SUMMARY OF THE PHD DISSERTATION

Treatment of drugs by pulsed laser ablation in gaseous environment

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1. Introduction

In recent decades and today, one of the most popular areas of drug research is the improvement of critical properties of active drugs. The two main goals of modern pharmaceutical technology research are to maximize the bioavailability of already well-proven active ingredients and to reduce adverse side effects and toxicity without altering the underlying mechanisms of action. In addition, in order to maximize efficacy, there is a need to develop new drug formulations that can be used in a targeted, spatially and temporally well-defined manner in different segments of the human body. The bioavailability of drugs is defined as the fraction of an administered drug that reaches the systemic circulation. This is mainly influenced by the solubility properties of the active ingredients. The slowest process of the drug's journey through the body is absorption, which can be accelerated by improving the dissolution properties of the drug and/or creating new alternative routes of administration (*e.g.*, dermal, pulmonary administration). Therefore, the improvement of dissolution properties is one of the most important tasks of pharmaceutical technology today, and many different methods have been developed to achieve this. These methods include for example, salt formation, various dispersion processes, nanosuspension, amorphization, and particle size reduction. The most promising of these are particle size reduction and amorphization, which can significantly increase the efficacy of drugs. A large group of active substances requiring such improvements are non-steroidal anti-inflammatory drugs (NSAIDs), as they are highly insoluble in water in their usual commercially available form. Innovative nanotechnology can be used to produce nanocomposite particles with specific (*e.g.*, magnetic or pH-sensitive) properties, which can be used for targeted drug delivery and controlled release. Nanocomposite particles form the core of many modern medical diagnostic and therapeutic applications and are also the focus of attention in today's research. The most commonly used magnetic nanoparticle is magnetite (Fe_3O_4) due to its high biocompatibility and superparamagnetic properties. The applicability of magnetic nanoparticles and nanocomposites is highly dependent on the size, shape and surface properties of the particles. [1,2]

For inorganic materials and simple polymers, amorphization and particle size reduction using lasers are already common practices. Using Pulsed Laser Ablation (PLA), we can reduce the particle diameter of certain materials down to the nanometer range. Furthermore, in recent years, inorganic nanocomposite particles have also been successfully produced using PLA. Pulsed laser deposition (PLD) can be used to produce thin films with specific physical properties (*e.g.* crystallinity) by carefully adjusting the experimental parameters. Lasers are one of the most preferred and most efficient tools in material processing and are widely used. Laser light can be considered a highly reliable, wear- and contact-free (sterile) "tool" that can produce much higher quality products much more economically than conventional machining methods. This is also true for the laser processing of organic and medical materials, where its greatest advantage is clearly its sterility. [3–5]

2. Experimental methods

In my dissertation, I describe a series of applications of pulsed laser pharmaceutical technology in a gas medium. All applications were based on the PLA method, which was used to reduce the particle size of some of the poorly water-soluble drug substances (ibuprofen, meloxicam, niflumic acid) and to modify the crystal structure of ibuprofen. In addition, I prepared magnetic nanocomposites containing magnetite nanoparticles and drugs.

The essence of PLA is to focus the high-energy laser pulse on the surface of the target. Due to the energy absorbed, high temperatures are generated in the target and material is ejected from it in small explosions in the direction of the surface normal. The properties of the particles ejected during ablation and the crater created in the target are determined by the optical (absorption), thermodynamic, topological and mechanical properties of the irradiated material, as well as by the parameters of the laser beam (wavelength, pulse time, energy density). PLA has been very successful in a variety of applications, such as nanometer-precision material processing in industry, the creation of nanoparticles and nanocomposites, and the restoration of works of art. However, its most successful applications for humans have been found in medicine, as it provides the basis for many modern medical treatments, such as the treatment of cancer cells, kidney

stone removal, and well-known eye correction surgeries. In my experiments, I irradiated drug tablets containing pure drug substance (or pure drug mixed with magnetite nano-powder) with three laser beams of different wavelengths (UV, visible and near-infrared). For UV irradiation I used a KrF excimer laser (LLG Twinamp, FWHM = 18 ns, λ = 248 nm, f = 10 Hz), while for the other two wavelengths I used the first and second harmonics of a Nd: YAG laser (Quantel, FWHM = 6 ns, λ = 532 nm / 1064 nm, f = 10 Hz). The tablets were prepared with a hydraulic press at a pressure of 175 MPa. During ablation, I varied the energy densities between 1.5 Jcm^{-2} and 15 Jcm^{-2}, differently for each drug. The produced particles were transported and collected with the help of 0.3-2 l/min N_2 gas flow. During the generation of nanocomposites, I also used an external magnetic field to study the magnetic properties of the resulting particles. The other method I use is PLD which is based on PLA. In PLD, not only the target is placed in the chamber, but a substrate is also placed in front of the target to collect the ablated particles. By varying the number of pulses hitting the target, a thin layer of virtually any thickness can be deposited on the substrate. PLD is usually performed under vacuum to increase efficiency. The great advantage of PLD is that by choosing the right laser parameters, it is possible to produce a thin layer of practically any material with good quality and arbitrary composition, while the process of layer building can be precisely controlled. Besides industrial applications, PLD is also used extensively in medical biology, where biocompatible coatings are made, and has recently shown promising results in the field of cell and tissue printing. Using KrF excimer laser (emitting in the UV range) , I managed to modify the crystal structure of ibuprofen by PLD. At different vacuum chamber pressures (1 - 10^{-4} mbar) the ablated particles were deposited on KBr substrates. I kept the energy density of the laser beam fixed at 3 Jcm^{-2} and created each thin layer with 10,000 pulses. The physical and chemical properties of the ablated drugs were investigated using different methods to make sure that the particles / thin films are suitable for the development of new drug formulations and for human testing. For this purpose, infrared and Raman spectroscopic measurements as well as scanning electron microscopy and energy dispersive X-ray spectroscopic studies were performed. Moreover, comprehensive size distribution studies were carried out on the produced particles in the range

of 10 nm to 10 μm. In order to get an idea of the formation mechanism of the resulting particles, fast imaging was performed using a pump-probe setup.

3. New scientific results

In my thesis, I investigated the applicability of laser irradiation for the development of a new type of pharmaceutical formulation process. I conducted extensive experimental studies to see how the active ingredients respond to the adjustment of laser parameters and other experimental conditions. I have inspected whether the chemical composition and/or any physical parameters (particle diameter, crystallinity, morphology) of the drug change during laser ablation, as these are key properties for its future therapeutic use. I interpreted the experimental results based on theoretical considerations, and summarize my findings thematically in the following thesis points:

3.1 Thesis points

T1. Particle size reduction of non-steroidal anti-inflammatory drugs (NSAIDs) by pulsed laser ablation (PLA).

Most NSAIDs have very low solubility in water. Therefore, to achieve the desired therapeutic effect, a relatively high intake of the drug is required, often leading to gastrointestinal complications. By reducing the particle size, the dissolution rate of the active substances can be increased, thus the amount of substance needed can be reduced and adverse side effects can be avoided.

T1/A. I have shown that pulsed laser ablation can be successfully applied to reduce the particle size of poorly water-soluble drugs (*e.g.*, ibuprofen, niflumic acid and meloxicam) by several orders of magnitude. Using laser beams of different wavelengths (532 nm; 1064 nm) and nanosecond pulse durations (6 ns for λ=532/1064 nm), nano- and submicrometer-sized drug particles could be prepared. Due to the increased surface-to-volume ratio of the resulting particles, the dissolution rate of the drugs has improved.

T1/B. Using FTIR and Raman spectroscopy, I have shown that the chemical composition of the particles ablated by VIS and IR laser pulses is identical to that of the original active ingredients. I have also shown that ablation with ultraviolet laser beam results in chemically degraded drug

particles. The chemical composition of the particles produced was independent of the energy density of the pulses in the investigated range (1.5-15 Jcm^{-2}).

T1/C. Particle size distribution studies over a wide size range (10 nm-10 μm) have shown that the average size of the generated particles falls in the submicrometer regime depending on the specific drug and the wavelength of the applied laser beam. **[S1] [S8]**

T2. Investigation of the drug particle formation mechanism during the ablation process.

Considering the relatively high energy density of laser pulses and the sensitivity (*e.g.* thermal stability) of drug substances at the same time, it is a legitimate question to ask, what the ablation mechanism could be, by which the particle size is reduced without any chemical damage. Therefore, I have studied the laser ablation process in detail.

T2/A. I have shown that the ejection of chemically intact drug particles is caused by secondary (photomechanical) processes during the ablation.

T2/B. Ellipsometric studies were performed to determine the optical absorption of the drug tablets. Based on these data, temperature calculations were performed within the absorption volumes. My results showed that the temperature in the absorption volume was higher than the decomposition temperature of the active pharmaceutical ingredient in all cases. Thus, the chemically non-degraded particles cannot originate from the primary processes of ablation.

T2/C. To monitor the ablation processes over time, a typical pump-and-probe setup was built. Using the recordings, I determined the velocity of the shock wave propagating outwards from the excited area and then, based on this, I calculated the recoil pressure exerted on the surface by the shock wave. The calculations revealed a significant recoil pressure (80-350 atm), indicating strong photomechanical effects responsible for the fracturing of the particles. **[S1] [S8]**

T3. Preparation of amorphous and mixed (partly amorphous/partly crystalline) phase ibuprofen thin films by pulsed laser deposition (PLD).

In addition to particle size reduction, amorphization is a well-known technique to increase the solubility and thus bioavailability of poorly soluble drugs. However, the production of amorphous phase often requires very complex procedures. Laser irradiation offers a relatively simple crystal engineering method for the production of amorphous phases of active pharmaceutical ingredients.

T3/A. Amorphous and mixed phase ibuprofen thin films were prepared by PLD using a UV laser beam (λ=248 nm). I varied the pulse durations of laser beams (18 ns /500 fs) and the pressure applied in the experimental chamber (1 bar-10^{-4} mbar).

T3/B. Infrared (FTIR) and Raman spectroscopy were used to determine the chemical composition and chemical homogeneity of the thin films. The material of the thin film produced with femtosecond pulses degraded, as did the one produced at atmospheric pressure.

Using nanosecond laser pulses, ibuprofen thin films with mixed (crystalline and amorphous) aggregates were successfully produced at high chamber pressures (10-10^{-1} mbar), while purely amorphous thin films were produced at lower pressures (10^{-2}-10^{-4} mbar).

T3/C. The crystallinity of the layers was studied by Scanning electron microscopy (SEM)and differential scanning calorimetry (DSC). The results verified our observations described in the previous paragraph (*i.e.*, the FTIR and Raman spectroscopy results were confirmed). **[S2] [S7]**

T4. Production of magnetic drug nanocomposite particles by pulsed laser ablation.

Magnetic drug nanocomposites can provide multifunctional theranostic platforms and allow for a combination of diagnostics, monitoring and therapeutics. Using magnetic nanocomposites for targeted delivery and controlled release of drugs, better treatment efficiency and less side effects can be achieved.

T4/A. I was the first to apply PLA to produce magnetic drug nanocomposite particles. Using target tablets composed of commercially available ibuprofen (particle size ~5 μm) and magnetite nanoparticles

(particle size ~50 nm), and applying Nd:YAG laser beams (532 and 1064 nm), I successfully produced magnetic ibuprofen-magnetite composite nanoparticles.

T4/B. Spectroscopic (FTIR, Raman, SEM-EDX) studies has shown, that the ablated particles contain both magnetite NPs and ibuprofen in its original chemical form (*i.e.*, without any chemical degradation).

T4/C. Particle size distributions obtained by SMPS revealed that some of the composite particles fall in the nanometer size range. It has also been found that the ibuprofen:magnetite ratio of the target and the wavelength of the laser have no significant effect on the size of the particles produced under the selected experimental conditions.

T4/D. The experiments described in the previous paragraphs (T4/A-C) were repeated in the presence of an external magnetic field. Fast photography studies demonstrated that the two ingredients (magnetite and ibuprofen) definitely merge during PLA, and composite particles are formed. **[S3]**

3.2 Additional point for thesis

Although the next chapter does not belong to the thesis points of the dissertation, it plays an essential role in the completion of it. The studies presented here confirm the improved critical properties of PLA-derived drug formulations compared to the reference materials.

3.2.1. Investigation of the dissolution properties and bioavailability of NSAIDs prepared by PLA method

Investigating the medical applicability of drug particles created by laser ablation, there is also a need for pharmaceutical technology measurements to provide information on the dissolution and toxicity properties of the new formulations. These measurements were carried out at the Institute of Pharmaceutical Technology and Pharmacovigilance of the University of Szeged.

1/A. For all three drugs used, the dissolution rate and solubility of the ablated and reference materials were studied in aqueous solution at pH 7.4 which is the typical pH of the gut and lungs. In all cases, the ablated particles showed higher dissolution rates than the starting materials. The solubility did not change significantly for ibuprofen and niflumic acid, while for meloxicam it doubled compared to the original value.

1/B. The cytotoxicity measurements of the new formulations were performed on A549 cells modelling the alveolar wall of the lungs. Studies have shown that particles induced by laser ablation are suitable for pulmonary drug delivery. The ablated ibuprofen and meloxicam showed half the toxicity of the reference substances, which is an outstanding result, while in the case of niflumic acid the ablated substance was slightly more toxic than the original. The anti-inflammatory effect of the samples was also studied, where we found that all of the new formulations were more effective in reducing inflammation than commercially available pure active ingredients.

Publications

Publications related to the thesis points

[S1]: Gera T, Nagy E, Smausz T, Budai J, Ajtai T, Kun-Szabó F, Homik Z, Kopniczky J, Bozóki Z, Szabó-Révész P, Ambrus R and Hopp B 2020 Application of pulsed laser ablation (PLA) for the size reduction of non-steroidal anti-inflammatory drugs (NSAIDs) *Sci. Rep.* 10 15806

[S2]: Gera T, Smausz T, Kopniczky J, Galbács G, Ambrus R, Szabó-Révész P and Hopp B 2019 Production of ibuprofen in crystalline and amorphous forms by Pulsed Laser Deposition (PLD) *Appl. Surf. Sci.* **493** 359–67

[S3]: Gera T, Smausz T, Ajtai T, Kurilla B, Homik Z, Kopniczky J, Bozóki Z, Szabó-Révész P, Ambrus R and Hopp B 2021 Production of ibuprofen-magnetite nanocomposites by pulsed laser ablation *J. Phys. D. Appl. Phys.* **54** 395401

Other publications

[S4]: Ludasi K, Jójárt-Laczkovich O, Sovány T, Hopp B, Smausz T, Andrásik A, Gera T, Kovács Z and Regdon jr G 2021 Anti-counterfeiting protection, personalized medicines − Development of 2D identification methods using laser technology *Int. J. Pharm.* **605** 120793

[S5]: Smausz T, Kondász B, Gera T, Ajtai T, Utry N, Pintér M, Kiss-Albert G, Budai J, Bozóki Z, Szabó G and Hopp B 2017 Determination of UV–

visible–NIR absorption coefficient of graphite bulk using direct and indirect methods *Appl. Phys. A* **123** 633

[S6]: Hopp B, Smausz T, Lentner M, Kopniczky J, Tápai C, Gera T, Csizmadia T, Ehrhardt M, Lorenz P and Zimmer K 2017 Stability investigation of laser darkened metal surfaces Appl. Phys. A Mater. Sci. Process. 123

Conference papers

[S7]: Gera T, Smausz T, Kopniczky J, Ambrus R, Szabo-Revesz P and Hopp B 2019 Production and Characterization of Ibuprofen Particle Layer Generated by Pulsed Laser Deposition (PLD) 2019 Conference on Lasers and Electro-Optics Europe & European Quantum Electronics Conference (CLEO/Europe-EQEC) vol Part F140- (IEEE) pp 1–1

[S8]: Gera T, Smausz T, Kopniczky J, Galbács G, Ambrus R, Szabó-Révész P and Hopp B 2021 Size reduction of drug particles by Pulsed laser ablation technique ed P Földi and I Magashegyi (Szeged: Szegedi Tudományegyetem Természettudományi és Informatikai Kar Fizikai Intézet) pp 59–64

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[3] Ashfold M N R, Claeyssens F, Fuge G M and Henley S J 2004 Pulsed laser ablation and deposition of thin films 23–31

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[5] Ortaç B, Şimşek E U and Kurşungöz C 2017 Nanoparticles, Nanocrystals, and Nanocomposites Produced with Pulsed Laser Ablation and Their Applications *Laser Ablation - From Fundamentals to Applications* (InTech)