NANOFIBRILLAR CELLULOSE IN CONTROLLED DRUG RELEASE FORMULATIONS

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New materials for stabilization - Nanofibrillar cellulose (NFC)

Hierarchical structure of wood biomass and the characteristics of cellulose microfibrils

Adapted from Isogai et al., Nanoscale, 2011
Surface area and morphology allow efficient binding of drugs and drug nanoparticles.

Nanofibrillar cellulose - morphology

High water uptake
Gel at ca. 1% solid content

Huge aspect ratios (10 nm thickness vs. 1 µm length) → huge surface areas

Different thicknesses & chemical modifications possible

Non-modified NFC
Modified NFC

Surface area and morphology allow efficient binding of drugs and drug nanoparticles.
Sustained release

• Certain therapies require constant drug release rate for extended time periods
  – Oral administration: 4 - 12 hours
  – Implants: days to months

• Important aspects
  – Control of burst release
  – Constant release rate
  – Processing
NFC matrices for sustained drug release

Release systems produced either by spray-drying or filtration

Fiber width ca. 4-20 nm

“Hornification”

R. Kolakovic et al., Int. J. Pharm., 2012.
NFC matrices for sustained drug release

Efficient drug entrapment into the fibrous matrix - Intact drug particles

## Production efficiency

<table>
<thead>
<tr>
<th>Batch</th>
<th>Production loss (%)</th>
<th>Drug surface fraction (%)</th>
<th>Calculated final loading (%)</th>
<th>Final loading based on DSC measurements (%)</th>
<th>Entrapment efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INDO20</td>
<td>1.1</td>
<td>14.0</td>
<td>17.7</td>
<td>17.1</td>
<td>88.5</td>
</tr>
<tr>
<td>INDO30</td>
<td>0.9</td>
<td>10.7</td>
<td>27.7</td>
<td>28.0</td>
<td>92.3</td>
</tr>
<tr>
<td>INDO40</td>
<td>0.8</td>
<td>11.7</td>
<td>37.0</td>
<td>35.7</td>
<td>92.5</td>
</tr>
<tr>
<td>ITRA20</td>
<td>&lt;0.1</td>
<td>1.5</td>
<td>19.8</td>
<td>19.6</td>
<td>99.0</td>
</tr>
<tr>
<td>ITRA30</td>
<td>&lt;0.1</td>
<td>1.2</td>
<td>29.8</td>
<td>28.1</td>
<td>99.3</td>
</tr>
<tr>
<td>ITRA40</td>
<td>&lt;0.1</td>
<td>2.0</td>
<td>39.5</td>
<td>38.4</td>
<td>98.8</td>
</tr>
<tr>
<td>BECLO20</td>
<td>&lt;0.1</td>
<td>16.6</td>
<td>17.3</td>
<td>17.2</td>
<td>86.5</td>
</tr>
<tr>
<td>BECLO30</td>
<td>&lt;0.1</td>
<td>14.8</td>
<td>26.8</td>
<td>26.4</td>
<td>89.3</td>
</tr>
<tr>
<td>BECLO40</td>
<td>&lt;0.1</td>
<td>28.2</td>
<td>32.4</td>
<td>31.2</td>
<td>81.0</td>
</tr>
</tbody>
</table>
Sustained release from NFC matrices

Sustained release for up to 100 days

Matrix remains intact during the dissolution

Matrix after dissolution
## Drug interactions with NFC

<table>
<thead>
<tr>
<th>Drug</th>
<th>Medium</th>
<th>Diffusion coefficient (cm²/s)</th>
<th>Bound amount (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beclomethasone dipropionate</td>
<td>1% HPBC</td>
<td>0.602x10⁻⁷</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>1% HPBC/pH 1.2</td>
<td>0.601x10⁻⁷</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>1% HPBC/pH 7.4</td>
<td>0.738x10⁻⁷</td>
<td>0.029</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>MQ</td>
<td>2.128x10⁻⁷</td>
<td>No binding</td>
</tr>
<tr>
<td></td>
<td>Phosphate buffer pH 5</td>
<td>1.710x10⁻⁷</td>
<td>No binding</td>
</tr>
<tr>
<td></td>
<td>Phosphate buffer pH 7.4</td>
<td>2.540x10⁻⁷</td>
<td>No binding</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.03M NaCl/pH 1.2</td>
<td>1.548x10⁻⁷</td>
<td>0.003</td>
</tr>
<tr>
<td>Nafarelin acetate</td>
<td>MQ</td>
<td>1.541x10⁻⁷</td>
<td>2.940</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>MQ</td>
<td>0.930x10⁻⁸</td>
<td>1.760</td>
</tr>
</tbody>
</table>

- Diffusion coefficients in NFC are molecule size dependent and ca. 10 times smaller than in water.
- Binding to NFC is dependent on the charge of the drug/NFC.
- Drug release is largely diffusion-limited.

Drug diffusion studies from anionic NFC hydrogels combined with freeze-drying

- Does the freeze-drying process affect drug release?
- Can the product be rehydrated with no change in rheology?
- Background information about the FibDex™ product
- 3% and 6.5% ANFC hydrogels were evaluated

Paukkonen et al., manuscript in preparation.
Freeze-drying and morphology

- Highly porous aerogel structure through freeze drying
- Excipients were used to prevent NFC hornification and agglomeration
  - Trehalose: cryoprotection through water-trehalose hydrogen-bond replacement
  - PEG6000: prevents the formation of insoluble aggregates, lowers surface tension of water, improves rehydration

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mass change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFC 6.5 %</td>
<td>6.77</td>
</tr>
<tr>
<td>NFC 6.5 % (exp)</td>
<td>4.11</td>
</tr>
<tr>
<td>ANFC 3 %</td>
<td>7.48</td>
</tr>
<tr>
<td>ANFC 6.5 % (exp)</td>
<td>5.81</td>
</tr>
<tr>
<td>BSA 1 % / ANFC 3 % (exp)</td>
<td>4.79</td>
</tr>
<tr>
<td>BSA 1 % / ANFC 6.5 % (exp)</td>
<td>6.05</td>
</tr>
<tr>
<td>MZ 2 % / ANFC 3 % (exp)</td>
<td>2.42</td>
</tr>
<tr>
<td>MZ 2 % / ANFC 6.5 % (exp)</td>
<td>4.98</td>
</tr>
<tr>
<td>NAD 1.7 % / ANFC 3 % (exp)</td>
<td>2.90</td>
</tr>
<tr>
<td>NAD 1.7 % / ANFC 6.5 % (exp)</td>
<td>4.79</td>
</tr>
<tr>
<td>KETO 3.4 % / ANFC 3 % (exp)</td>
<td>1.96</td>
</tr>
<tr>
<td>KETO 3.4 % / ANFC 6.5 % (exp)</td>
<td>3.84</td>
</tr>
</tbody>
</table>
Rheological properties

Comparing hydrogels to rehydrated hydrogels (FD)

- Frequency measurements showed broken fiber network structure on Nadolol (no effect on other APIs)
- Crosslinking with cations increases the viscosity (but not drug release rate)
Drug release

<table>
<thead>
<tr>
<th>Compound*</th>
<th>Diffusion coefficients ($10^{-8} \text{ cm}^2/\text{s}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3% NFC</td>
</tr>
<tr>
<td>Ketoprofen (-)</td>
<td>53.9</td>
</tr>
<tr>
<td>NAD (+)</td>
<td>286.24</td>
</tr>
<tr>
<td>Metronidazole (ø)</td>
<td>716.99</td>
</tr>
<tr>
<td>BSA (-)</td>
<td>9.28 (± 2.46)</td>
</tr>
<tr>
<td>Lysozyme (+)</td>
<td>3.53 (± 0.31)</td>
</tr>
<tr>
<td>4 kDa FITC-dextran (ø)</td>
<td>59.44 (± 11.23)</td>
</tr>
</tbody>
</table>

For comparison:
- $D$ in water for LZ is $95 \times 10^{-8} \text{ cm}^2/\text{s}$ and for BSA is $46 \times 10^{-8} \text{ cm}^2/\text{s}$.
- For small molecules $D$ in water is typically $600 - 900 \times 10^{-8} \text{ cm}^2/\text{s}$.
Poorly soluble drugs

• Even up to 70% of new drug entities in pharmaceutical industry research pipelines are poorly soluble in water.

• Drug nanocrystals to improve the dissolution rate the most direct approach available
New materials for stabilization - Hydrophobins

Traditional surfactants can be replaced with a naturally occurring protein, hydrophobin (HFBI or HFBII, class II)

Small protein (7.5 kDa, 2 nm) with several aliphatic amino acid residues concentrated in a single patch

- Surfactant-like properties
- Water dispersible

Forms strong films on hydrophobic substrates
Preparation of nanoparticles

Bottom-up anti-solvent precipitation

1) Drug dissolved in a water miscible organic solvent

2) Sudden oversaturation in water causes precipitation

3) HFB proteins in water stop particle growth at 50-200 nm

H. Valo et al., ACS Nano, 2010
Itraconazole nanoparticles with HFBII coating

Nanoparticle production from poorly soluble drugs was simple.
Dissolution rate considerably improved.

Surface functionalization with protein-cellulose adhesion

CBD-HFBI fusion protein can be used to bring cellulose to a w/o interface

Similarities to a drug/water interface

Functionalization of the nanoparticles – Cellulose binding

Cellulose binding domain acts as a “bio-gluе” between the cellulose nanofibers and drug nanoparticles

Functionalization of the nanoparticles – Cellulose binding

Increased stability (3-14 days $\rightarrow$ 1 year).
No effect on dissolution rate by NFC.
HFB-NFC formulations - *in vivo* performance

Huge improvement in bioavailability, even higher than the positive control.

H. Valo et al., *J. Controlled Release*, 2011
Nanofibrillar cellulose - sources

Other sources besides trees exist

- Fruits (quince, red pepper, strawberry, etc.)
- Bacterial sources (Nata-de-Coco)

Fiber diameter 5-7 nm, bundles 20 nm

3 nm fibers

HFB-NFC formulations
- Role of the cellulose source

Source and modification of the cellulose makes a difference on the release profiles
→ Immediate or sustained release possible

Fruit cellulose  Bacterial cellulose  TEMPO oxidized wood cellulose

Drug releasing emulsions

- Drug releasing HFBII-NFC o/w-emulsions were evaluated for
  - Stability
  - Drug release rate of poorly water soluble drugs
  - The effect of the two different NFC grades, native (non-charged) and oxidized (anionic).

- HFBII stabilized the o/w interface and NFC was added to modify the viscosity of the continuous phase by a polymer network that limits drop collision.
HFBII as a surfactant in emulsion stabilization

- Ultrasound sonication at 10% oil phase and 90% aqueous phase with stabilizers.
- The minimum amount of the protein needed to gain stable emulsion droplets with the sonication method was 0.1% HFBII → extremely low compared to 10% oil phase

<table>
<thead>
<tr>
<th>Formulation (HFBII %)</th>
<th>Day 1: Diameter (nm)</th>
<th>Day 28: Diameter (nm)</th>
<th>Day 1: PDI</th>
<th>Day 28: PDI</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS-1 (0.01)</td>
<td>2013 ± 33</td>
<td>4623 ± 1879</td>
<td>0.517 ± 0.014</td>
<td>1 ± 0</td>
<td>25 ± 0.6</td>
</tr>
<tr>
<td>HS-2 (0.05)</td>
<td>537.6 ± 11.7</td>
<td>696.8 ± 208.4</td>
<td>0.415 ± 0.028</td>
<td>0.492 ± 0.219</td>
<td>24.8 ± 0.2</td>
</tr>
<tr>
<td>HS-3 (0.1)</td>
<td>339.0 ± 1.6</td>
<td>324.9 ± 39.0</td>
<td>0.440 ± 0.012</td>
<td>0.349 ± 0.035</td>
<td>31.4 ± 1.0</td>
</tr>
<tr>
<td>HS-4 (0.125)</td>
<td>301.4 ± 7.3</td>
<td>194.1 ± 0.9</td>
<td>0.443 ± 0.024</td>
<td>0.144 ± 0.027</td>
<td>30.2 ± 1.0</td>
</tr>
<tr>
<td>HS-5 (0.15)</td>
<td>345.0 ± 4.7</td>
<td>183.6 ± 1.3</td>
<td>0.520 ± 0.017</td>
<td>0.129 ± 0.006</td>
<td>32.7 ± 1.1</td>
</tr>
</tbody>
</table>
Emulsion stability index (ESI)

• The stability of the emulsions was evaluated by following the emulsion phase separation for 1 month.

\[ ESI = 1 - \frac{V_{w,\text{separated}}}{V_{\text{total}}} \]

• Higher NFC and HFB concentrations stabilized the emulsions more effectively.

• The best stability could be achieved by combining HFBII with adequate amounts of NFC-N or NFC-O.
Morphology of the emulsions

- Stabilization of emulsions was confirmed with cryo-TEM.

Emulsion droplets had a wide size distribution from below 100 nm to few micrometers.

HFBII-NFC-O formulations were more stable upon dilution than emulsions done with HFBII-NFC-N.
Drug release from NFC-stabilized emulsions

- **HFBII** controlled the drug release to some extent, but it was beneficial to add NFC into the formulations to gain well defined release profiles.

- Drug release from HFBII-NFC-N formulations was close to an immediate release profile.

- Sustained drug release profiles from HFBII-NFC-O formulations.

- The amount of NFC-N or NFC-O did not affect significantly the drug release rates, but the grade of NFC fibers did.
Conclusions

- Nanofibrillar cellulose looks like a promising material for controlled drug release applications.
- Sustained release profiles are possible due to efficient entrapment and slower mass transport (and binding) in NFC matrices.
- Drug nanocrystals for immediate release of poorly soluble drugs can be entrapped and stored in NFC hydrogels and aerogels.
- Freeze-drying can be used to dry the NFC hydrogels with minimal changes upon rehydration.
- Generally-Regarded-as-Safe.
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