Hydrophobin-nanofibrillated cellulose stabilized emulsions for encapsulation and release of BCS class II drugs

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ABSTRACT

The purpose of this study was to construct biopolymer-based oil-in-water emulsion formulations for encapsulation and release of poorly water soluble model compounds naproxen and ibuprofen. Class II hydrophobin protein HFBII from *Trichoderma reesei* was used as a surfactant to stabilize the oil/water interfaces of the emulsion droplets in the continuous aqueous phase. Nanofibrillated cellulose (NFC) was used as a viscosity modifier to further stabilize the emulsions and encapsulate protein coated oil droplets in NFC fiber network. In our previous study it has been shown that hydrophobin proteins and NFC can be used successfully to stabilize oil-in-water emulsions (Varjonen et al., Soft Matter 7, 2011, 2402-2411). In this study the use of these materials has been extended for stabilization of pharmaceutical emulsions. The potential of both native and oxidized NFC were studied for this purpose. Various emulsion formulations were prepared and the abilities of different formulations to control the drug release rate of naproxen and ibuprofen, used as model compounds, were evaluated. The optimal formulation for sustained drug release consisted of 0.01% of drug, 0.1% HFBII, 0.15% oxidized NFC, 10% soybean oil and 90% water phase. By comparison, the use of native NFC in combination with HFBII resulted in an immediate drug release for both of the compounds. The results indicate that these NFC originated biopolymers are suitable for pharmaceutical emulsion formulations. The native and oxidized NFC grades can be used as emulsion stabilizers in sustained and immediate drug release applications. Furthermore, stabilization of the emulsions was achieved with low concentrations of both HFBII and NFC, which may be an advantage when compared to surfactant concentrations of conventional excipients traditionally used in pharmaceutical emulsion formulations.

Keywords

1   Nanofibrillated cellulose; Hydrophobin; Emulsions; Drug release; Ibuprofen; Naproxen

2   **Abbreviations**

3   **BC**, bacterial cellulose; **BCS**, biopharmaceutical classification system; **DLS**, dynamic light

4   scattering; **GIT**, gastrointestinal tract; **HFBII**, class II hydrophobin protein HFBII; **IBU**, ibuprofen;

5   **NAP**, naproxen; **NFC**, nanofibrillated cellulose; **NFC-N**, native nanofibrillated cellulose; **NFC-O**,

6   TEMPO oxidized nanofibrillated cellulose; **PDI**, polydispersity index; **pI**, isoelectric point

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8   **1. Introduction**

9   Oral administration route is the preferred drug delivery route for chronic therapy due to its high patient

10   compliance and ease of administration when compared to the parenteral routes. The bioavailability

11   of orally administered poorly water soluble biopharmaceutical classification system (BCS) class II

12   drugs is limited by their solubility in the varying conditions of the gastrointestinal tract (GIT) (Khadra

13   et al., 2015). Consequently this may result in poor bioavailability. Several formulation strategies have

14   been developed to tackle the solubility limitation including soluble complexes, emulsions and self-

15   emulsifying systems (Fahr, 2007;Singh et al., 2011). In lipid based drug delivery systems the drug

16   molecules are solubilized in the formulation, which is beneficial for consistent bioavailability of

17   poorly water soluble drugs. Emulsion formulation strategies have been used to increase the aqueous

18   solubility of poorly soluble drugs and to control the required bioavailability of these drugs (Gao et

19   al., 1998;Gorain et al., 2014;He et al., 2011).

20   Emulsions are widely used as pharmaceutical dosage forms as the emulsified materials may obtain

21   advantages such as improved bioavailability and masking of poor organoleptic properties when

22   compared to other dosage forms. Typically pharmaceutical emulsions are used for oral, topical or

23   intravenous administration routes. However, emulsions are thermodynamically unstable and

24   therefore must be stabilized by addition of an emulsifying agent. Several emulsifying agents can be

25   used depending on the type of emulsion that is produced (Strickley, 2004). The use of traditional

26   pharmaceutical emulsifiers may be limited by their concentration dependent toxicity (Davis et al.,

27   1970;Gloxhuber, 1974). In general, orally administered nonionic and anionic surfactants are low in

28   toxicity but compatibility with skin and mucous membranes is concentration dependent (Gloxhuber,

29   1974).

30   Typically used nonionic surfactants in pharmaceutical dosage forms include Tweens, Spans,

31   Cremphors and Pluronic block copolymers. However, these compounds may interfere with the

absorption of certain drugs due to the inhibition of various efflux pumps and transporters present in the GIT (Rege et al., 2001;Rege et al., 2002;Woodcock et al., 1992). The drug absorption may be increased if the surfactants affect the functionality of efflux pumps. This may affect the desired bioavailability levels of certain drug compounds that are substrates for efflux pumps or transporters in the GIT. In food industry proteins in addition to emulsifiers are widely used in emulsion stabilization (Wilde et al., 2004). Proteins stabilize emulsions due to their interfacial properties and the ability to form viscoelastic layers at interfaces. Natural biopolymers, such as proteins, offer an alternative to synthetic excipients used in the stabilization of pharmaceutical emulsions.

Development of nanotechnology has enabled the production of nanocellulose from the natural polymer cellulose. Nanocellulose term applies to nanofibrillated cellulose (NFC), bacterial cellulose (BC) and cellulose nanocrystals (Klemm et al., 2011). Plant materials such as wood pulp are commonly used starting materials for NFC preparation. Typically the production includes mechanical fibrillation of native cellulose before and/or after enzymatic or chemical pretreatment. The diameter of native NFC (NFC-N) fibers is typically 5-60 nm and the length is several micrometers. The NFC-N surface chemistry, hemicellulose content, fiber dimensions and the proportions of crystalline and amorphous regions may vary depending on the origin of cellulose as well as nanofibrillation processing (Klemm et al., 2011;Lin and Dufresne, 2014). NFC-N from plant materials may have hemicelluloses and lignin as accompanying impurities on the surface of the fibrils (Fall et al., 2011). Hemicellulose contains carboxyl groups and on the surface of NFC-N it introduces a surface charge to the NFC fibers due to the deprotonation of carboxyl groups.

Cellulose can be generally considered to be biocompatible due to moderate, if any, foreign responses in vivo (Märtson et al., 1999;Miyamoto et al., 1989). Additionally, NFC has been shown to be nontoxic in various *in vitro* cell models (Hannukainen et al., 2012;Hua et al., 2014;Vartiainen et al., 2011). Plant derived NFC-N and BC have been used in various biomedical applications such as drug delivery, skin tissue repair and tissue bioscaffolds for cellular culture (Kolakovic et al., 2012;Kolakovic et al., 2013;Malinen et al., 2014;Rouabhia et al., 2014). Plant derived native NFC-N is a promising matrix material for controlled drug release applications due to its high surface area to its volume ratio and the multiple carboxyl binding sites on the surface of NFC-N fibers that can interact with drug molecules via electrostatic interactions (Kolakovic et al., 2013). The electrostatic binding of molecules with carboxyl groups present on the surface of NFC-N is pH dependent. Electrostatic interactions between negatively charged carboxyl groups present in NFC-N and positively charged drugs has been seen as the main binding mechanism for these drugs (Kolakovic et al., 2013). However, drug interactions with NFC driven by other mechanisms such as aromatic π

stacking and hydrogen bonding might still take place regardless of the charge of the molecule. The surface of NFC can be activated via TEMPO [(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl] oxidation to contain negatively charged carboxyl groups as binding sites for various macromolecules such as peptides and enzymes (Weishaupt et al., 2015). This approach enables the immobilization of drugs and macromolecules by electrostatic interactions due to the high density of negative charges on the surface of TEMPO oxidized NFC (NFC-O). The NFC-O fibers have typically 3-4 nm diameter and the length in the range of few micrometers (Saito et al., 2006;Saito et al., 2007). Furthermore, NFC-O has been shown to be nontoxic in *in vitro* cell models (Alexandrescu et al., 2013;Hua et al., 2014).

Hydrophobin proteins (HFBs) are a group of small about 10 kDa amphiphilic fungal proteins that are capable of lowering the interfacial tension by self-assembly into monolayers at hydrophobic/hydrophilic interfaces such as air-water (a/w) and oil-water (o/w) (Linder et al., 2005;Linder, 2009;Paananen et al., 2013). Due to this property HFBs have the ability to convert hydrophobic surfaces into hydrophilic ones and vice versa (Lumsdon et al., 2005;Szilvay et al., 2007). HFBs are nontoxic by themselves which is a prerequisite for pharmaceutical excipients (Ebbole, 1997). Additionally, a surface layer of HFB on the airborne fungal spores has been shown to prevent immune recognition (Aimanianda et al., 2009). This is an attractive feature for materials in pharmaceutical applications. Based on differences in biophysical properties and hydropathy patterns HFBs are divided into class I and class II HFBs (Linder, 2009). Class II *Trichoderma reesei* HFBs (e.g., HFBI, HFBII and HFBIII) are more water soluble than class I HFBs and can be easily dissolved at rather high concentrations (at least 100 mg/ml) in aqueous solutions (Kisko et al., 2008;Linder et al., 2005). The amphiphilic nature of HFBs is attributed to the existence of a hydrophobic patch on the surface of otherwise fairly hydrophilic surface of HFB monomers (Linder et al., 2005). The overall structure therefore resembles the structure of surfactants with one hydrophobic and one hydrophilic part. The amphiphilicity makes HFBs suitable surface coating materials for pure drug crystals as well as drug carrier materials. HFBs have been successfully used as surfactants, encapsulating agents for nutraceuticals and coatings for drug nanoparticles (NPs) (Israeli-Lev and Livney, 2014;Sarparanta et al., 2012;Valo et al., 2010). HFBII coating on the surface of drug NPs has been shown to introduce mucoadhesive properties to drug NPs in the GIT (Sarparanta et al., 2012). For poorly water soluble drugs, mucoadhesive formulations are beneficial for improving drug bioavailability due to the prolonged time at the site of drug absorption.

Recently NFC has been used in combination with HFBs to produce emulsions, stabilize drug nanocrystals and to produce aerogel matrices for HFB coated drug NPs (Valo et al., 2011;Valo et al., 2013;Varjonen et al., 2011). It has been shown in our previous study that HFBs and NFC can be used

successfully to stabilize o/w emulsions (Varjonen et al., 2011). Surface active HFB fusion proteins that contained cellulose-binding domains from cellulolytic enzymes were used. By combining the fusion protein HFBs and NFC emulsions that contained tightly packed thin NFC films at the oil/water interface were obtained. In this study *Trichoderma reesei* HFBII and NFC were used for the stabilization of pharmaceutical emulsions. Due to the interesting surface properties, low toxicity and good biocompatibility of both NFC and HFBII, these materials were selected for the production of o/w emulsion formulations.

The emulsions were used for the encapsulation and release of poorly water soluble BCS II drugs naproxen (NAP) and ibuprofen (IBU). HFBII was used as a surfactant to stabilize the o/w interface of the emulsions, whereas NFC was added to modify the viscosity of the continuous aqueous phase. The addition of NFC polymer increases the viscosity of the continuous phase in o/w emulsions as well as w/o/w emulsions and creates a polymer network that limits drop collision, thus reducing the coalescence phenomena (Carrillo et al., 2015;Dimic-Misic et al., 2013;Garti, 1999;Xhanari et al., 2011). Furthermore, the bending of hydrophilic NFC at the o/w interface favors positive curvature towards the organic oil-phase and results in water continuous o/w emulsions (Binks, 2002). These properties were exploited for emulsion stabilization. The effect of the amount of NFC, HFBII and their combinations on the emulsion stability was studied. After the preliminary formulation optimization, the most suitable formulations were selected for the drug release studies. The two different grades of NFC, native and oxidized, were expected to induce differences in the drug release profiles.

2. **Materials and methods**

2.1 Materials

Hydrophobin class II protein HFBII from *Trichoderma reesei* was provided by VTT Technical Research Centre of Finland, Finland. Native (Growdex® 1.73%) and oxidized (0.56%) nanofibrillated cellulose hydrogels (NFC-N and NFC-O, respectively) were kindly provided by UPM-Kymmene Corporation, Finland. Cellulose kraft pulp was chemically modified and fibrillated to form NFC hydrogels (Saito et al., 2006;Saito et al., 2007). Due to birch based raw material the NFC-N used in this work contained some soluble hemicelluloses. This hemicellulose fraction attributes to the slightly anionic surface charge of NFC-N. Higher negative charge of NFC-O is generated by the carboxyl groups directly on the fiber surface (carboxylic acid content 1.02 mmol/g pulp). 5 mg/ml stock solutions of HFBII and NFC-N were prepared in ultrapure water. NFC-O was used at the provided concentration of 5.6 mg/ml. Soybean oil (batch no. MKBN8800V) and ethyl oleate (batch no.

BCBL9739V) were purchased from Sigma-Aldrich, USA and UK respectively. NAP and IBU were purchased from Sigma-Aldrich, USA and China respectively. 1 mg/ml stock solutions of IBUand NAP were prepared in soybean oil. Solubility of IBUand NAPin soybean oil was confirmed by nephelometry (data not shown) (Nepheloscan Ascent, Labsystems, Finland). Acetonitrile was of analytical grade, Sigma-Aldrich, Germany. Phosphate salts used in the preparation of 0.1 M pH 7.4 phosphate buffer were of analytical grade, Sigma-Aldrich, Germany. Whatman 0.4 µm pore size Track-Etch polycarbonate membranes were used as semipermeable membranes in the diffusionstudies, Sigma-Aldrich, Germany.

2.2 Methods

2.2.1. Emulsion preparation method

Emulsions were prepared from combinations of ultrapure water, soybean oil, ethyl oleate, HFBII 5 mg/ml stock solution, NFC-N 5 mg/ml stock solution and NFC-O 5.6 mg/ml stock solution. To prevent aggregation in an aqueous environment, HFBII stock solution was sonicated first for 10 minutes before preparing the emulsions. All formulations were premixed by repeated pipetting in eppendorf tubes prior to ultrasound-sonication. The final emulsion volume was 1 ml for all emulsion formulations and the volume of the oil phase was 10%. The ultrasound-sonication based method for the preparation of emulsions was described in our previous study (Varjonen et al., 2011). The same method was used in this study with minor modifications. The sonication was performed with a high intensity ultrasound processor (Sonics, USA) equipped with a 2 mm stepped microprobe (power 750 W, frequency 20 kHz and amplitude 30%). During 4 x 30 seconds of sonication the samples were kept in an ice bath. After the sonication the samples were homogenized in an ultrasonic bath for 1 hour. For high-shear mixing tests, a process time of 1-4 minutes was used (25 000rpm, Ultra-Turrax T8, IKA WERKE, Germany). In NAP and IBU formulations the drug was dissolved in soybean oil in 1 mg/ml concentration prior to the emulsion preparation. Table 1 summarizes the different formulations and their specific contents.

1 **Table 1.** Emulsion formulation codes and contents. NAP and IBU containing formulations were
2 prepared based on preliminary formulations.

Formulation code	HFBII (mg/ml)	NFC-N (mg/ml)	NFC-O (mg/ml)	Soybean oil (10 %)	Ethyl oleate (10 %)	NAP (mg/ml)	IBU (mg/ml)
C_NS-1		0.1		×			
C_NS-2		0.5		×			
C_NS-3		1		×			
C_NS-4		1.25		×			
C_NS-5		1.5		×			
C_NE-1		0.1			×		
C_NE-2		0.5			×		
C_NE-3		1			×		
C_NE-4		1.25			×		
C_NE-5		1.5			×		
HS-1	0.1			×			
HS-2	0.5			×			
HS-3	1			×			
HS-4	1.25			×			
HS-5	1.5			×			
HC$_N$S-1	1	0.5		×			
HC$_N$S-2	1	1		×			
HC$_N$S-3	1	1.5		×			
HC$_N$E-1	1	0.5			×		
HC$_N$E-2	1	1			×		
HC$_N$E-3	1	1.5			×		
HC$_O$S-1	1		0.5	×			
HC$_O$S-2	1		1	×			
HC$_O$S-3	1		1.5	×			
HC$_O$E-1	1		0.5		×		
HC$_O$E-2	1		1		×		
HC$_O$E-3	1		1.5		×		
NAP-1	1	1		×		0.1	
NAP-2	1		1	×		0.1	
NAP-3	1	1.5		×		0.1	
NAP-4	1		1.5	×		0.1	
NAP-5	1			×		0.1	
IBU-1	1	1		×			0.1
IBU-2	1		1	×			0.1
IBU-3	1	1.5		×			0.1
IBU-4	1		1.5	×			0.1
IBU-5	1			×			0.1

H = HFBII
C_N = NFC-N (native nanofibrillated cellulose)
C_O = NFC-O (TEMPO oxidized nanofibrillated cellulose)
S = soybean oil
E = ethyl oleate
IBU= ibuprofen formulation
NAP= naproxen formulation

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2.2.2. Emulsion droplet size and zeta-potential measurements

HFBII stabilized emulsions were diluted 1:10 with dilute HFBII (0.01 mg/ml in ultrapure water) solution to avoid the possible breakage of HFBII protein coated emulsion droplet o/w interface surface structures. The zeta-potential values of emulsion droplets were measured by a ZetaSizer Nano-ZS (Malvern Ltd., UK) in water at 25°C in a folded capillary cell (Malvern Ltd., UK). Dynamic light scattering (DLS) measurements for hydrodynamic diameter and size distribution of emulsion droplets were also measured by a ZetaSizer Nano-ZS (Malvern Ltd., UK) in water at 25°C in disposable polystyrene cuvettes (Sarstedt,Germany). Size measurements were performed at day 1 and at day 28 after the emulsion preparation, whereas zeta-potential measurements were performed only at day 1.

2.2.3. Emulsion stability index (ESI)

The stability of the emulsions was evaluated by following the emulsion phase separation. Qualitative emulsion stability index (ESI) values were used to evaluate the phase separation of emulsions during 1 month storage at 4°C in glass vials (Varjonen et al., 2011). Digital camera images were taken to follow the phase separation of clear aqueous phase from the opaque emulsion phase. The volume fraction of opaque emulsion phase was determined from the separation of the clear aqueous phase. The digital camera images were analyzed with ImageJ software (National Institutes of Health, Bethesda, USA). With ImageJ, a line was drawn from the top to the bottom of the emulsion in the glass vial. Maximum and minimum intensities along the line length that represented the total volume were determined in order to estimate emulsion separation. The distance values in pixels (x-axis) were plotted against gray values (y-axis). The distance values were normalized to represent the total emulsion volume (x-axis). The gray values were normalized to represent emulsion/water separation (y-axis). Above or at a value of 0.5 (at a scale of 0-1) at y-axis, the corresponding volume fraction at x-axis was considered to be unseparated opaque emulsion. Below the value of 0.5 at y-axis, the corresponding volume fraction at x-axis was considered to be separated clear aqueous phase. ESI values were calculated from the volume of separated water and total volume as follows.

$$ESI = 1 - \frac{V_{w,separated}}{V_{total}}$$

ESI value 1 corresponds to completely unseparated emulsion whereas 0 is a value for completely separated system. The ESI values were calculated at day 1, 3, 7, 14, 21 and 28 after the emulsion preparation.

2.2.4 Viscosity measurements

The shear rate viscosity of ultrapure water, 0.1 % and 0.15 % NFC-N and NFC-O was measured at 4°C with HAAKE Viscotester iQ Rheometer (Thermo Fisher Scientific, Karlsruhe, Germany) equipped with a Peltier system for temperature control. Results were analyzed with HAAKE RheoWin 4.0 software (Thermo Fisher Scientific). Cup and double-gap cylinder geometry was used with a 4.0 mm gap and 2.64 ml sample volume. Before each measurement, the samples were allowed to rest for 2 min at 4°C. Shear viscosity was measured by increasing the shear rate from 100 to 1900 1/s. All measurements were performed in triplicate.

2.2.5. Screening of oils for emulsions

The solubility of NAP and IBU in soybean oil and ethyl oleate was measured. An excess amount of NAP and IBU were added to both oils, and then mixed with magnetic stirring. After stirring for 72 h at 25 °C, the equilibrated samples were centrifuged for 10 min at 14 000 g to remove excess amount of undissolved drug. Then the supernatant was filtered through a 0.2 µm pore size Minisart syringe filter, Sartorius, Germany. Filtered supernatants were diluted first with methanol/acetonitrile (50:50) and then further with acetonitrile/phosphate buffer pH 2 (40/60). The concentrations of NAP and IBU were determined with Ultra performance liquid chromatography (UPLC) instrument Acquity UPLC (Waters, USA).

2.2.4. Cryo-TEM and light microscopy

Optical microscopy images of emulsions were taken with an optical microscope (Leica DMLB, Germany) and a microscope coupled digital camera as a quality control. Emulsion samples were diluted 1:10 for light microscopy imaging. A single droplet of an emulsion was dropped between the object plate and the cover glass for optical imaging. Emulsions were further imaged and characterized with 200 kV Tecnai F20 Cryo-TEM microscope (FEI Company, Holland). Before freezing, the 300 mesh copper grids (Quantifoil R2/2, Holey Carbon Film) were treated with glow discharge treatment. The samples were frozen as thin films with automatic plunge freezing and bare grid technique (Leica EM GP, Germany) using liquid nitrogen cooled ethane.

2.2.5. In vitro drug release studies

In vitro drug release studies were performed in side-by-side-diffusion cells (Laborexin, Finland). Whatman 0.4 µm pore size Nuclepore Track-Etch polycarbonate membrane filters were clamped between two identical halves of diffusion cells. The exposed surface area of the clamped membrane was 0.785 cm^2. The diffusion cells were kept at 37°C by a surrounding water socket to mimic physiological conditions. 0.8 ml of drug containing emulsion (0.1 mg/ml of drug) was transferred into the donor compartment, which was filled to 3.5 ml final volume with 0.1 M phosphate buffer pH

7.4. The acceptor compartment was filled with 3.5 ml of 0.1 M pH 7.4 phosphate buffer. Free drug solutions of NAP and IBU were used as drug release controls through the membrane. The control samples in the donor compartment contained 0.08 mg of drug per 3.5 ml volume corresponding to 100 % of drug in the emulsions. 200 µl samples were collected from the acceptor compartment and replaced with 200 µl of fresh buffer at 0.17, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h. The free drug solutions of NAP and IBU were used as controls.

NAP and IBU concentrations from the *in vitro* drug release samples were analyzed with Ultra performance liquid chromatography (UPLC) instrument Acquity UPLC (Waters, USA). For NAP, the column was a BEH Shield column 1.7 µm (2.1 x 50 mm) (Waters, USA) at 30°C. The flow rate was 0.5 ml/min and the injection volume 5 µl. The detection was performed at a wavelength of 231 nm. During the gradient run, the mobile phase consisted of a mixture of acetonitrile and 15 mM phosphate buffer pH 2 in 30:70 ratio at 0 - 3 min. After 3 min, the mobile phase ratio was changed to 70:30. The retention time of NAP was 1.49 min. The linear concentration of NAP was established in the range of 0.1 - 10 µg/ml and LOQ for NAP was 0.007 µg/ml. For IBU the column was a HSS-C18 column 1.8 µm (2.1 x 50 mm) (Waters, USA) at 30°C. The flow rate was 0.5 ml/min and the injection volume 5 µl. The detection was performed at a wavelength of 219 nm. During the isocratic run the mobile phase consisted of a mixture of acetonitrile and 15 mM phosphate buffer pH 2 in 60:40 ratio. The retention time of IBU was 0.85 min. The linear concentration of IBU was established in the range of 0.1 - 25 µg/ml and LOQ for IBU was 0.09 µg/ml.

3. Results and discussion

3.1. Emulsion droplet size and zeta-potential measurements

Measurements of emulsion droplet diameters and zeta potential values were performed in order to estimate the minimum amount of the protein needed to gain stable emulsion droplets with the sonication method (Varjonen et al., 2011). Sonication produces emulsions with ultrasound-driven mechanical vibrations. Droplet size, polydispersity index (PDI) and zeta potential measurements were performed for HFBII stabilized and 10% soybean oil containing preliminary formulations. These HS-1 to HS-5 formulations contained 0.1–1.5 mg/ml of HFBII as the sole emulsion stabilizer (see Table 1). DLS measurements were not possible with the samples containing NFC as the light scattering by the long fibers distorted the signal too much.

Higher HFBII concentrations in the aqueous phase reduced the emulsion droplet sizes effectively from 2013 nm to 345 nm and increased the zeta potential values from 25 mV to 32.7 mV (Table 2). Based on DLS and zeta potential measurements it was concluded that HFBII concentrations between

1-1.5 mg/ml (HS-3–HS-5), in the aqueous phase of the emulsions, resulted in good colloidal stability at day 1. These formulations had smaller droplet sizes and higher zeta potential values over 30 mV when compared to the formulations with smaller amounts of HFBII. However, the PDI values were quite high for all formulations indicating that the size distribution of droplets was wide in all emulsions. It might be possible to obtain monodisperse emulsion droplets with other techniques like homogenization (Maa and Hsu, 1999). At day 28 the emulsion droplet size had increased significantly for formulations with low amount of HFBII (0.1-0.5 mg/ml) when compared to day 1 results. The amount of HFBII in these emulsions was therefore insufficient. For formulations that contained higher amounts of HFBII (1-1.5mg/ml) a decrease in the droplet size at day 28 was observed. This might have been caused by the loss of excess amount of protein at the o/w interface. As HFBII is known to self-associate and form tetramers in an aqueous solution at the concentration range 0.5-10 mg/ml (Kisko et al., 2008).

Table 2. DLS and zeta potential measurements at day 1 and 28 after the preparation (mean ± S.D., n=3). Preliminary formulations HS-1 to HS-5 contained 0.1 - 1.5 mg/ml of HFBII as the sole emulsion stabilizer. Higher HFBII concentrations reduced the emulsion droplet sizes and increased the zeta potential values. PDI values were relatively high for all formulations indicating polydispersity. Abbreviations: H=HFBII and S=soybean oil.

Formulation	Day 1: z-average diameter (nm)	Day 28: z-average diameter (nm)	Day 1: PDI	Day 28: PDI	Day 1: Zeta Potential (mV)
HS-1	2013 ± 33	4623± 1879	0.517 ± 0.014	1 ± 0	25 ± 0.6
HS-2	537.6 ± 11.7	696.8 ± 208.4	0.415 ± 0.028	0.492 ± 0.219	24.8 ± 0.2
HS-3	339.0 ± 1.6	324.9 ± 39.0	0.440 ± 0.012	0.349 ± 0.035	31.4 ± 1.0
HS-4	301.4 ± 7.3	194.1 ± 0.9	0.443 ± 0.024	0.144 ± 0.027	30.2 ± 1.0
HS-5	345.0 ± 4.7	183.6 ± 1.3	0.520 ± 0.017	0.129 ± 0.006	32.7 ± 1.1

HS-3 formulation contained 1 mg/ml of HFBII and the emulsion droplet size did not change drastically during 28 days. Therefore, 1 mg/ml HFBII concentration was selected to be used in the combination formulations with NFC fibers. HFBII has been shown to adsorb to hydrophobic interfaces, such as hydrophobic polymer films and o/w interphase of an emulsion, and to lower the interfacial tension even at low concentrations (Lumsdon et al., 2005). 0.2 mg/ml HFBII concentration in the aqueous phase has been shown to stabilize o/w emulsions effectively. These results support our finding that HFBII can be used as a surfactant to stabilize emulsions at extremely low concentrations. Furthermore HFBs are nontoxic, which is an advantage when HFBs are used as pharmaceutical excipients (Aimanianda et al., 2009;Ebbole, 1997).

The sonication method produced submicron emulsion droplets with high zeta potential values (Table 2). Therefore, the emulsion quality was considered to be sufficient for the evaluation of HFBII and NFC materials in the stabilization of pharmaceutical emulsions. Different sonication times and high-shear mixing were tested with HS-3 formulation in order to evaluate further whether the emulsion droplet size and PDI values could be decreased. Based on the results it was observed that a sonication time of 2 minutes was optimal (Table 3). Longer sonication times or high-shear mixing resulted in over processing and significant increase in the droplet size. The size range of droplet diameters was in micrometers with high-shear mixing as opposed to nanometers obtained with sonication. Therefore, high-shear mixing was not suitable as a preparation process. Furthermore, it was observed that it was beneficial to mix all formulation components gently by repeated pipetting prior to sonication while preparing the formulations that contained NFC in addition to HFBII. The addition of NFC after the sonication of HFBII and other formulation components (oil and water) was tested, but this approach led to fast phase separation in a matter of minutes.

Table 3. The effect of the preparation process on emulsion droplet size and PDI values (mean ± S.D., n=3). HS-3-formulation was prepared with sonication and high-shear mixing techniques with different mixing durations. Sonication technique produced overall smaller emulsion droplet diameters than the high-shear mixing technique.

| Preparation method	Day 1: z-average diameter (nm)	Day 28: z-average diameter (nm)	Day 1: PDI	Day 28: PDI
2 min sonication	339.0 ± 1.6	324.9 ± 39.0	0.440 ± 0.012	0.349 ± 0.035
3 min sonication	415.3 ± 6.1	366.0 ± 6.2	0.796 ± 0.046	0.310 ± 0.008
4 min sonication	550.0 ± 18.7	480.0 ± 11.8	0.937 ± 0.045	0.540 ± 0.027
5 min sonication	605.9 ± 55.3	357.0 ± 35.0	0.834 ± 0.113	0.550 ± 0.090
1 min high-shear mixing	4117 ± 468	nd	0.319 ± 0.052	nd
2 min high-shear mixing	4327 ± 202	nd	0.372 ± 0.073	nd
4 min high-shear mixing	6388 ± 120	nd	0.123 +0.029	nd

3.2. NFC and HFBII as emulsion stabilizers

3.2.1. Formulations and emulsion stability index (ESI)

Formulation optimization process was performed by evaluating the ability of different formulation components and their combinations to stabilize the emulsion. Emulsions of soybean oil or ethyl oleate and water in the presence of HFBII (1 mg/ml) and/or NFC were studied (see Table 1 for details). Soybean oil and ethyl oleate are pharmaceutically acceptable oils that have been used successfully in a variety of pharmaceutical formulations (Chen et al., 2006;Cui et al., 2009;Gorain et al., 2014;Wulff-Pérez et al., 2009). However, soybean oil has a broader use as it is FDA approved for intravenous,

oral and topical formulations. The stability of different formulations were evaluated by following the phase separation of emulsions with ESI (Fig. 1A-F). The best material combinations were selected for IBU and NAP formulations based on the highest ESI values of preliminary formulations.

The ability of NFC-N (0.1-1.5 mg/ml) to stabilize the emulsions increased when a higher amount of NFC-N was used in combination with soybean oil (Fig. 1A-B). NFC-N formulations with soybean oil were more stable than with ethyl oleate during the 28 day period, which could be observed as higher ESI values and less phase separation. The stability of the soybean oil based emulsions increased in direct proportion to the amount of NFC-N, whereas the effect of NFC-N amount on the stability of ethyl oleate emulsions could not be seen as clearly. It has been previously shown that a continuous NFC-N matrix forms in the aqueous phase of o/w emulsions when NFC-N concentration is increased to 1 mg/ml (Varjonen et al., 2011). This was reportedly followed by association of oil droplets to the NFC-N fibrils most likely caused by the emulsification process. Best stability was gained in our study when at least 1.5 mg/ml NFC-N was used with soybean oil in emulsion stabilization. Reportedly the addition of NFC increases the viscosity of the continuous phase of o/w emulsions and creates a polymer network that limits drop collision, thus reducing the coalescence phenomena (Carrillo et al., 2015;Dimic-Misic et al., 2013;Garti, 1999;Xhanari et al., 2011). Therefore, the stability of NFC-N containing emulsions depended on the concentration of NFC-N due to the increased viscosity of the water phase as well as the benefits gained from increasing the amount of NFC for encapsulation of the oil droplets.
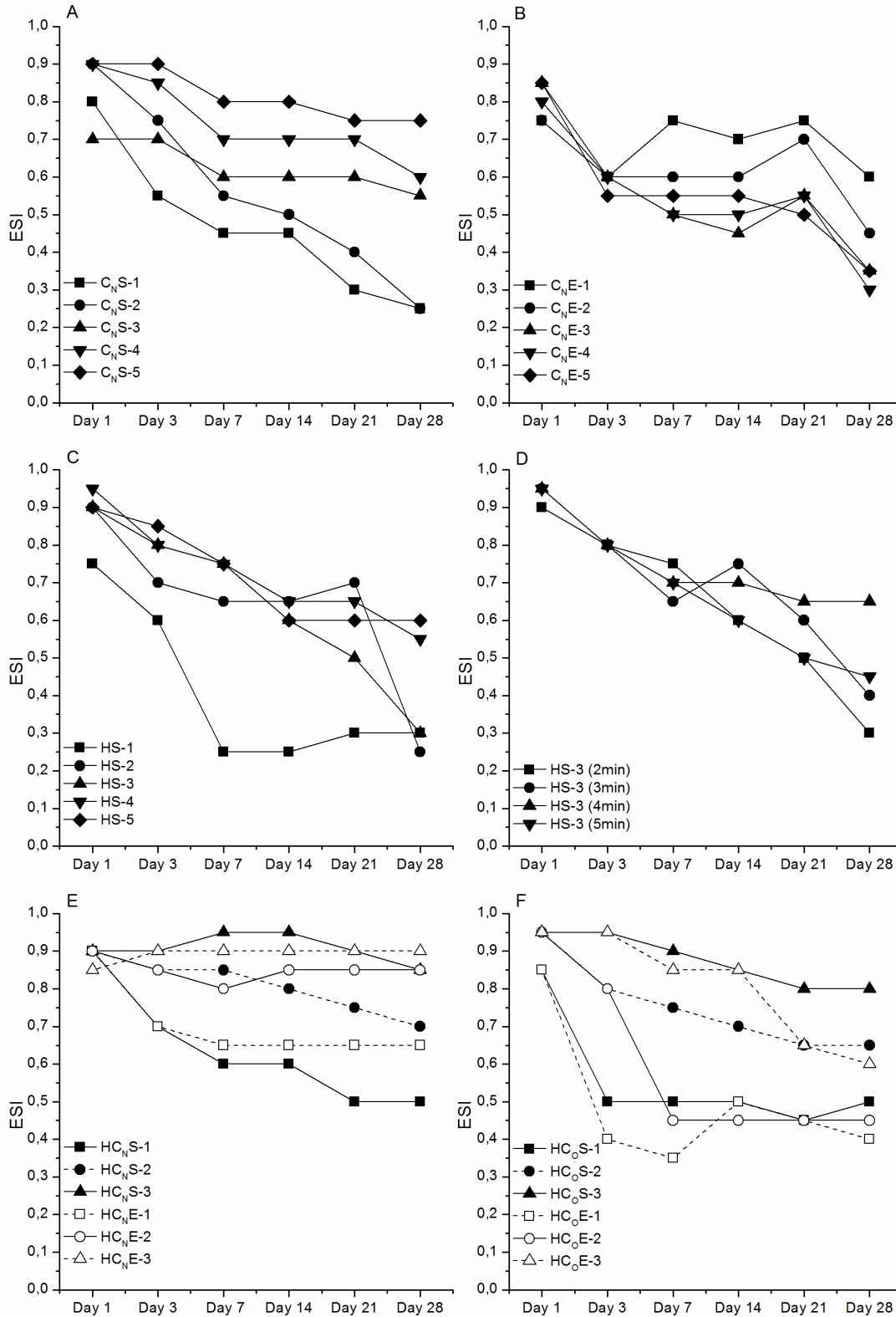
Figure 1. Emulsion stability index (ESI) values for different emulsion formulations (n=1): A) NFC-N with soybean oil, B) NFC-N with ethyl oleate, C) HFBII with soybean oil, D) 1 mg/ml HFBII (HS-3) with soybean oil prepared by different sonication times, E) HFBII-NFC-N formulations with soybean oil or ethyl oleate, F) HFBII-NFC-O formulations with soybean oil or ethyl oleate. Abbreviations: H=HFBII, C_N= NFC-N (native nanofibrillated cellulose), C_O= NFC-O (TEMPO oxidized nanofibrillated cellulose), S=soybean oil and E=ethyl oleate.

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When HFBII (0.1-1.5 mg/ml) was used for emulsion stabilization only the formulation that contained the smallest amount of HFBII (0.1 mg/ml) was clearly unstable (Fig. 1C). The most stable formulations contained 1.25-1.5 mg/ml of HFBII, which indicated clearly that the stability of these emulsions increased in direct proportion to HFBII content. However, 1 mg/ml HFBII concentration (HS-3 formulation) was selected to be used in combination with NFC due to the size and PDI measurements that clearly indicated 1.25 and 1.5 mg/ml concentrations to be excessive (Table 2). The stability of 1 mg/ml HFBII stabilized HS-3 formulation series that was prepared with different sonication times was tested (Fig. 1D). Differences in emulsion stability evaluated by phase separation were quite small. By contrast, the emulsion droplet size and PDI value increased when sonication time was increased. Regarding the overall emulsion stability 2 min sonication time was optimal.

The stability of the emulsions was increased when adequate amounts of HFBII and NFC-N or NFC-O were used in combination (Fig. 1E-F). The ESI values were higher than when sole emulsion stabilizers were used in the formulations. We tested 0.5, 1 and 1.5 mg/ml concentrations of NFC-N and NFC-O in combination with 1 mg/ml HFBII and evaluated the impact of the two oils, soybean oil and ethyl oleate, on ESI values. Formulations with low amounts (0.5 mg/ml) of NFC-N or NFC-O proved to be quite unstable as the emulsion phase separation was quite fast already at day 3. The most stable formulations had 1.5 mg/ml of NFC-N or NFC-O in combination with 1 mg/ml of HFBII. Relatively stable emulsions were obtained when the surfactants like properties of HFBII were combined with NFC that can be used to modify the viscosity of the water phase in addition to droplet encapsulation. Based on the emulsion stability data, the HFBII concentration was kept at a constant 1 mg/ml and two different NFC-N and NFC-O concentrations, 1 mg/ml and 1.5 mg/ml, were selected for the drug containing formulations in the release studies.

Shear viscosities of 1 mg/ml (0.1 %) and 1.5 mg/ml (0.15 %) of NFC-N and NFC-O were measured as a function of shear rate in order to evaluate the effect of NFC concentration on the viscosity of aqueous continuous phase in emulsions (Fig. 2). NFC-N at low concentrations of 0.1% and 0.15% did not affect the viscosity significantly as the shear rate did not affect the viscosity and the viscosity profile was similar to pure water. NFC-O had approximately ten times higher viscosity values than NFC-N at 100 (1/s) shear rate. Furthermore, moderate shear thinning was observed for NFC-O. It has been shown for both NFC grades in several previous studies that the concentration affects the viscosity and intense shear thinning behavior is observed (Benhamou et al., 2014;Bhattacharya et al., 2012;Nechyporchuk et al., 2014;Pääkkö et al., 2007). NFC hydrogels can be classified as pseudoplastic materials in this regard.

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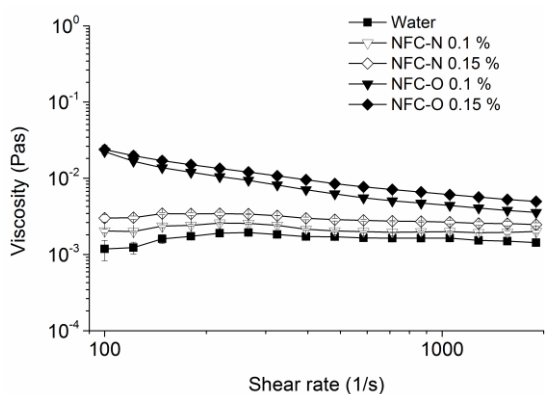
Figure 2. Shear rate viscosities of water, 0.1 % and 0.15 % of NFC-N and NFC-O at 4 °C (mean ±
S.D., n=3).

The low shear viscosity of 0.1% and 0.15% NFC-N and NFC-O was not surprising as there was a
very small amount of fibers to form the NFC network in the aqueous environment. Upon exposing
increased shear rate the weakly bonded network falls apart easily and individual fibers start to flow.
However, as NFC-O had slightly higher shear viscosity at 4 °C, which was also used as the storage
temperature of emulsions, it seems that NFC-O may be more effective in emulsion stabilization due
to modified viscosity of the continuous phase even at low concentrations. The effect of low NFC-N
concentration on viscosity was minimal. The emulsion stabilizing effect of NFC-N at 0.1 - 0.15 %
concentration may be attributed to the reduced emulsion droplet movement rather that the increased
viscosity of the continuous phase.

The solubility of NAP and IBU in soybean oil and ethyl oleate was measured to find the drug
solubilization capacity of the oils (Table 4). Ethyl oleate had a higher solubilization capacity than
soybean oil with regard to both drugs. The solubility of NAP was 2.8 g/L in soybean oil and 5.1 g/L
in ethyl oleate. For IBU, the solubility was 46.2 g/L in soybean oil and 98.7 g/L in ethyl oleate. The
different drug solubilization capacity of soybean oil and ethyl oleate is attributed to the structural
differences of these oils (Chen et al., 2006;Date and Nagarsenker, 2008;Sharif Makhmalzadeh et al.,
2012). Vegetable origin soybean oil contains triglycerides with long hydrocarbon chains and is a high
molecular volume oil, whereas fatty acid ester ethyl oleate is a lower molecular volume oil than
soybean oil (Date and Nagarsenker, 2008). Soybean oil with a 1563 Å^3 molecular volume has been
shown to have a slightly lower solubilizing capacity for lipophilic drugs than ethyl oleate with a 600
Å^3 molecular volume (Malcolmson et al., 1998). However, the solubility of both drugs in tested oils
was significantly higher than the aqueous solubility. Therefore, the emulsion formulations containing
either of these oils can be used to effectively solubilize both NAP and IBU. Soybean oil was selected
for the oil phase in the emulsions and for the solubilization of poorly water-soluble model drugs NAP

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1 and IBU as it proved to be slightly better than ethyl oleate with regard to ESI values. 10% of oil phase

2 and 90 % of aqueous phase with 0.1 mg/ml drug loading was used for all emulsions. The drug amount

3 in the emulsions can be improved by a higher amount of drug in the oil phase or by a higher amount

4 of the oil phase. However, these conditions were chosen for the release studies in order to evaluate

5 the performance of the emulsion stabilizing excipients. Further studies are needed to optimize the

6 amount of NFC and HFBII for emulsions with a higher volume of oil phase.

7 **Table 4.** Solubility values for NAP and IBU in aqueous media, soybean oil and ethyl oleate.

| Compound	Molar mass (g/mol)	pKa	logP	Solubility in aqueous media	Solubility in soybean oil	Solubility in ethyl oleate
NAP	230.3	4.15	3.18	15.9 mg/L, MQ[a] 2.8 - 3.6 g/L, pH=7.4[b]	2.8 g/L	5.1 g/L
IBU	206.3	4.85	3.97	21 mg/L, MQ[c] 6 g/L, pH=7.4[d,e]	46.2 g/L	98.7 g/L

8 [a]Yalkowsky et al., 2003; [b]Mora and Martínez, 2006; [c]Yalkowsky and Dannenfelser, 1992;

9 [d]Lerdkanchanaporn et al., 2001; [e]Levis et al., 2003

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11 *3.2.2. Characterization with optical microscopy and cryo-TEM*

12 HFBII stabilized emulsion droplets could clearly be observed in optical microscopy as well as cryo-

13 TEM images. Optical microscopy images of formulations that were selected for the drug release

14 studies were taken after 1:10 dilution as a quality control (Fig. 3 A-C). The formulations contained

15 HFBII and combinations of HFBII-NFC-N and HFBII-NFC-O as stabilizers. NFC-O stabilized

16 emulsions seemed to be more stable upon dilution in light microscopy images than NFC-N stabilized

17 (Fig. 3B-C). NFC-O stabilized emulsions had more uniform distribution of emulsion droplets in

18 evenly distributed fiber network whereas NFC-N stabilized emulsions formed clusters of NFC-N and

19 emulsion droplets upon dilution. Emulsion droplets with diameters in the micrometer range were

20 observed with optical microscopy.
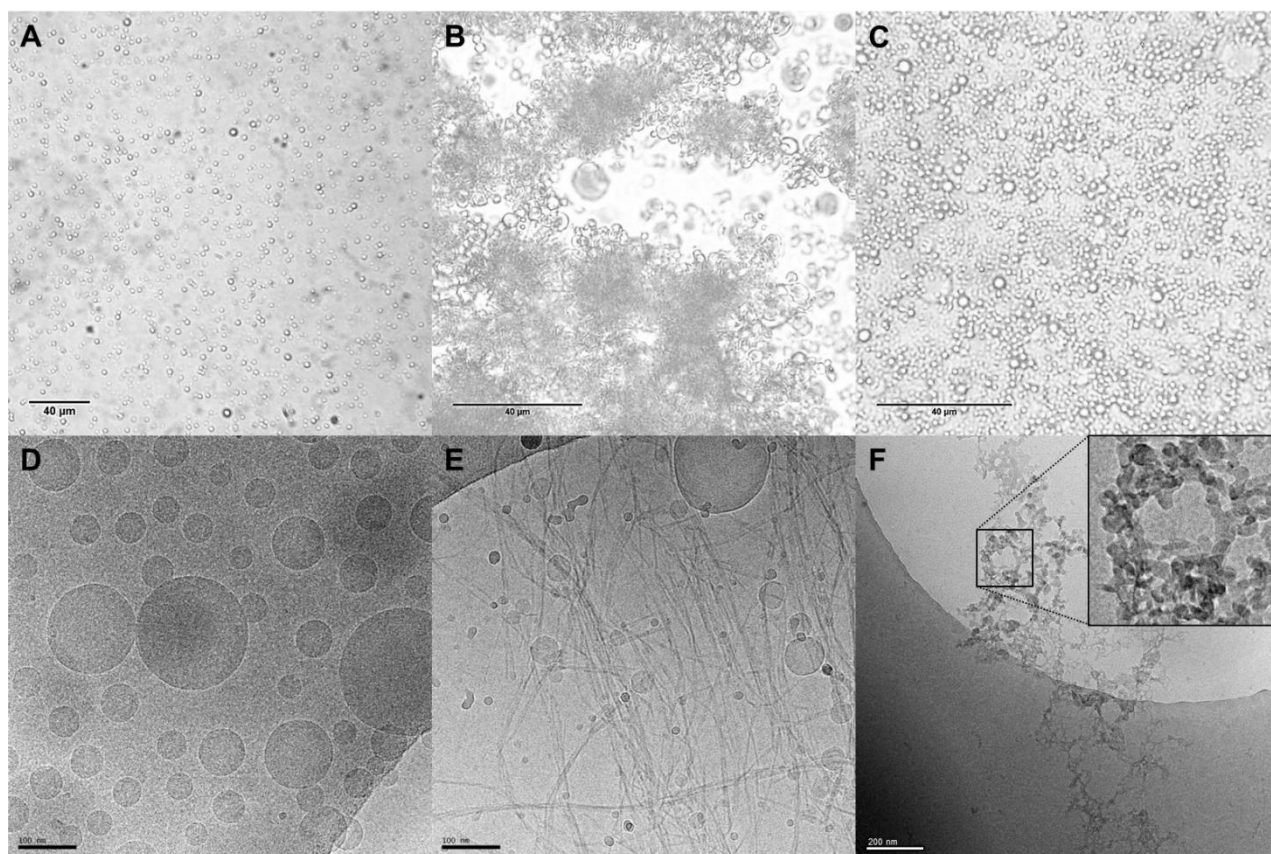
Figure 3. Light microscopy and cryo-TEM images of HFBII, HFBII-NFC-N (1:1 mass ratio) and HFBII-NFC-O (1:1 mass ratio) stabilized emulsions. Upper image row (A-C) contains light microscopy images of respective formulations, whereas lower image row (D-F) contains cryo-TEM images. The emulsion droplets of HFBII-NFC-N and HFBII-NFC-O formulations were within the entangled NFC fibers. For solely HFBII stabilized emulsions only spherical emulsion droplets were observed. Scale bar is 40 µm for A-C, 100 nm for D-E and 200 nm for F image.

The spherical morphology of the protein coated emulsion droplets as well as the wide size distribution in all formulations was confirmed with cryo-TEM (Fig. 3D-F). For HFBII-NFC-N formulation the fibrous NFC matrix surrounded the emulsion droplets, whereas for solely HFBII stabilized emulsion only the protein coated spherical emulsion droplets were observed. The NFC-O fibers surrounding the protein coated oil droplets consisted of shorter NFC-O fibers as observed in cryo-TEM images (Fig. 3F) as opposed to the longer NFC-N fibers (Fig. 3E). NFC-O fibers are typically shorter than NFC-N fibers due to the oxidation process that reduces the fiber length (Benhamou et al., 2014). It was observed that the emulsion droplets had a tendency to exist as clusters when stabilized with the shorter NFC-O fibers. The expected stabilization mechanism, a combination of HFBII stabilized oil droplets with NFC fiber network, of the emulsions was confirmed with cryo-TEM.

3.3. In vitro drug release studies

In vitro drug release studies were done in diffusion cells through a 0.4 µm pore size membrane. Therefore, lag times were observed in the release rate profiles at the beginning of the drug release studies. The model compounds NAP and IBU both have three distinctive diffusion steps from the o/w emulsions in our experimental setup that need to be considered when interpreting the drug release results. These steps include: 1) diffusion across the HFBII coated oil droplet interfacial film, 2) diffusion through the water phase of the diluted emulsion in the donor compartment and 3) diffusion from the donor compartment across the membrane to the receptor solution.

The drug release profiles of NAP and IBU from solely HFBII stabilized formulations were compared to NFC containing formulations. The amount and grade of NFC was investigated in terms of effect to drug release rate. The cumulative drug release of NAP and IBU from the HFBII, HFBII-NFC-N and HFBII-NFC-O stabilized emulsions are presented in Figure. 3. NAP (pKa 4.15) and IBU (pKa 4.85) are both weak acids and poorly soluble in low pH values but have a high solubility above pH 6.8 due to the ionization (Álvarez et al., 2011;Khadra et al., 2015). Therefore, the diffusion of NAP and IBU across the HFBII coated oil droplet interfacial films and NFC fiber matrix was evaluated by using the free drug solutions of NAP and IBU as positive controls for immediate release. The *in vitro* drug release experiments, conducted under sink conditions, indicated that different formulation compositions had an impact on the drug release rate when compared to the free drug solution controls of NAP and IBU. Major differences in the drug release rate were observed depending on the grade of NFC fibers used in the formulations.

NAP release was fast from HFBII-NFC-N formulations and 64-70% was released during 8 h in comparison to 85% of the free drug through the membrane (Fig. 4). The drug release was slightly slower than for the positive control of immediate release (free drug solution), but still close to an immediate drug release profile. Sustained drug release profile for NAP was obtained with the HFBII-NFC-O formulations as only 18-27% was released during 8 h. The drug release of NAP from HFBII formulation (NAP-5) resembled a sustained release profile with the 38% of drug release during 8 h. The amount of NFC-N or NFC-O in the formulation affected only slightly the drug release rates of NAP. However, the grade of NFC fibers used in the formulations with HFBII had a significant impact.
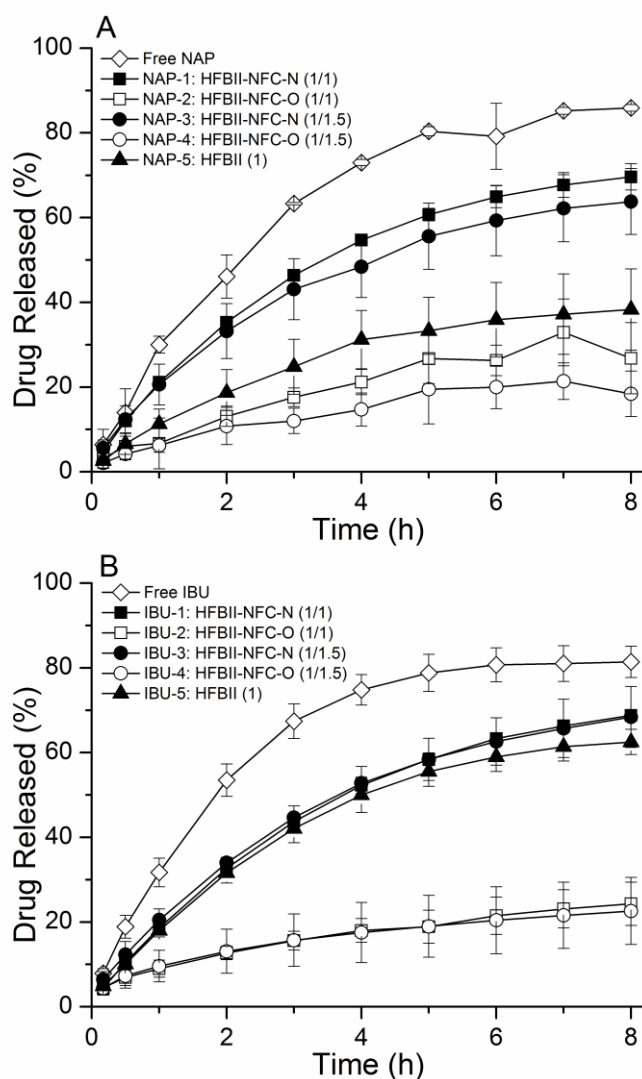
Figure 4. Drug release studies with NAP and IBU: A) Cumulative NAP release (%) from different formulations (mean ± S.D., n=3-5), B) Cumulative IBU release from different formulations (mean ± S.D., n=4-5). Mass ratios of HFBII to NFC-N or NFC-O are in brackets.

The drug release results of NAP and IBU from different formulations were rather similar (Fig. 4). IBU release was fast from the HFBII-NFC-N and HFBII formulations. 62-67% of IBU was released in 8 h, while 80% of the free drug had passed through the membrane in the same time. Sustained drug release profiles were obtained for IBU with HFBII-NFC-O formulations as only 20-23% of IBU was released during 8 h. The amount of NFC-N or NFC-O in the formulation did not affect significantly the IBU release rates, but the grade of NFC fibers did. Overall, the model drugs were released in a sustained manner from the HFBII-NFC-O formulations. By comparison, immediate release profiles were gained with the HFBII-NFC-N formulations. It was expected that different release profiles would be gained with these materials as it has been previously reported that the drug interactions with

HFBII and different NFC-grades has an impact on the drug release rate (Kolakovic et al., 2013;Valo et al., 2010;Valo et al., 2013). Especially pH dependent electrostatic interactions between drug molecules and NFC fibers seem to play an important role with regard to drug release (Kolakovic et al., 2013). It is possible that some drug interactions existed with both NFC grades driven by aromatic π stacking and hydrogen bonding. However, physical interactions such as viscosity, stability and the surface barrier of the emulsion droplets were most likely more critical in determining the drug release rates.

At pH 7.4 both NFC grades are negatively charged, but NFC-O has a greater negative charge due to a greater distribution of carboxyl groups directly at the fiber surface (Saito et al., 2007;Weishaupt et al., 2015). HFBII has an isoelectric point (pI) of 6.7 and a negative net charge at higher pH values (Krivosheeva et al., 2013). However, it is possible that the protein coated oil droplets may have had a positive charge at pH 7.4 as the formation of tight interfacial HFBII film reportedly reduces the surface area and ionizable functional groups exposed to water resulting in a shift of the pI (Basheva et al., 2011). The measured zeta potential values of HFBII stabilized preliminary formulations indicated positive net charge at neutral environment of ultrapure water (Table 2). It is likely that the sustained release profiles of NAP and IBU from HFBII-NFC-O formulations were attributed to a greater diffusion barrier at the o/w-interphase that was present due to electrostatic attraction between negatively charged NFC-O and positively charged HFBII. As NFC-N has a lower negative charge, the effect was not as pronounced resulting in an immediate drug release profiles.

Futhermore, NFC-O stabilized emulsions were more stable upon dilution (Fig. 3B-C, 10x dilution) and the emulsion structure remained more intact than with NFC-N. This was probably attributed the high surface density of negative charges of NFC-O fibers due to multiple carboxyl groups on the fiber surface (Benhamou et al., 2014). Since the emulsions were slightly diluted (4x) in the release experiments, the electrostatic repulsion between NFC-O fibers may have stabilized the emulsion droplets of HFBII-NFC-O formulations more effectively in addition to electrostatic attractive interactions between HFBII and NFC-O. Furthermore, NFC-O had a higher viscosity than NFC-N (Fig.2) indicating that NFC-O was a more effective grade for emulsion storage stabilization due to increased viscosity of the continuous phase. In theory, the higher viscosity of HFBII-NFC-O formulations may have prolonged the drug release, but upon dilution the effect should have been minimal. We believe that the sustained drug release properties of HFBII-NFC-O formulations were at least partially attributed to the electrostatic interactions between HFBII and NFC-O, which stabilized the emulsion structure.

For solely HFBII protein stabilized formulations an immediate and sustained drug release profiles were observed for IBU and NAP respectively. IBU has a higher solubility at pH 7.4 than NAP (see Table 4.). The solubility difference at pH 7.4 may be the reason for the differences in the drug release rates of these molecules. The solubility of the diffusing drug molecules can reportedly have a higher effect on drug release rate than the interfacial film properties of the emulsion droplets (Llinàs et al., 2013). Consequently, the solubility of the ionized drug molecules, in the pH 7.4 release medium, can affect the diffusion rate from the inner oil phase and overall drug release. Therefore, it was concluded that while the HFBII formulations were successful in controlling the drug release to some extent it was beneficial to add NFC into the formulations to gain well defined release profiles. Furthermore, the storage stability of the emulsions was improved when NFC was used in the stabilization together with HFBII (Fig. 1).

HFB proteins have hydrophobic and hydrophilic patches directly at their surfaces (Lumsdon et al., 2005;Paananen et al., 2013;Szilvay et al., 2007). The amphiphilic nature of HFBII has been exploited for hydrophilic and hydrophobic interactions with interfaces. Previously the ability of HFBs to modify hydrophobic interfaces into hydrophilic ones has been used in nanoencapsulation of hydrophobic nutraceuticals, stabilization of emulsions and hydrophobic drug nanoparticles (Israeli-Lev and Livney, 2014;Valo et al., 2010;Varjonen et al., 2011). Here HFBII was mainly used to stabilize the o/w interphase of pharmaceutical emulsions and to aid in the solubilization of BCS class II drugs in the oil phase of emulsions. Some hydrophobic and hydrophilic interactions between HFBII and the model drugs may have existed, but the effect on drug release profiles was minimal. Similar release profiles were observed from the HFBII and HFBII-NFC-N formulations apart from NAP with HFBII. The release profiles of IBU and NAP were close to an immediate release profiles from HFBII-NFC-N. At the low concentrations of 1 mg/ml for HFBII and 1 - 1.5 mg/ml for NFC-N in the formulations, these materials were better suited for emulsion storage stabilization than to provide controlled release. However, immediate release profile after oral administration is beneficial in many pharmaceutical applications such as analgesia.

Based on the *in vitro* drug release studies, it was clearly shown that the use of native or oxidized NFC as an emulsion stabilizer results in different drug release profiles. Morphology and the stability of the different formulations most likely had an impact on drug release results. Drug release profiles were affected only moderately by the viscosity of the emulsions or by the interactions of the drug molecules with NFC fibers and HFBII proteins. Clearly both NFC grades, native and TEMPO oxidized, can be used as emulsion stabilizers. However, further studies are needed in order to evaluate a concentration range where HFBII may act as a surfactant and provide sustained release from the inner oil phase.

The oxidized NFC grade seems promising excipient for sustained release formulations as an emulsion stabilizer due to its greater extent to modify the drug release rate. The native NFC grade in turn seems more suitable for formulations that benefit from an immediate drug release profile. As an increasing number of drug candidates suffer from poor aqueous solubility, the emulsion formulation is an option for solubilization of lipophilic drugs and enhancement of the adequate absorption from the GIT following oral administration.

4. Conclusions

This study has shown that a combination of natural biopolymers HFBII and nanofibrillated cellulose can be used to tailor emulsions for immediate and sustained drug release applications. The low toxicity of these materials is a significant benefit in drug delivery applications via the oral route when compared to synthetic traditional surfactants. The formulations that contained HFBII and oxidized nanofibrillated cellulose proved to be effective in controlling the drug release rate, which indicated the suitability of these materials for controlled drug release applications. Formulations with HFBII and native nanofibrillated cellulose were more suitable for immediate drug release applications. The drug release profiles or our poorly soluble model compounds, naproxen and ibuprofen, could be tuned by the choice of the nanofibrillated cellulose grade.

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