Particle size reduction of meloxicam drug by pulsed laser ablation

Summary of the PhD dissertation

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1. SUMMARY

1.1 Introduction

For medicines, it is optimal to achieve the desired effect as quickly as possible, using the lowest possible dose and minimizing side effects. Once an active pharmaceutical ingredient (API) has been developed, excipients are added and drug formulation techniques are used to optimize the product and make it suitable for commercialization. According to the Biopharmaceutical Classification System (BCS), about 40% of marketed API-s and 90% of those under development are poorly water soluble [1]. This trend in drug development is a challenge for the pharmaceutical industry, as poor solubility limits bioavailability in most cases.

Among the potential solution options, different particle size reduction and amorphization techniques are quite common. Reducing particle size increases the active surface area, which generally improves the dissolution rate and transport characteristics, allowing human cells to take up the drug more rapidly [2], [3], whereas in the case of amorphization, the higher energy state due to the absence of a crystal lattice and the increased mobility within the system result in higher solubility [4].

Pulsed laser ablation can be used to produce micro- and nanometer-sized particles from a bulk material, the crystallinity of which can also change as a result of irradiation. During ablation, a focused laser pulse is absorbed in the target, which causes a cloud of material to form perpendicular to the target surface, which may contain electrons, ions, atoms, molecules, melt droplets and solid particles [5]. In addition to these primary products, there may also be secondary particles that are produced by the impact of the ablation shock wave on the target. The optical, thermal and morphological properties of the target are significant in this process. Examples of both organic and inorganic particle production was demonstrated using the right selection of laser parameters and experimental conditions. [6] The generated particles need to be analyzed primarily in terms of their chemical composition, namely whether they have been degraded by irradiation, whether by-products have

been formed and, if so, which compounds were generated and in what quantities. Secondly, the size, size distribution, morphology and crystallinity of the particles should be investigated. In addition to stoichiometry and size, it is important to analyze and characterize the mechanism of particle generation and to carry out pharmaceutical applicability studies.
In my work, I have studied laser ablation of pure active pharmaceutical ingredient tablets as a particle size reduction and amorphization technique. In my dissertation, I present the experiments and results obtained on the laser ablation of meloxicam, a poorly water-soluble non-steroidal anti-inflammatory drug. In order to provide a background, I first give a literature overview of the motivation behind these experiments, present the studied drug and describe the methods of solubility enhancement and their results, with particular emphasis on particle size reduction and amorphization. Next, I present the scientific background of laser ablation, highlighting publications on the irradiation of active pharmaceutical ingredients. After presenting the objectives of my dissertation, I describe the properties of the investigated material and target, the parameters of laser ablation and the preparation of samples for analysis. For each investigating technique used, I briefly describe the theoretical background and the equipment design and finally the applied parameters. I then present the results of the measurements carried out on the chemical analysis, the size and morphology of particles generated by nanosecond and femtosecond pulses, the study of the particle generation process and the pharmaceutical applicability. For convenience, the results of each investigation are immediately followed by a discussion of their evaluation and the conclusions drawn from them. I conclude my thesis by summarizing my scientific achievements.

1.2. Objectives and studies

In my research, I investigated pulsed laser ablation as a particle size reduction and amorphization technique to improve the bioavailability of water-insoluble or poorly water-soluble active pharmaceutical ingredients. As a model drug, meloxicam, a non-steroidal anti-inflammatory drug with very low

water solubility, was chosen due to its relatively high melting point and wide applicability.

A series of systematic experiments were carried out under various experimental conditions (laser parameters, media) during the ablation of meloxicam tablets in order to find the ideal parameter window. I aimed to investigate whether this method could be used to produce pure meloxicam particles of ideal size for pulmonary delivery (in the size range 0.5–5 μm [7]) without chemical damage.

First, I investigated the nanosecond pulsed laser ablation of meloxicam in distilled water medium in relation to the applied wavelength and fluence. The generated particles were analyzed comprehensively in terms of chemical composition, morphology and size distribution, as well as pharmaceutical applicability. In addition to characterizing the particles, I also aimed to describe the particle generation process.

Secondly, I irradiated meloxicam targets with femtosecond pulses in distilled water and in air, analyzing the effect of the applied fluence and repetition rate. For these particles, stoichiometry, size reduction, morphology, thermal and crystallinity characteristics were also studied. I analyzed the influence of the medium on the model of the particle generation mechanism.

In summary, my aim was to provide an experimental and theoretical basis for the applicability of laser ablation induced size reduction of APIs, thus enabling the improvement of the effectiveness of water-insoluble or poorly water-soluble drugs, the development of alternative drug formulations and application of drug delivery routes.

1.3. New scientific results

The rising number of poorly water-soluble active substances in the pharmaceutical industry has led to the development of a number of processes to improve solubility and thus bioavailability. These mechanisms include particle size reduction, which achieves the improvement through increased active surface area, and amorphization, which achieves the desired outcome through the dissolution of the crystal lattice.

In my research, I have investigated whether laser ablation, which is widely used in nanotechnology, can be applied as a particle size reduction and

amorphization technique for active pharmaceutical ingredients. To test the effectiveness of the laser ablation method, meloxicam, a non-steroidal anti-inflammatory drug with poor water solubility, was chosen as a model drug. The generated particles were subjected to extensive chemical, morphological and pharmaceutical applicability studies, additionally the underlying mechanism was also investigated. The results of my work are summarized in the following thesis points.

T1. Nanosecond pulsed laser ablation of a meloxicam target in distilled water (PLAL): particle generation and characterization

I have investigated the effect of nanosecond laser ablation parameters on particle generation. I have generated particles by irradiating a meloxicam tablet target with different wavelengths (λ = 248, 532 and 1064 *nm*) and fluences ($\sim$F = 4–13 J/cm^2).

T1/A I have analyzed the PLAL generated particles and the reference meloxicam powder by FTIR and Raman spectroscopy. Comparing the results, I have found that laser ablation did not induce significant changes in the spectra, the generated particles predominantly retained their chemical composition. HPLC-MS measurements performed by our collaborating partners confirmed that only a small amount of degradation was detectable and meloxicam impurity B was the only identifiable by-product. **[S1] [S3]**

T1/B The morphology and size distribution of the generated particles were characterized by scanning electron microscopy and crystallinity was investigated by XRPD. I have demonstrated that the PLAL technique can be used as a particle size reduction technique: particles resembling broken, fragmented crystal pieces, ranging from a few hundred nanometers to a few micrometers in size were produced, which is the optimal size range for pulmonary drug delivery. No detectable amorphization took place during laser ablation and the particles remained crystalline in structure, like the initial material. **[S1] [S3]**

T1/C The spectrophotometric extinction studies provided information on the parallel occurrence of sedimentation and dissolution processes, the time evolution of which is an important parameter for further formulation design. I have demonstrated that from the laser ablation generated sample more meloxicam can be dissolved than from the same amount of the reference powder. This was confirmed by the solubility tests performed by our collaborating partners: compared to the reference, the solubility of the laser ablation produced samples increased by several-fold (4-8-9-fold). **[S1] [S3]**

T1/D Based on the cytotoxicity and anti-inflammatory effect measurements performed by our collaborating partners on the samples I have generated and prepared, I have concluded that the anti-inflammatory effect of the PLAL generated particles was significantly greater than that of the initial commercially available meloxicam. On the basis of the determined solubility, cytotoxicity and anti-inflammatory properties, it is expected that the same therapeutic effect can be achieved at a significantly lower drug dose using the particles produced by the PLAL technique. **[S3]**

T2. Characterizing the particle generation process/ mechanism of the nanosecond pulsed laser ablation of a meloxicam target in distilled water

T2/A I have developed an approximate thermal model to describe the temperature change in the irradiated target, using the absorption coefficient determined by ellipsometry measurements and the modified Lambert-Beer law. The thermal calculations have indicated that due to the irradiation the top layer of the target ($\sim$100 *nm* – a few μm) could reach a temperature of approximately 10^3–10^5 K which is highly above the melting/ degradation temperature of the material. **[S1]**

T2/B In order to explore and describe the ablation process, I have built a pump-probe fast photography system to monitor the evolution of the shock wave after laser ablation into an acoustic wave, the formation and pulsating size changes/ rebounding dynamics of the cavitation bubbles, and the particle release into the liquid medium. Based on the measurements I have confirmed

the previous hypothesis that the mechanical disintegrating, shredding effect of the shock wave is the dominant mechanism in particle generation. **[S3]**

T2/C Based on our experiments, I have determined that the mechanism of meloxicam particle generation by nanosecond PLAL process consists of the following main steps: a) The energy of the laser pulse is absorbed by the surface layer of the target, where the material is heated, it evaporates and decomposes. b) The rapid changes in temperature, pressure and the expansion result in the generation of a shock wave and the emerging recoil forces tear out particles from the residual surface. c) The propagation velocity of the shock wave decreases to the acoustic wave range and a cavitation bubble is formed in the liquid. d) The pulsating size changes/ rebounding dynamics of the cavitation bubble occur, which can lead to further size reduction. e) After the collapse of the cavitation bubble, the generated particles are dispersed in the liquid medium. **[S1] [S3]**

T3. Femtosecond pulsed laser ablation of a meloxicam target in distilled water (PLAL): particle generation and characterization

I have investigated the effect of femtosecond laser ablation parameters on particle generation. I have generated particles by irradiating a meloxicam tablet target in different media (distilled water and air), with different fluences (∼F = 0.7–1.9 J/cm^2) and repetition rates (f = a few *Hz* or 1 *kHz*).

T3/A The FTIR studies have shown that regardless of the applied fluence and repetition rate, in the case of ablation in air (PLA) the positions of the characteristic FTIR peaks were identical with the reference although their relative intensities varied, while for ablation in water (PLAL) the spectra matched well with the spectrum of the initial material in all their properties. The Raman spectra of the laser ablation generated particles matched the reference spectrum for both media. Based on the HPLC-MS measurements performed by our collaborating partners, I have concluded that both PLA and PLAL samples contained meloxicam impurity B as a by-product, which, being

a fragment of the meloxicam molecule, is difficult to detect by FTIR and Raman spectroscopy. **[S2] [S4]**

T3/B The morphology of the particles was characterized using scanning electron microscopy. I have observed significant differences between particles produced in air and in distilled water media. I have demonstrated that in the case of PLA, the change of the repetition rate affected the morphology of the particles. In this case, predominantly spherical particles were obtained over a wide range of sizes (~0.1 – a few μm in diameter) with a varied surface texture (smooth or covered with crystal blocks). In contrast, in the case of PLAL, some of the particles were crystalline in nature, resembling broken glass pieces, while there were also amorphous-like particles with rounded edges and shapeless, aggregated/ agglomerated mass as well. **[S2] [S4]**

T3/C As the result of the low repetition rate laser ablation in air (PLA) I have produced predominantly sub-micrometer particles and concluded that changing the energy density within the investigated range only affected the yield, not the size distribution. This was confirmed by the SMPS measurements performed by our collaborating partners. **[S2]**

T3/D I have compared the XRPD and DSC curves of the laser ablation generated samples with those of the reference material. In the XRPD spectra of PLA and PLAL samples of the same weight, the position of the characteristic peaks did not change, while their intensities decreased significantly compared to the reference. Based on the examination of the DSC curves of the samples, I have observed that their endotherm melting peaks shifted towards lower temperatures, flattened and broadened. In both measurements, the PLA samples deviated more from the reference than the PLAL samples. Together with the results of the FTIR and SEM studies, the changes suggest that partial amorphization may have occurred, while significant part of the generated material retained its initial polymorphic form, which was the enolic form/ form I. **[S2] [S4]**

T3/E Based on the concentration measurements performed by our collaborating partners on the samples I have generated and prepared, I have verified that the solubility of both PLA and PLAL generated particles has improved compared to the initial powder. **[S4]**

T4. Characterizing the particle generation process/ mechanism of the femtosecond pulsed laser ablation of a meloxicam target in distilled water and in air

T4/A Based on thermal calculations, I have demonstrated that at the applied fluences, for both PLA and PLAL, the temperature of the surface layer of the target significantly exceeded the melting temperature of meloxicam. I have verified with heating experiments that the melting and decomposition temperatures of meloxicam do coincide at normal atmospheric pressure and that degradation can be detected by FTIR and Raman spectroscopy. I have aimed to describe the mechanism based on a synthesis of scanning electron microscopy images of the generated particles and the ablated tablet surfaces, as well as other analytical studies and literature. I have concluded that the medium in which the ablation took place fundamentally determines the nature of the particle generation process. **[S2] [S4]**

T4/B In the case of ablation in air, both the spherical shape of the generated particles and the surface of the ablated tablet were indicating previous melting, which led me to conclude that thermal effects were dominant in the particle generation process. The chemical analysis confirmed that the generated particles were not degraded despite melting. This was presumably the result of the specific pressure and temperature parameters of femtosecond laser ablation, which allowed a phase transition (melting) below the decomposition temperature. **[S2] [S4]**

T4/C I have described the formation of nanoaggregate chains for an organic molecule, meloxicam, a phenomenon previously described only for elementary compounds. In addition, I have demonstrated the production of

excipient-free meloxicam spheres, which are aerodynamically and dimensionally ideal for pulmonary delivery. **[S2] [S4]**

T4/D Based on the presence of crystalline-like, amorphous-like particles and aggregated/ agglomerated mass in the samples generated by ablation in distilled water medium, I have concluded that several mechanisms were involved in the ablation process. As a mechanical effect, the ablation induced shock wave could tear out particles from the surface of the bulk material, and in addition, we can observe indications of a thermal effect. At the increased repetition rate, the interaction of particles already in the suspension with the laser irradiation, i.e., laser fragmentation, may also be significant. **[S2] [S4]**

2. PUBLICATIONS

MTMT identifier: 10073972

2.1 List of peer-reviewed articles published in international journals related to the thesis points

[S1] B. Hopp, E. Nagy, F. Peták, T. Smausz K., J. Kopniczky, Cs. Tápai, J. Budai, I. Z. Papp, Á. Kukovecz, R. Ambrus, and P. Szabó-Révész, "Production of meloxicam suspension using pulsed laser ablation in liquid (PLAL) technique," J Phys D Appl Phys, vol. 51, no. 16, p. 165401, Mar. 2018, doi: 10.1088/1361-6463/aab4be.

[S2] E. Nagy, A. Andrásik, T. Smausz, T. Ajtai, F. Kun-Szabó, J. Kopniczky, Z. Bozóki, P. Szabó-Révész, R. Ambrus, and B. Hopp, "Fabrication of Submicrometer-Sized Meloxicam Particles Using Femtosecond Laser Ablation in Gas and Liquid Environments," Nanomaterials, vol. 11, no. 4, p. 996, Apr. 2021, doi: 10.3390/nano11040996.

[S3] E. Nagy, Z. Homik, T. Smausz, J. Kopniczky, M. Náfrádi, T. Alapi, D. Kokai, K. Burián, P. Szabó-Révész, R. Ambrus, and B. Hopp, "A comprehensive analysis of meloxicam particles produced by nanosecond laser ablation as a wet milling technique," Sci Rep, vol. 12, no. 1, pp. 1–13, Dec. 2022, doi: 10.1038/s41598-022-16728-9.

[S4] E. Nagy, J. Kopniczky, T. Smausz, M. Náfrádi, T. Alapi, J. Bohus, V. Pajer, P. Szabó-Révész, R. Ambrus, and B. Hopp, "A comparative study of femtosecond pulsed laser ablation of meloxicam in distilled water and in air," Sci Rep (Under Review), Jan. 2023, doi: 10.21203/RS.3.RS-2506430/V1.

2.2 List of additional peer-reviewed articles published in international journals

[S5] R. Ambrus, P. Szabó-Révész, T. Kiss, E. Nagy, T. Szűcs, T. Smausz, and B. Hopp, "Application of a suitable particle engineering technique by pulsed laser ablation in liquid (PLAL) to modify the physicochemical properties of poorly soluble drugs," J Drug Deliv Sci Technol, vol. 57, p. 101727, Jun. 2020, doi: 10.1016/j.jddst.2020.101727.

[S6] T. Gera, E. Nagy, T. Smausz, J. Budai, T. Ajtai, F. Kun-Szabó, Z. Homik, J. Kopniczky, Z. Bozóki, P. Szabó-Révész, R. Ambrus, and B. Hopp, "Application of pulsed laser ablation (PLA) for the size reduction of non-steroidal anti-inflammatory drugs (NSAIDs)," Sci Rep, vol. 10, no. 1, pp. 1–13, Dec. 2020, doi: 10.1038/s41598-020-72865-z.

2.3 Conference presentations and posters

[S7] E. Nagy, F. Peták, T. Smausz K., T. Bellák, A. Nógrádi, and B. Hopp, "Sejtmintázatok kialakítása lézeres technikával", Kvantumelektronika 2018: VIII. Szimpózium a hazai kvantumelektronikai kutatások eredményeiről, p-34, 2018.

[S8] E. Nagy, F. Peták, T. Smausz K., R. Ambrus, P. Szabóné R., and B. Hopp, "Gyógyszerhatóanyag aprítás folyadék alatti lézeres ablációval", Kvantumelektronika 2018: VIII. Szimpózium a hazai kvantumelektronikai kutatások eredményeiről, p-33, 2018.

[S9] E. Nagy, F. Petak, T. Szucs, T. Smausz, J. Kopniczky, R. Ambrus, P. Szabo-Revesz, and B. Hopp, "Production of Drug Particles using Pulsed Laser Ablation in Liquid (PLAL) Technique," in 2019 Conference on Lasers and Electro-Optics Europe & European Quantum Electronics Conference (CLEO/Europe-EQEC), pp. 1–1., 2019, doi: 10.1109/CLEOE-EQEC.2019.8873211.

[S10] E. Nagy, Z. Homik, T. Smausz, J. Kopniczky, R. Ambrus, P. Szabó-Révész, and B. Hopp, “Laser ablation of meloxicam in liquid environment,” in International Workshop on Laser-Induced Breakdown Spectroscopy, pp. 84–88, 2020.
[S11] T. Smausz, E. Nagy, T. Gera, Z. Homik, J. Kopniczky, T. Ajtai, R. Ambrus, P. Szabó-Révész, M. Ehrhardt, K. Zimmer, P. Lorenz, and B. Hopp, “Study of the fragmentation of solid drug particles during ablation with different pulse length lasers,” in International Workshop on Laser-Induced Breakdown Spectroscopy, pp. 32–37, 2020.

[S12] T. Gera, E. Nagy, T. Smausz, Z. Homik, J. Kopniczky, J. Budai, T. Ajtai, R. Ambrus, P. Szabó-Révész, and B. Hopp, “Size reduction of drug particles by pulsed laser ablation technique,” in Kvantumelektronika 2021, pp. 59–63, 2020.

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[5] D. Péter *et al.*, “D2 kurzus: OPTIKAI ALAPOK AZ ELI-ALPS TÜKRÉBEN II.”

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