and the slopes from the fits shown in Fig. 7 for spray dried particles containing indomethacin, metoprolol tartrate and verapamil hydrochloride.

Sample fitted	r^2	Slope ($k \times 10^4$)
INDO20	0.993	78
INDO30	0.985	78
INDO40	0.985	79
METO20 1st	0.980	223
METO20 2nd	0.923	39
METO30 1st	0.986	207
METO30 2nd	0.921	48
METO40 1st	0.976	162
METO40 2nd	0.944	35
VERA20	0.964	48
VERA30	0.962	73
VERA40	0.950	74

Table 5 gives the values of the coefficients and slopes for all the batches. The release rate constant k for all the indomethacin and verapamil hydrochloride samples had similar values, which indicates that drug loading did not influence the diffusion rate. Further, the solubility of the drug in the dissolution medium did not primarily determine the diffusion rate, since the verapamil hydrochloride samples had significantly higher k values than METO20, and both the drugs are freely soluble in water (Table 4). Also, the solubility of indomethacin is very low compared to verapamil hydrochloride and metoprolol tartrate, but indomethacin dissolution rate was almost the same as with verapamil hydrochloride. Therefore, the difference in the diffusion rate and, hence, in the dissolution rate, is most probably due to the different affinities of the drugs to the NFC.

4. Conclusions

Drug loaded microparticles with NFC as matrix structure former were produced by spray drying method. This led to the production of microparticles with diameters of approximately 5 μm, in which the drugs existed mainly in an amorphous form. A portion of the drug was dried separately or located at the particle surface, which affected the final drug loading. Drug release studies showed sustained drug release profiles over a period of two months. The drug release curves were fitted to a mathematical model describing the release kinetics from a spherical matrix. The fittings showed that different drugs have different release kinetics, which is a consequence of the combination of several factors including different solubilities of the drugs in the chosen medium and different affinities of the drugs to NFC. As a conclusion, NFC microparticles can

sustain drug release by forming a tight fiber network and thus limit drug diffusion.

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