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Published in:
A C S Biomaterials Science & Engineering

Link to article, DOI:
[10.1021/acsbiomaterials.9b00495](https://doi.org/10.1021/acsbiomaterials.9b00495)

Publication date:
2019

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Mazzoni, C., Tentor, F., Antalaki, A., Jacobsen, R. D., Mortensen, J., Slipets, R., Ilchenko, O., Keller, S. S., Nielsen, L. H., & Boisen, A. (2019). Where Is the Drug? Quantitative 3D Distribution Analyses of Confined Drug-Loaded Polymer Matrices. *A C S Biomaterials Science & Engineering*, 5(6), 2935-2941.
<https://doi.org/10.1021/acsbiomaterials.9b00495>

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Controlled Release and Delivery Systems

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ACS Biomater. Sci. Eng., **Just Accepted Manuscript** • DOI: 10.1021/acsbmaterials.9b00495 • Publication Date (Web): 10 May 2019

Downloaded from <http://pubs.acs.org> on May 14, 2019

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Where is the drug? - Quantitative 3D distribution analyses of confined drug-loaded polymer matrices

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ABSTRACT

To enhance oral bioavailability of poorly soluble drugs, microfabricated devices can be utilized. One example of such devices is microcontainers. These are cylindrical in shape with an inner

cavity for drug loading and with only the top side open for release. Supercritical CO₂ (scCO₂) impregnation is an interesting technique for loading drugs into polymeric matrices in e.g. microcontainers since it avoids the use of organic solvents and is cheap. One of the main drawbacks of this technique is the unknown three dimensional drug distribution in the polymer matrix. The aim of this study was to investigate the loading of two poorly soluble drugs, naproxen and ketoprofen, by scCO₂ impregnation into confined polymer matrices of different sizes. Three different sizes of microcontainers (small, medium and large) and thereby, different surface areas accessible for impregnation, were compared. From *in vitro* studies, the amount of naproxen and ketoprofen loaded into the different microcontainers and their corresponding release profiles were seen to be similar. A custom-made Raman microscope facilitated volumetric Raman maps of an entire microcontainer filled with polyvinylpyrrolidone (PVP) and scCO₂ impregnated with either naproxen or ketoprofen. In all microcontainer sizes, the drugs were only detected in the top layer of the polymer matrix, explaining the observed similar release profiles. Using X-Ray Powder Diffraction and Raman spectroscopy, the solid state form of the drugs was evaluated, showing that ketoprofen was amorphous in all microcontainer sizes. Naproxen was found not to be crystalline neither amorphous, but in a less ordered configuration than the crystalline state. In conclusion, volumetric Raman mapping is a powerful technology for imaging drug distribution and drug crystallinity in polymers and allowed us to conclude that i) scCO₂ impregnation depth does not depend on surface area and ii) impregnated drugs are non-crystalline.

KEYWORDS: Microdevices, polymer matrix, drug distribution, poorly soluble drug, supercritical CO₂ impregnation, Raman spectroscopy

1. INTRODUCTION

Among the different administration routes for drugs, oral delivery is preferred by patients since the drug can be self-administered leading to high compliance.¹ However, oral drug delivery is often challenging due to e.g. harsh conditions in the stomach and poor permeability over the intestinal wall.¹ Many drugs are classified as poorly water soluble in the biopharmaceutics classification system (BCS, class II and IV).^{2,3} For oral delivery of poorly soluble drugs, solubility and dissolution rate need to be improved to obtain an acceptable bioavailability. One approach for achieving this, is to convert the drug to its amorphous form.⁴ Here, the long range order in the crystal lattice is lacking and the disordered structure results in improved solubility and dissolution rate.^{4,5} The disadvantage of the amorphous form is its physical and chemical instability. It can convert back to its metastable or stable counterpart during storage and/or dissolution.⁶ There are various techniques to improve the physical stability of the amorphous form e.g. co-amorphization of two drugs⁷ or use of polymers as excipients.⁸ Another approach for protecting the amorphous drugs is the use of microcontainers.^{5,9} Microcontainers are cylindrical, polymeric microdevices with an inner cavity for drug loading and with only the top side open. Previously, confinement of the amorphous poorly soluble drug indomethacin reduced the re-crystallization rate by 1.8 fold compared to unconfined bulk samples.⁵ In particular, using microcontainers with cavity diameters of 174 μm , 29.0 ± 2.6 % of the amorphous indomethacin crystallized over a period of 30 days compared to microcontainers with diameters of 223 μm where 38.3 ± 1.5 % crystallized. This indicates that microcontainers with smaller diameters enhance the stability of the amorphous drug loaded inside.⁵ Unconfined indomethacin crystallized within a few days. In addition to the stabilization properties, microcontainers have been used for improving oral drug delivery by protecting the drug from the harsh gastric

environment and providing a release in the small intestine.^{10–12} Furthermore, it has been demonstrated that microcontainers adhere to the intestinal mucus layer leading to higher relative oral bioavailability in rats of model drugs such as ketoprofen and furosemide compared to controls.^{13,14}

In spite of the advantages of utilizing microdevices for oral drug delivery, loading drugs into the small cavities can be challenging since all of the well-known techniques for preparing oral formulations, such as tableting, cannot be used. Supercritical CO₂ (scCO₂) impregnation is one of the techniques that can be used for loading drugs into polymer-filled microcontainers. The critical point of CO₂ is 31.1°C and 73.8 bar and due to those mild conditions, this technique is suitable for drug loading. In addition, it can be used in combination with various polymers.^{15,16} It has previously been demonstrated that the hydrophilic polymer polyvinylpyrrolidone (PVP) can be loaded into microcontainers as a polymer matrix and impregnated by scCO₂ with the drug ketoprofen.^{17,18} It was found that ketoprofen was in its amorphous form after impregnation in the PVP matrix inside the microcontainers.¹⁴ However, the influence of the size of the confined polymer volumes loaded by supercritical impregnation has never been investigated. One of the main challenges for systematic studies of drug loading with this technique has been the unknown three-dimensional (3D) drug distribution in the polymer matrix after CO₂ impregnation. Therefore, it has not been possible to understand the influence of the parameters on the release profiles and the drug-polymer interactions.^{16,17,19}

In literature, the distribution of impregnated or encapsulated material has been studied with various techniques. Polymeric membranes have been examined with energy dispersive X-ray analyses, obtaining a two-dimensional map,¹⁹ and this technique has also been successfully used for 3D mapping of nanoparticles.²⁰ Dispersive X-ray Absorption Spectroscopy (μED-XAS)

tomography has been utilized and was able to resolve both 2D and 3D spatial distribution of chemical species from different iron mineral standards.²¹ Alternatively, Raman spectroscopy has been used to evaluate the distribution of a drug inside a 3D printed tablet.²² Previously, a 2D map of a cross section of tablets using Raman spectroscopy has been obtained, understanding the distribution of three different components in an area of 4 x 4 mm.²³ Raman spectroscopy has successfully been used as a quantification technique in case of inkjet-printed pharmaceuticals requiring, however, the sectioning of the sample prior to analyses in order to measure a cross section.²⁴ Cross sectional mapping with Raman spectroscopy is a destructive method and in case of a confined polymer matrix (i.e. for microcontainers) this application is not possible. Furthermore, for investigations with Raman spectroscopy, the polymer and drug normally have a relatively low transparency under laser excitation. For reaching an acceptable Raman signal at the bottom of samples as deep as e.g. a microcontainer reservoir, a highly sensitive method for confocal Raman microscope has been developed.

The aim of this study was to investigate the loading of two BCS class II drugs, naproxen and ketoprofen, using scCO₂ impregnation into confined polymer matrices of different sizes. For this purpose, three different sizes of microcontainers (small, medium and large) and thereby, different surface areas accessible for impregnation were compared. Furthermore, the quantity and solid state form of ketoprofen and naproxen loaded into the microcontainers were evaluated. Finally, the 3D distribution of the drugs in 225 µm deep polymer matrices was analyzed by confocal Raman microscopy.

2. EXPERIMENTAL SECTION

2.1. MATERIALS

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3 Silicon (Si) wafers (4-in, b100N n-type) were provided by Okmetic (Vantaa, Finland). SU-8
4 2075 and SU-8 developer were purchased from Microresist Technology GmbH (Berlin,
5 Germany). Polyvinylpyrrolidone (PVP) (Molecular weight of 10,000 Da), ketoprofen powder
6 ($\geq 98\%$, racemate) and phosphate buffer saline (PBS) were obtained from Sigma-Aldrich (St.
7 Louis, MO, USA). Naproxen was purchased from Fagron (Newcastle upon Tyne, England).
8 Deionized water (18.2 m Ω) was acquired from Merck KGaA (Darmstadt, Germany).
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10 11 12 2.2. METHODS

13 14 15 2.2.1. FABRICATION OF MICROCONTAINERS

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17 Squared chips of microcontainers with dimensions of 12.8 x 12.8 mm² were fabricated on Si
18 wafers in the epoxy-based photoresist SU-8 using a similar procedure as described previously.⁵
19 Three different sizes of microcontainers were produced having three different cavity diameters
20 (small, medium and large) and the same cavity height. The number of microcontainers per chip
21 for the three different sizes was chosen to keep the total polymer surface exposed to the scCO₂
22 per chip constant, and thereby also the total polymer volume constant. The dimensions of the
23 microcontainers were measured using an Alpha-Step IQ Stylus Profilometer (KLA-Tencor
24 Corporation, Milpitas, USA) and an optical microscope.
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40 41 42 2.2.2. LOADING OF NAPROXEN AND KETOPROFEN INTO MICROCONTAINERS 43 USING SUPERCRITICAL CO₂ IMPREGNATION

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45 The microcontainers on Si chips were manually filled with PVP powder. Excess powder in
46 between the microcontainers was blown away using an air gun in a similar setup as described
47 previously.¹⁴ One chip of each size (small, medium and large) was placed within a supercritical
48 CO₂ chamber, together with 4.8 $\pm$ 0.1 mg (n=3) of ketoprofen powder or 6.0 $\pm$ 0.03 mg (n=3) of
49 naproxen. The impregnation with ketoprofen was conducted by bringing CO₂ to its supercritical
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state at 120 bar and 45°C keeping it under stirring for 1 h. The impregnation with naproxen was performed bringing CO₂ to 100 bar and 40°C. These parameters were chosen to have a solubility in the supercritical CO₂ of 0.06 g/L for both drugs.^{25,26} The pressurization and depressurization rates were 3.9 bar/min and 2.5 bar/min, respectively, for both drugs. The chips with microcontainers were weighed before and after filling with PVP to determine the amount of polymer loaded into the microcontainers. A tabletop Scanning Electron Microscope (SEM) TM3030Plus (Hitachi High-Technologies, Tokyo, Japan) was used to visualize the microcontainers after filling with PVP and after the impregnation process.

2.2.3. *IN VITRO* RELEASE OF KETOPROFEN OR NAPROXEN FROM MICROCONTAINERS

For determining the release of ketoprofen or naproxen over time, a μ -Diss profiler (pION INC, Woburn, MA), equipped with *in situ* UV probes with a path length of 10 mm for ketoprofen and 5 mm for naproxen was used. The release studies were performed in PBS at pH 6.5 for 120 min. Standard curves of either ketoprofen or naproxen were obtained before each release experiment. In order to prepare the standard curves, aliquots of a stock solution of ketoprofen (5 mg/mL in ethanol) or naproxen (3 mg/mL in ethanol) were added to known volumes of PBS, and the absorbance was assessed in a wavelength range of 250-260 nm for ketoprofen and at a wavelength of 230 nm for naproxen.

For release experiments, the chips with drug-loaded microcontainers were attached to cylindrical magnets and placed inside glass vials. 10 mL of PBS buffer were added to the vials immediately before starting an experiment. All the release studies were run at 37°C stirring the chips at 100 rpm. The experiments were performed in triplicates for each drug and for each size

of microcontainers, the data are presented as mean (normalized by the quantity of PVP filled) $\pm$ SD.

2.2.4. THREE-DIMENSIONAL DISTRIBUTION OF DRUGS IN MICROCONTAINERS

Volumetric Raman microscopy was used to evaluate the distribution of ketoprofen or naproxen in the microcontainers. The microscope collected Raman spectra in the range of 350-2400 cm^{-1} with a spectral resolution of 2.5 cm^{-1} under the excitation of a 785 nm laser. The laser power was 35 mW, and the diffraction limited spot size was equal to 1.7 μm with the use of a 100x/0.75 HD DIC Zeiss microscope objective. The chip of microcontainers was placed on the surface of a custom-made Peltier stage and kept at 8°C during Raman measurements. These Raman spectra were studied performing a non-negative least squares analysis to obtain quantitative chemical response, visualized as voxel based 3D images.^{27,28}

2.2.5. SOLID STATE ANALYSES OF THE DRUGS LOADED INTO MICROCONTAINERS

X-Ray Powder Diffraction (XRPD) was used to determine the solid state form of ketoprofen or naproxen in the microcontainers. An X'Pert PRO X-ray diffractometer (PANalytical, Almelo, The Netherlands, MPD PW3040/60 XRD; Cu KR anode, $\lambda = 1.541 \text{ \AA}$, 45 kV, 40 mA) was utilized. A starting angle of 5° 2 θ and an end angle of 28° 2 θ were employed for the scans with a scan speed of 0.67335° 2 θ /min and a step size of 0.0262606° 2 θ . Data were collected using X'Pert Data Collector software (PANalytical B.V.). The diffractograms of naproxen or ketoprofen loaded in the microcontainers were compared to the pure crystalline drugs. In addition, Raman microscopy was used to investigate the solid state form of the drugs. The spectra measured from the naproxen or ketoprofen loaded into the microcontainers were collected as described in “Three-dimensional distribution of the drugs in microcontainers”

section. For the spectra of crystalline and amorphous ketoprofen, naproxen and PVP the laser power was 35 mW and the exposure time was 2 s. The amorphous ketoprofen was prepared by melting the crystalline ketoprofen powder at 98°C on a heating plate followed by immediate measurements of the sample.

2.2.6. STATISTICS

All data are expressed as mean ± standard deviation (SD). Statistical analyses were carried out, where relevant, using Student t-tests (GraphPad Prism, La Jolla, CA, USA, version 7.04). P-values below 5 % ($p < 0.05$) were considered statistically significant.

2.3. RESULTS AND DISCUSSION

2.3.1. FABRICATION OF MICROCONTAINERS

Cylindrical microcontainers with three different sizes were successfully fabricated (Table 1 and Figure 1). The cavity depth of the microcontainers with the different sizes was kept constant at 225 µm. The number of microcontainers per chip was chosen to keep the total polymer volume and the total surface area exposed to the supercritical CO₂ similar for the different sizes (Table 1). Due to this, it was possible to compare the influence of the microcontainer size on quantity and distribution of the poorly soluble model drugs loaded with supercritical CO₂ impregnation.

Table 1. Numbers showing the dimensions of the SU-8 microcontainers, amount of microcontainers per chip and total polymer surface area per chip. The data represents mean ± SD in 8 replicates.

Sample	Internal microcontainer diameter [µm]	Number of microcontainers per chip	Total polymer surface area exposed to scCO ₂ per chip [mm ²]
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Small	97 ± 6	1024	31 ± 4
Medium	191 ± 9	256	30 ± 3
Large	413 ± 5	64	34 ± 1

Figure 1. SEM images of SU-8 microcontainers in the size of a) small, b) medium and c) large having an internal diameter of $97 \pm 6 \mu\text{m}$, $191 \pm 9 \mu\text{m}$ and $413 \pm 5 \mu\text{m}$, respectively.

2.3.2. LOADING OF KETOPROFEN OR NAPROXEN INTO MICROCONTAINERS
USING SUPERCRITICAL CO₂ IMPREGNATION

Every chip was manually filled with approximately 0.9 mg of PVP powder (Figure 2a), the amount varied slightly for the different sizes (Table 2). Followed by filling with PVP, one chip per size was then simultaneously loaded with ketoprofen or naproxen. An SEM image of the medium size microcontainers after scCO₂ impregnation with ketoprofen can be seen in Figure 2b. In a previous study, the amount of PVP filled per chip was higher.¹⁴ This is due to the fact that, even if the medium size microcontainers have similar dimensions to that used in the previously reported study, the number of microcontainers is here reduced from 625 to 256.

Figure 2. SEM images of medium sized microcontainers a) filled with PVP and b) loaded with ketoprofen using scCO_2 impregnation. These images are representative examples of the PVP filling and the drug loading with the supercritical impregnation method.

2.3.3. *IN VITRO* RELEASE OF KETOPROFEN OR NAPROXEN FROM MICROCONTAINERS

The quantity of ketoprofen or naproxen loaded into the microcontainers with different sizes was evaluated in order to assess if there was an influence of the dimension of the surface exposed to scCO_2 . The quantity of the loaded ketoprofen or naproxen in small, medium or large microcontainers was obtained from the release studies (Table 2 and Figure 3). The release profiles of the small, medium and large microcontainers loaded with ketoprofen showed similar

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3 release profiles without any significant differences (Figure 3a). The same behavior was observed
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5 in the case of naproxen (Figure 3b).
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8 The total amount of ketoprofen loaded in the small size microcontainers compared to the
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10 medium and large microcontainers was not significantly different (p-value: 0.4049 and p-value:
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12 0.3667, respectively). No significant difference was observed between the loaded quantity of
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14 ketoprofen in the medium and in the large microcontainers (p-value: 0.8098). The same
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16 similarities, as observed for ketoprofen, were found for the total amount of loaded naproxen. In
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18 fact, the amount of naproxen in the small size microcontainer was not statistically different to the
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20 amount of drug in the medium or large microcontainers (p-value: 0.1071 and p-value: 0.2431,
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22 respectively). Comparing the medium with the large size microcontainers, the total amount of
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24 loaded naproxen also did not result in statistically different drug loadings (p-value = 0.3286).
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31 Since the solubility of the two drugs in the scCO₂ was set to be the same, the release
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33 experiments allowed for comparison of loading the two poorly water-soluble drugs into the
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35 microcontainers with three different sizes. Within the first 10 min, 90 % of ketoprofen or
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37 naproxen was released (Figure 3) from all sizes of microcontainers even if the release from small
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39 microcontainers loaded with naproxen showed a larger variability. No statistical difference in the
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41 loaded amount of ketoprofen or naproxen was discernible, independent of the size of the
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43 microcontainers. Comparing the loaded amount of ketoprofen and naproxen in small
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45 microcontainer sizes, the p-value was equal to 0.4374. For medium and large microcontainer
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47 sizes, the p-values corresponded to 0.0642 and 0.1351, respectively.
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51 Consequently, there was no difference in loading a BCS class II drug such as ketoprofen or
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53 naproxen in a polymer matrix (PVP) having smaller or larger surfaces exposed to the scCO₂.
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This suggests that the size of the microcontainer opening has no influence on the quantity of drug loaded into the microcontainers. Furthermore, both BCS class II drugs were released with similar kinetics from the different sizes of microcontainers.

Table 2. Amount of ketoprofen or naproxen loaded in the three different sizes of microcontainers. The data represents mean $\pm$ SD in triplicates.

	Amount of PVP filled per chip [mg]	Total amount of ketoprofen loaded per chip [μ g]	Total amount of naproxen loaded per chip [μ g]
Small	0.94 ± 0.36	128.3 ± 65.9	89.7 ± 40.95
Medium	0.82 ± 0.1	91.3 ± 20.1	160.54 ± 42.8
Large	1.03 ± 0.17	86.1 ± 28.7	128.23 ± 26.50

Figure 3. Release profiles of a) ketoprofen) and b) naproxen from small, medium and large microcontainers performed on a μ -Diss profiler in PBS at pH 6.5. The inserts represent the same profiles zoomed in on the first 10 min. The graphs represent mean $\pm$ SD in triplicates.

2.3.4. THREE-DIMENSIONAL DISTRIBUTION OF DRUGS IN MICROCONTAINERS

It was possible to obtain 3D maps of polymer and drug-loaded microcontainers down to a depth of 225 μm (the entire height of the microcontainer) using our custom-made Raman microscopy technique. To avoid heating of the sample, due to relatively high absorption of the laser, the temperature was kept constant at 8°C. To distinguish the various materials (PVP,

ketoprofen/naproxen, SU-8 or Si) in the samples, a chemical decomposition was performed on the spectra (Figure 4). In Figure 4, the same microcontainer 3D map reconstruction is shown in three different perspectives: an overview, a cross section view and a top view.

For all sizes of microcontainers loaded with either ketoprofen or naproxen, the drug was mainly impregnated in the top layers of the polymer matrix confined within the microcontainer walls. The results obtained in the *in vitro* release studies showed that both drugs reached 90 % of release within 10 min. The fast release could be explained by the fact that the drugs were mostly in the top part of the polymer matrix and not deep inside the microcontainer cavity. It is important to notice that the drug was distributed with the same morphology as PVP. It can therefore be speculated that, in a more porous polymer matrix, the drug could have penetrated deeper during the supercritical impregnation. In the top view of the microcontainers, it is possible to notice that both drugs were homogenously distributed on the PVP. Furthermore, ketoprofen and naproxen were absent at the edge of the microcontainers meaning that both drugs were preferentially deposited in the PVP matrix and not in microcontainer material, SU-8. This technique can be useful to analyze polymer matrices for drug delivery in tissue engineering since the drug depth in the polymer matrix affects the release kinetic of the drug.²⁹ A pharmaceutical application, in which this technique can also be successfully used, is the characterization of tablet coatings as the presence of holes or different thicknesses can change the solubility kinetics of the tablet.³⁰

Figure 4. Volumetric Raman maps of ketoprofen or naproxen loaded into the microcontainers. The overview, the lateral view and the top view can be seen from left to right for each of the different sizes of microcontainers: small, medium and large from top to bottom. Ketoprofen or naproxen are represented in red, PVP in green, SU-8 in yellow and Si in black. The scale bars correspond to 50 μm .

2.3.5. SOLID STATE ANALYSES OF THE DRUGS LOADED INTO MICROCONTAINERS

It has previously been shown that loading ketoprofen in a PVP matrix led to its conversion into its amorphous form.^{14,18} In Figure 5a, the diffractograms from XRPD of the small, medium and large size of microcontainers loaded with ketoprofen showed a halo, distinctive of an amorphous form. This indicated that the loaded ketoprofen was amorphous. In the case of naproxen loaded into the PVP matrix in the different sizes of microcontainer, the diffractograms also showed a halo for the small microcontainers (Figure 5b). For the medium and large microcontainers, the halo still appeared, but with few peaks comparable to those of the crystalline diffractogram of naproxen. Probably, a low crystallization of the drug occurred in the medium and large microcontainers. In literature, studies have showed that when naproxen has been combined with excipients or other drugs, a stable amorphous form could be obtained^{6,31} despite the high tendency of naproxen to recrystallize.³² In particular, Liu et al. showed that naproxen was amorphous even after 4 months when it was thermally treated and combined with PVP.⁶ A connection between the microcontainers size and the stability of the amorphous form of naproxen may therefore exist. This confirms what has previously been shown: smaller sizes of microcontainers prolong the stability of the amorphous form of indomethacin (BCS class II drug).⁵

Figure 5. (a) XRPD diffractograms of crystalline and amorphous ketoprofen, PVP, small, medium and large microcontainers filled with PVP followed by impregnation with ketoprofen. (b) XRPD diffractograms of crystalline naproxen, PVP, small, medium and large microcontainers filled with PVP followed by impregnation with naproxen.

The results obtained by means of XRPD were confirmed by Raman spectroscopy for both drugs (Figure 6). The spectra from the microcontainers loaded with ketoprofen were similar to an amorphous ketoprofen spectrum, confirming that ketoprofen is amorphous when loaded in PVP matrices by scCO_2 impregnation (Figure 6a).¹⁴ Due to the instability of naproxen, it was not possible to obtain a Raman spectrum of its amorphous form. Therefore, the peak-shifts were analyzed (Figure 6b). In particular, the peaks at 1626, 1390 and 740 cm^{-1} in the crystalline naproxen spectrum are shifted to 1630-1632, 1387-1389 and 742 cm^{-1} in the spectra corresponding to microcontainers loaded with naproxen meaning that naproxen loaded in the

microcontainers is not in its crystalline form. The Raman signal from large microcontainers loaded with naproxen, at the wavenumber of 740 cm^{-1} , showed a larger peak shift compared to the other sizes of microcontainers (Figure 6b zoom-in). This might be due to the fact that the Raman spectra were acquired from a random spot within a microcontainer in which also the contribution from other materials might be measured. Previously, the same peak-shifts have been considered, together with other techniques, to show the amorphous state of naproxen when co-milled with cimetidine.³³

Figure 6. a) Raman spectra of PVP, crystalline and amorphous ketoprofen, small, medium and large microcontainers filled with PVP followed by impregnation with ketoprofen. b) Raman spectra of PVP, crystalline naproxen, small, medium and large microcontainers filled with PVP followed by impregnation with naproxen. The zoom-in areas show the peak-shifts in naproxen loaded in microcontainers compared to its crystalline form.

CONCLUSIONS

In this study, the influence of the surface exposed to scCO_2 was evaluated when loading two poorly water soluble drugs in a PVP polymer matrix confined in microcontainers. The release studies showed that the amount of loaded naproxen or ketoprofen was the same, when keeping the total surface area constant, and the release profiles were similar having 90 % of the drug released within 10 min. For microcontainers of different sizes, the loaded amount of drug nicely correlated with the surface area of the PVP matrix exposed to supercritical CO_2 during impregnation. To evaluate the 3D distribution of the drug in the polymer matrix in the microcontainers, a custom-made Raman microscope allowed obtaining volumetric Raman maps of the complete microcontainer volume. In the small, medium and large microcontainers, ketoprofen or naproxen were impregnated in the top of the polymer matrix explaining the fast release observed in the release studies. Moreover, the solid state form of the drugs was evaluated, showing that ketoprofen was amorphous in all microcontainers sizes and naproxen, despite its instability, was found only to be partly crystalline.

SUPPLEMENTARY INFORMATION

In the supplementary information, SEM images of microcontainers are shown. In Figure S1, SEM images of small and large microcontainers filled with PVP are presented, and SEM images

of the different sizes of microcontainers loaded by scCO₂ impregnation with either ketoprofen or naproxen are represented in Figure S2 and S3, respectively.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Danish National Research Foundation (DNRF122) and Villum Fonden (Grant No. 9301) for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN). Nanna Bild, DTU Nanotech, is acknowledged for the drawings of the graphical abstract.

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Where is the drug? - Quantitative 3D distribution analyses of confined drug-loaded polymer matrices

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