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Summary of the Ph.D. thesis

DEVELOPMENT OF MODERN DRUG IDENTIFICATION TECHNOLOGIES USING LASER TECHNOLOGY, AGAINST DRUG COUNTERFEITING

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1. INTRODUCTION

Medicines have always been high value-added products, have a high intellectual, scientific and technical value, and are expensive to manufacture. It is understood that the production and distribution of such special products are subject to strict quality assurance, thus ensuring the safety of medicines for users and prescribers and recommenders (doctors, pharmacists).

The production of drugs is officially regulated by law, it requires a Manufacturing Drug License, and these processes are controlled by the authorities. Good manufacturing practice (GMP) gives the minimum standard that a medicines manufacturer must meet during production. It has the force of law, requiring that manufacturers, processors, and packagers of drugs ensure that their products are effective and safe with adequate purity. This protects the consumer from purchasing a product that is not effective or even dangerous.

Unfortunately, it is not surprising that counterfeiters have entered the market for such high-value-added products in the hope of making a quick profit.

Substandard and falsified (SF) medicines pose a serious threat to global public health. According to the World Health Organization (WHO), it is estimated that 1 in 10 medical products is substandard or falsified in low- and middle-income countries where health systems are weak or non-existent. It is threatening that over 50% of medicines purchased over the Internet are counterfeit in the cases when sites conceal their actual physical address.

The European Union (EU) has a strong legal framework for medicines. At the end of the distribution chain, only licensed pharmacies and approved retailers are allowed to sell medicines, including legitimate sale via the Internet. For EU Member States, since February 2019, it has been mandatory to satisfy the requirements of Commission Delegated Regulation EU 2016/161 and Directive 2011/62/EU of June 2011. Serialisation prevents SF medicinal products from entering the legal distribution chain. It involves tracing an individual product from the manufacturer through the wholesaler and via the pharmacy to the patient, by a unique two-dimensional (2D) identification that is put on each box of prescription drugs. The 2D code should include the product code, the batch number, the serial number, the expiry date, and the national identification number if required by the Member State where the product is placed on the market.

SF medical products may fail to treat the illness for which they were produced, or they can cause harm to patients, and this can lead to a loss of confidence in medicines, healthcare providers, and health systems. All regions of the world are affected.

It has to be emphasized that, in addition to all kinds of protection and coding, the most important strategy that can be adopted for the patients' safety is to organize communication and education campaigns to inform them and to educate the public on the safe use of Internet pharmacies. People must be taught to be able to differentiate between legal and illegal medication suppliers.

2. AIMS

The aim of this research is to support the regulation by developing a technology for marking an individual traceable code directly on the surface of the medicine, besides the obligatory identification code that has been on each box of drugs in the EU since 2019. Anyone with a camera-enabled phone and a suitable application should be able to authenticate these uniquely marked drugs, thus helping in the fight against counterfeiters.

The main steps in the experiments were as follows:

- Selection of the dosage form to be coded
- Choosing the instrument for marking
- Selection of lasers
- Determination of coatings required for lasering
- Selection of tablet coating materials
- Laser ablation with different lasers
- Comparison of the effects of different lasers on the coated tablet sample (physico-chemical tests, analytical analyses)

3. MATERIALS AND METHODS

3.1. MATERIALS

Tablet samples for laser marking preformulation were original tablets from the legal supply chain: Sinecod (GSK), Telfast (Sanofi), Klacid (Abbott), furthermore Eudraguard® control (EudrC) and HPMC coated placebo tablets.

QR codes were ablated on the model tablets with active ingredient Ibuprofen DC 85 (Ibu): 16.66% (w/w), and excipients were: talc 3% (w/w), crospovidone (Kollidon CL-M) 5% (w/w), magnesium stearate 1% (w/w), microcrystalline cellulose (Vivapur 102) 74.33% (w/w), used as received.

Several types of coatings have been studied during selection of the lasers: an aqueous-based enteric coating solution was prepared. It consisted of 52% w/w dry substance of a neutral copolymer based on ethyl acrylate and methyl methacrylate with a ratio of 2:1 (EudrC dispersion 30% w/w (Evonik Nutrition & Care GmbH, Germany)), 16% w/w talc, 28% w/w alginic acid sodium salt, 4% w/w glycerol, and distilled water. Coatings were coloured with 1% w/w patent blau 85 (blue), 3% w/w gelborange (orange), 1.5% w/w azorubin (cherry) or 1.5% w/w iron oxide red (red).

Several types of coatings have been studied, both free polymer films and coated tablets. Coating materials used: ethyl acrylate and methyl methacrylate with a ratio of 2:1 neutral copolymer (Eudraguard® control, Evonik Nutrition & Care GmbH, Germany), HPMC-based coatings with conventional dyes (Sepifilm PW Red (SPW-R), PW Green (SPW-G) and PW White (SPW-W)), and coatings with natural colouring (SEPIFILM™ NATurally COLoured Pink (SNC-P) and SEPIFILM™ NATurally COLoured Green (SNC-G); Seppic S. A., Paris La Defense, France) and anionic polymethacrylate (Eudragit L30 D55 (E-L30 D55), Evonik Evonik Nutrition & Care GmbH).

3.2. METHODS

3.2.1. Preparation of API content tablets

For the Ibu containing tablet (see the composition in Chapter 3.1.3), the pressing procedure was the following: the ingredients of the tablet were homogenized with a Turbula mixer (Willy A. Bachofen Maschienenfabrik, Switzerland) for 8 minutes, and for 2 minutes after the addition of the lubricant. The homogenous powder mixture was compressed with a Korsch EK0 (E. Korsch Maschienenfabrik, Germany) single punch eccentric tablet press.

3.2.2. Tablet coating procedure

The spray coating process was performed using a 4M8 Pancoat (Pro-C-epT, Zelzate, Belgium) perforated coating pan.

3.2.2.1. Coating with EudrC dispersion

The spray coating process was performed on a batch of 500 g tablets. The process was divided into three stages. The coating parameters are shown in Table 1.

Table 1. Coating parameters of placebo tablets with EudrC.

Step	Inlet air temperature (°C)	Exhaust air temperature (°C)	Tablet bed temperature (°C)	Drum speed (rpm)	Air flow rate (m^3/min)
Warm-up	50		until 30	5	0.50
Coating	49±2	32±2	30±2	18	0.50
Drying & cooling	40	27	25	5	0.50

For the application of the atomized spray coating solution, 0.8 mm spray nozzle was used for 140 min, with an atomizing air pressure of 1.5 bars and an air flow rate of 0.50 m^3/min. The drying and cooling processes together lasted for 30 min.

3.2.2.2. Preparation of free films

Experiments were carried out on free films that were sprayed on the surface of polyethylene balls (Primary Balls Kft., Budaörs, Hungary) with an outer diameter of 2.5 cm. Thereafter the film was removed from the ball, marked by laser and examined. 35 pieces of balls were coated at the same time in four stages. The coating parameters are shown in Table 2. A 0.8 mm spray nozzle was used for the application of the atomised spray coating solution for 55 min, with an atomising air pressure of 2.0 bars and an air flow rate of 0.70 m^3/min. The drying and cooling process lasted for 15 min each.

Table 2. Coating parameters of balls with Sepifilm.

Step	Inlet air temperature (°C)	Exhaust air Temperature (°C)	Ball temperature (°C)	Drum speed (rpm)
Warm-up	60	N/A	Until 50	3
Coating	50-55	40-42	45	9
Drying	40	30	27	3
Cooling	25	25	25	3

3.2.2.3. Coating procedure of tablets with API content

330 g of placebo tablets and 70 g of Ibu containing tablets were coated together at the same time in order to save material and time. During spray coating, 2 layers of coating were put on the tablets. The first layer was E-L30 D55. A 0.8 mm spray nozzle was used for the application of the atomised spray coating solution for 75 min, with an atomising air pressure of 1.0 bar, a spray rate of 3 g/min and an air flow rate of 0.70 m^3/min. The drying and cooling process lasted for 15 min. The other coating parameters are shown in Table 3.

Table 3. Coating parameters of E-L30 D55.

Step	Inlet air temperature (°C)	Exhaust air Temperature (°C)	Tablet temperature (°C)	Drum speed (rpm)
Warm-up	60		Until 50	3
Coating	45-55	40-45	30-35	15
Drying	50	38-40	35-37	3
Cooling	25	25	25	3

The second layer was the HPMC-based ready-to-use coating formula. A 0.8 mm spray nozzle was used for the application of the atomised spray coating solution for 45 min in the case of Sepifilm PW coating, with an atomising air pressure of 2.0 bars, a spray rate of 2 g/min and an air flow rate of 0.70 m^3/min. The drying and cooling process lasted for 15 min. The other coating parameters for the second layer are shown in Table 4.

Table 4. Coating parameters of Sepifilm films.

Step	Inlet air temperature (°C)	Exhaust air Temperature (°C)	Tablet temperature (°C)	Drum speed (rpm)
Warm-up	60		Until 50	3
Coating	55	40-42	35	9
Drying	40	30	27	3
Cooling	25	25	25	3

3.2.3. Irradiation by laser

5 different types of lasers were used for the irradiation of the films and the tablets, at the Department of Optics and Quantum Electronics, University of Szeged.

3.2.3.1. Nd:YAG laser

Wavelength: 1064 nm, power: 1–2.6 W, frequency: 1 kHz.

3.2.3.2. Semiconductor laser

Continuous wave semiconductor laser, wavelength: 405 nm, spot size: 73 μm, power: 1000 mW, irradiation time: 15–20 ms.

3.2.3.3. ArF 193nm, UV excimer laser (ArF laser)

Wavelength: 193 nm, energy: 3±0.2 mJ, fluence: 444 mJ/cm^2, FWHM: 20 ns, spot size: 375 μm, using a simple square-shaped mask.

3.2.3.4. KrF 248 nm, UV excimer laser (KrF laser)

Wavelength: 248 nm, energy: 0.5 mJ, number of impulses: 10, spot size: 100 μm, FWHM: 700 fs, fluence: 6.37 J/cm^2. The central part of unfocused 4 cm x 4 cm pulses was cut out by an aperture.

3.2.3.5. Titan-Sapphire Femtosecond Laser (femto laser)

Wavelength: 800 nm, energy: 0.62 mJ, number of impulses: 20, spot size: 110 μm, FWHM: 135 fs, repetition rate: 200Hz, fluence: 6.52 J/cm^2.

3.2.4. Test methods for laser ablated films and tablets

3.2.4.1. Light microscope

Measurement of final coating thickness was determined by light microscopy using a LEICA Image Processing and Analysis System (LEICA Q500MC, LEICA Cambridge Ltd., Cambridge, United Kingdom). 4–4 tablets of the API containing tablets were cut in half along the middle of the tablet band, and each was measured at 10 places and averaged.

3.2.4.2. Digital microscope

The surface morphology of the ablated film was observed by using a Digital Microscope (KEYENCE, VHX-6000). This instrument enables the creation of a precise 3D image by analysing small changes in texture after capturing numerous images at different heights and different angle positions. Data were evaluated by HDR playback image playback software developed by KEYENCE.

3.2.4.3. Surface profilometer

Profilometry measurements were performed using a Veeco, Dektak 8 Advanced Development Profiler®. The tips employed had a radius of curvature ~2.5 μm, and the force applied to the surface during scanning was ~30 μN.

3.2.4.4. Determination of the ablation threshold

The ablation threshold indicates the minimal laser energy required to remove the material from the substrate (i.e., tablet surface). The threshold value is a fundamental parameter for laser fine-tuning. In most of the cases, the laser operates close to the threshold but with slightly higher energy to be effective, and also to avoid unwanted side effects, such as the thermal distortion of the material. The characteristics of the ablation holes were examined with a surface profilometer. The laser parameters required for ablation were determined from the data obtained using a profilometer.

3.2.4.5. Scanning electron microscope (SEM)

The ablated tablets were observed by using a SEM (SEM, Hitachi, Japan S4700). The measurements were performed at a magnification of 30–5000, applying 10.0 kV electron energy and 1.3–13 MPa air pressure.

3.2.4.6. Raman spectroscopy

Raman spectroscopy was used for the examination of the samples treated by lasers. Spectra were acquired with a Thermo Fisher DXR Dispersive Raman spectrometer (Thermo Fisher Scientific Inc., MA, USA), a diode laser operating at a wavelength of 780 nm and equipped with a CCD camera. Raman measurements were carried out with a laser power of 4 mW (Ibu), 6 mW (EudrC), 12 mW (E-L30 D55, SNC-P, SPW-R, SPW-W, SPW-G) and 24 mW (SNC-G) at a slit aperture size of 25 µm, using automated cosmic ray and fluorescence corrections. OMNIC 8 software was used for data collection. Data were evaluated by Spectragryph - optical spectroscopy software.

3.2.4.7. Thermal gravimetric analysis (TGA)

The TGA of the samples was carried out with a Mettler-Toledo TGA/DSC1 instrument (Mettler-Toledo GmbH, Switzerland). The start temperature was 25 °C, the end temperature was 500 °C, the applied heating rate was 10 °C/minute. Nitrogen atmosphere was used. 5 ± 1 mg samples were measured into aluminum pans (40 µl). The TG curves were evaluated with Mettler-Toledo STAR^e Software.

3.2.4.8. Mass spectrometry (MS)

The gas analysis of the tablet coating material was carried out with the Thermo Star (Pfeiffer Vacuum, model ThermostarTM GSD 320, Germany) quadrupole mass spectrometer (maximum 300 amu) for gas analysis, which was coupled to the TG instrument. The measurements were carried out in a flow of nitrogen atmosphere. The connection between the TG and the mass spectrometer was made by means of a heated silica capillary, which was maintained at 120 °C. Ions with various mass numbers were determined with the SEM MID measurement module of the Quadera software.

3.2.4.9. In vitro drug disintegration

In the disintegration studies, the tablets were tested according to the standard method of the European Pharmacopoeia with an Erweka model ZT71 apparatus (Erweka, Germany). First, in 900 ml of artificial enzyme-free gastric juice (pH = 1.22) were put the tablets, and after 2 hours, the medium was changed to a phosphate buffered saline solution (pH = 6.82).

3.2.4.10. In vitro drug dissolution

In the present study, the investigation of drug release kinetics from marked tablets was carried out with an Erweka DT 700 (Erweka GmbH, Germany) dissolution tester according to the standards of the European Pharmacopoeia. A rotating basket method was used for the dissolution tests, where the rotation speed was 100 rpm, the dissolution medium was 900 ml of artificial enzyme-free gastric juice (pH = 1.22) for 2 hours, and then it was replaced with 900 ml of phosphate buffered saline solution (pH = 6.82) for 1 hour. The temperature was maintained at 37± 0.5 °C. As a sample, 5 ml of the dissolution medium was taken manually at predetermined intervals without being replaced. The absorbance of ibuprofen DC85 was analyzed at 222 nm, using a spectrophotometer (Genesys 10S UV-VIS, Thermo Fisher Scientific Inc., MA, USA). Four tablets were tested, and samples were taken at the following time intervals: at 120 min in the case of gastric juice, and after changing to intestinal fluid at 5, 10, 15, 30, 45 and 60 min.

4. RESULTS AND DISCUSSION

4.1. Choosing the pharmaceutical form to code

In my Ph.D. work, tablets were chosen for coding as oral drug delivery is the most preferred and convenient route of pharmaceutical drug administration. They are physically and chemically stable, simple-to-use, have sustainable production

4.2. Selecting the tool to use for marking

There is a wide range of options for labelling medicines, and several aspects had to be taken into account when making the choice. From among many different options, printing is one of the most attractive methods for marking, as the capital equipment cost is relatively low. However, the clear printing pattern may easily be affected by the environmental conditions of the process room, uniformity, temperature, and drying of the ink. The printing process necessitates contact between the substrate and some form of ink carrier, toner reservoir, or stamp, so it could be a source of contamination. High printing speed is required for some of the fastest production lines and that can result in a loss of image quality and the risk of unreadable codes. Furthermore, the ink formulation has to be designed with respect to its viscosity and surface tension to guarantee continuous printing and high reproducibility of the forming droplets. Printability is also affected by the surface roughness of tablets, which may cause problems such as mottled appearance, blur, or dirt of the inks. Also, organic solvents which are harmful to the employees' health and the environment are often used for the inks.

In the present study, laser ablation was chosen for marking as it overcomes the drawbacks of ink printing technologies. The laser coding technology is a non-contact method that avoids the problem of contact contamination. It allows the authentication of tablets.

4.3. Selection criteria for the lasers

When selecting lasers, the aim was to compare as many different types of instruments as possible to give a broad overview of the effects of different lasers on drugs.

The continuous mode semiconductor (diode) laser ablates with a photothermal effect and operates at different wavelengths. The pulsed mode excimer laser works with photochemical ablation, which seems to have a more gentle effect on the materials. However, it uses gas mixtures, usually noble gas and halides, and the running costs are high due to the maintenance and equipment costs. Although the near-infrared femtosecond laser operates at high wavelengths, its pulse is ultra-short, therefore its thermal effect is negligible, and no or just little chemical or thermal damage occurs during the material removal. In addition, it can create very fine structures, it is more accurately adjustable, faster and also cleaner. As the study progressed, it became clear that certain lasers had a detrimental effect on the sample, so this aspect had to be considered in the selection process.

4.4. Determination of the application of coatings required for lasering

When laser coding is applied, it should be considered that the tablet has to be coated. At least one coloured layer should be applied to the drug, even if it already has a functional coating on, as the 2D code is created by ablating the coating. In cases when tablets have a coloured

coating (for example, for improved swallowability or identification purposes), that layer could be marked. When a functional coating is needed because of the therapy, an extra coating is required on top of it to enable marking without the loss of coating functionality.

In this experiment, the final plan is to put two coatings on the tablet surface in different colours, a functional one (gastric resistant) and a second one for marking. After the laser ablation of the upper film layer, the differently coloured code could be read even by the patient using a smartphone with the appropriate application.

4.5. Laser ablation with different lasers

In the following, I would like to present the experiments and tests performed with different lasers.

4.5.1. Nd:YAG laser

The preformulation study began using an inexpensive, more widely used pulsed Nd:YAG laser on original tablets. It was found that the intervention burned the tablet's coating during testing, so the device was not part of our further research. The result of the treatment is presented in Fig. 1. The phenomenon can be explained by the fact that due to its longer wavelength (1064 nm), it has a higher heat effect, which can lead to thermal decomposition.

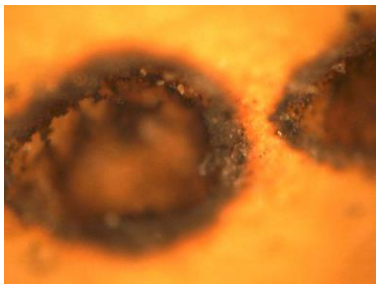

Fig. 1. Nd:YAG laser treated original tablet.

4.5.2. Semiconductor laser

A semiconductor laser was chosen next. The continuous mode laser ablates with a photothermal effect and has the advantages of being compact, efficient, with a quick modulation response and reliability. It is relatively small in size, and it has a low cost. In addition, it operates at different wavelengths.

4.5.2.1. The semiconductor laser treated EudrC coating

A photograph of the semiconductor laser treated tablet is shown in Fig. 2A and a microscopic image in Fig. 2B. The laser beam has blackened the coating, and it is likely to have been burnt. The SEM image (Fig. 2C) shows that the semiconductor laser caused significant damage to the coating structure during treatment. Holes are seen in every 200 μm on the film, surrounded by a wide range of burn traces. There are blistering, snow-flake like crystals around the holes, melting, and recrystallization. These may be the result of water loss or melting of the coating material.

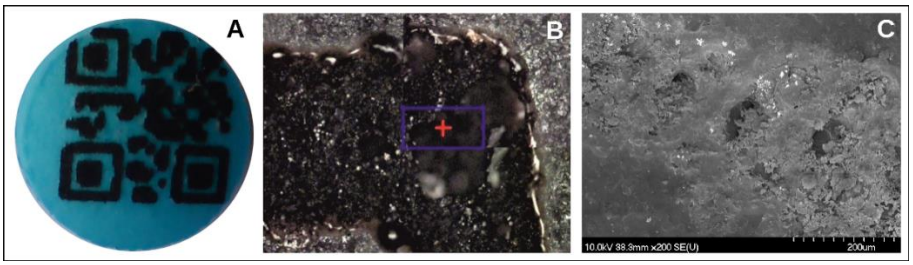

Fig. 2. Semiconductor laser treated EudrC coated tablet. A: Photomicrograph of the laser treated tablet, B: Microscopic image, C: SEM image (magnification 200×).

The Raman studies showed that the spectrum of the semiconductor laser treated EudrC film (Fig. 3, curve C) was completely changed, it smoothed, compared to the spectrum of the untreated film (Fig. 3, curve B). It is assumed that this is the result of the combustion shown in Fig. 2.

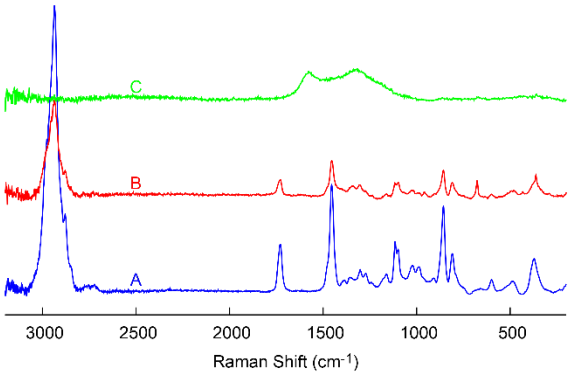

Fig. 3. Raman spectra. A: dispersion of EudrC, B: untreated film of EudrC, C: semiconductor laser treated film of EudrC.

4.5.2.2. Semiconductor laser treated Sepifilm coating

Since the semiconductor laser is a cheap and easy-to-use, it was found worthwhile to test the laser on materials of other compositions. In view of the growing demand for natural materials, HPMC-based, ready-to-use coating formulas were chosen, which had been coloured with natural colours (Sepifilm ™ Naturally Colored coatings), and for comparison, conventionally coloured coating formulas were also examined (SPW-R, SPW-G, and SPW-W).

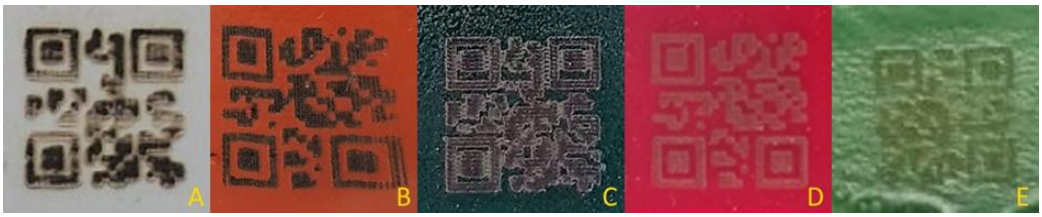

Fig. 4. Photographs of HPMC-based coating films treated by semiconductor laser. A: SPW-W, B: SPW-R, C: SPW-G, D: SNC-P, E: SNC-G.

The experiments were carried out on free films that were sprayed on the surface of polyethylene balls, and thereafter the film was removed from the ball, marked by laser and examined. The result of the treatment by semiconductor laser is black burst signals (Fig. 4ABC) or fading of the colour of coatings, which may be seen in Fig. 4D and E, respectively. Raman studies were performed to clarify the nature of the changes on the treated surface.

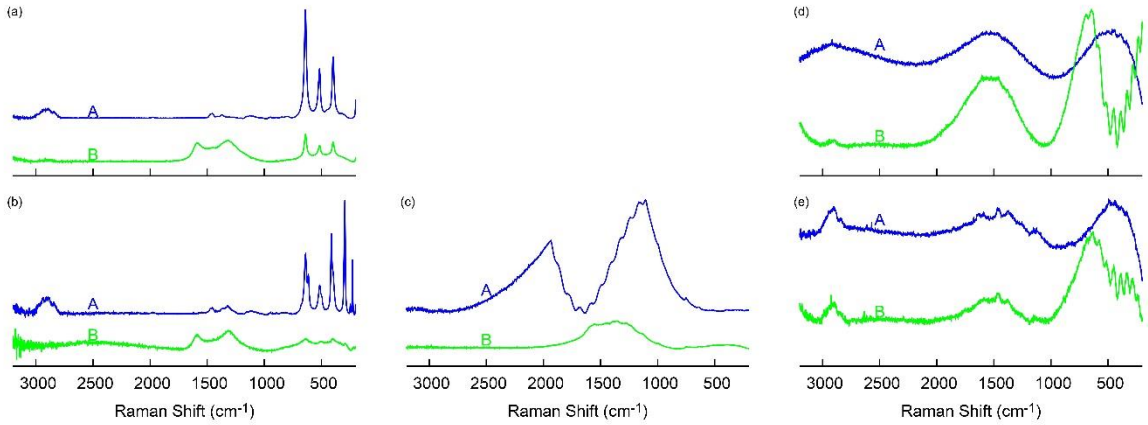

Fig. 5. Raman spectra of Sepifilm coatings treated with semiconductor laser. (a): SPW-W, (b): SPW-R, (c): SPW-G, (d): SNC-P, (e): SNC-G (A: original, B: semiconductor laser treated film).

The spectra of free films and laser treated films are summarised in Fig. 5. Coatings (SPW-G SNC-P, SNC-G) containing natural extract of colouring foodstuffs (e.g. extracts of fruits, algae), where the exact composition is not known, made analysis difficult, as exhibited severe fluorescence during the measurement (Fig. 5(c)(d)(e)). This effect could not be corrected by photobleaching. In all cases, the spectra of the films treated with semiconductor lasers changed, which is probably due to the product degradation that had occurred during the laser treatment.

In the further research, the lasers that do not produce a heat effect during ablation were tested.

4.5.3. ArF 193 nm UV excimer laser

Based on the literature data, the UV excimer laser was chosen for the next experiment as it works by photochemical ablation, so it has a negligible heat effect, which minimizes the chemical degradation of the material during the process.

4.5.3.1. ArF laser treated EudrC coating

The experiment started with the EudrC coating, as in the previous study. A simple square-shaped mask was used during the treatment, which resulted in a 1 mm^2 square-shaped ablation hole. The effect of different number on the coating film was investigated. Fig. 6B shows the micrograph, and Fig. 6C the SEM image of the square "imprint", where no major degradation is visible, and the film structure is relatively intact.

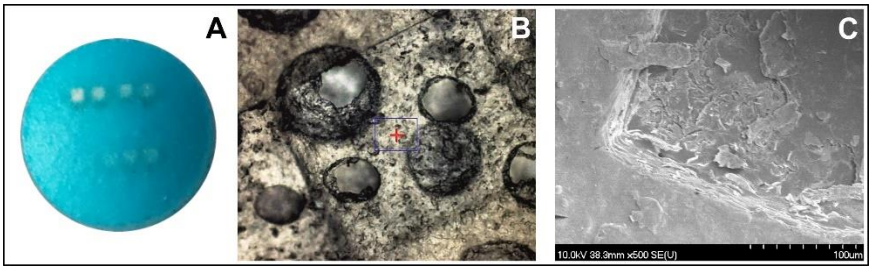

Fig. 6. ArF laser treated EudrC coated blue tablet. A: Photograph, square-shaped holes from bottom line left: 10, 20, 30, 40, from upper line right: 50, 60, 70, 80 impulses, B: microscopic picture of one ablated square, C: SEM micrograph of the ablated square (magnification of 500×).

The extents of the ablated holes were measured with a surface profilometer. The results are shown in Fig. 7. The curve on the left shows the depth of holes which were made with increasing pulse rate on the surface of a red tablet. The fitted line on the right shows a nearly linear relation between the applied number of impulses and ablation depth.

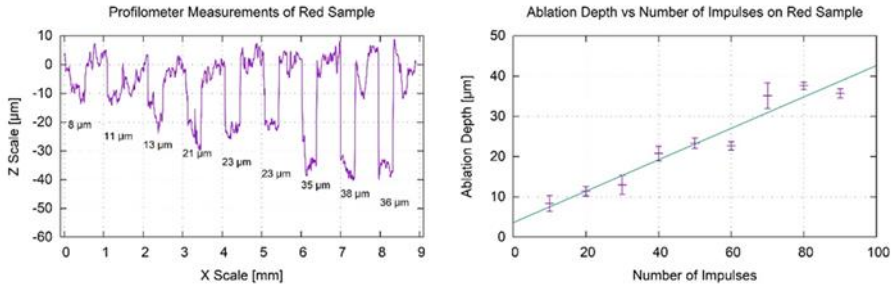

Fig. 7. Profilometer analysis of the series of the ablated holes on the EudrC coated red tablet surface. Left: the curve shows the depth of the ablated holes made by growing numbers of ArF laser pulses, right: the figure shows a near linear relation between the applied number of impulses and ablation depth.

The results of Raman spectroscopy showed that there was no significant difference between the spectra of the original film (curve B in Fig. 8) and the EudrC film treated with ArF laser (curve C in Fig. 8).

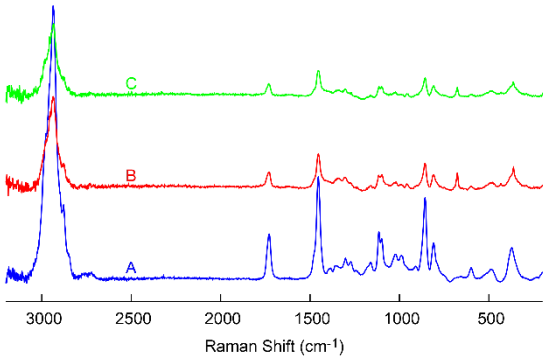

Fig. 8. Raman spectra. A: EudrC dispersion, B: EudrC free film, C: ArF laser ablated EudrC film.

Furthermore, thermoanalytical studies show that the TGA and DTG curves of the same samples run together, as shown in Fig. 9, indicating that the material has not changed during laser treatment.

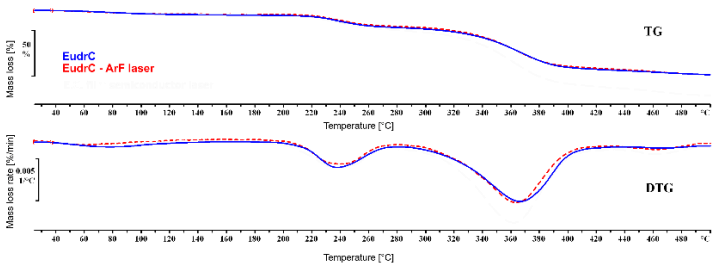

Fig. 9. TG and DTG curves of EudrC film, and EudrC film treated with ArF laser.

Taking into account the results, it can be concluded that no chemical lesions were observed on the EudrC coating treated with ArF laser.

4.5.3.2. ArF laser treated Sepifilm coating

After promising results, the laser was also tested on HPMC based coatings. Using the square-shape mask, a significant difference was observed in SPW-R and SPW-G coatings. White and black particles appeared in the ablation square, which are shown in Fig. 10AB. This phenomenon had been seen previously neither on the semiconductor laser treated surface (Fig. 4), nor on the naturally coloured SNC-P and SNC-G coatings (Fig. 10CD).

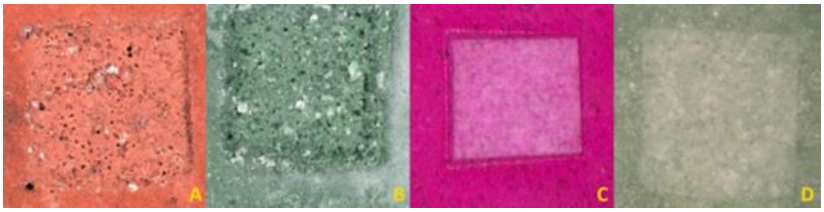

Fig. 10. 3D digital microscope images of coating films treated by ArF laser. A: SPW-R, B: SPW-G, C: SNC-P and D: SNC-G.

Conventionally coloured coatings, SPW-R and SPW-G contain TiO_2 and talc, for better coverage, while the new naturally coloured film formulations do not contain the excipients in question.

Each material has its characteristic ablation threshold. This value is specific to the material, to the type of laser, to the ablation method,to the wavelength and to the fluence. In the studies of Laude et al., the ablation of talc started at 250 mJ/cm^2 fluence, at 248 nm wavelength, so it is likely that talc was ablated during the laser treatment in this study. The ablation of TiO_2 requires higher wavelength, or fluence than the other ingredients of the coating material need, and it is likely to require higher fluence than ArF laser can provide. In the present case, the laser was used at 193 nm wavelength and 444 mJ/cm^2 fluence, whereas literature data about the ablation threshold of TiO_2 are available only at 248 nm wavelength and 1.44 J/cm^2 or 910 mJ/cm^2 fluence. The threshold value for the current investigation was still below the ablation threshold of TiO_2 and was not enough for its removal, but it was enough for the removal of the rest of the coating. Therefore, it can be stated the presence of these excipients can disturb the 2D code recognition.

Furthermore, according to literature data, irradiation of TiO_2 with a 248 nm wavelength KrF laser induced a colour change from white to dark blue, i.e., the crystal structure changed from anatase to rutile. Furthermore, Kato et al. studied the mechanism of printing film-coated tablets containing TiO_2 by using a tripled Nd: YVO4 UV laser printing machine (wavelength of 355 nm). They marked clear numbers and letters on the surface of the tablet by turning the colour of the TiO_2 particles in the film from white to black, causing the film to turn gray on the lasered surface. This is the result of the appearance of many black particles in the white film. It was assumed that in the present case the white and black points in Fig. 10AB are also associated with the laser irradiated TiO_2.

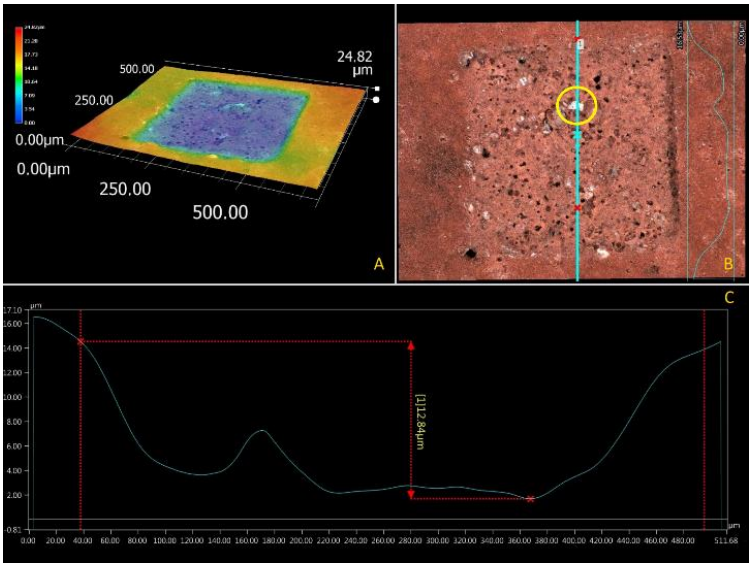

Fig. 11. Surface analysis by 3D microscope of the ArF laser treated region of SPW-R coating. A: 3D surface graph, B: top view with the original colours of the ablation hole, the white TiO_2 particle is marked with a yellow circle, C: profile analysis.

The remaining particle size can be about half of the ablation depth, as can be seen from the 3D microscope measurements in Fig. 11. The designated line where the measurement was taken passes through the ablation hole (Fig. 11B) and the corresponding profile of the ablation hole is shown in Fig. 11C.

As in the semiconductor laser study, Raman spectroscopy was used to investigate whether any chemical changes occurred during the laser ablation. In this case, fluorescence also interfered with the measurement. As it can be seen in Fig. 12, in all cases, the spectra of the raw films and the lasered films run together, so it can be concluded that the ArF laser did not cause considerable alteration in the coatings.

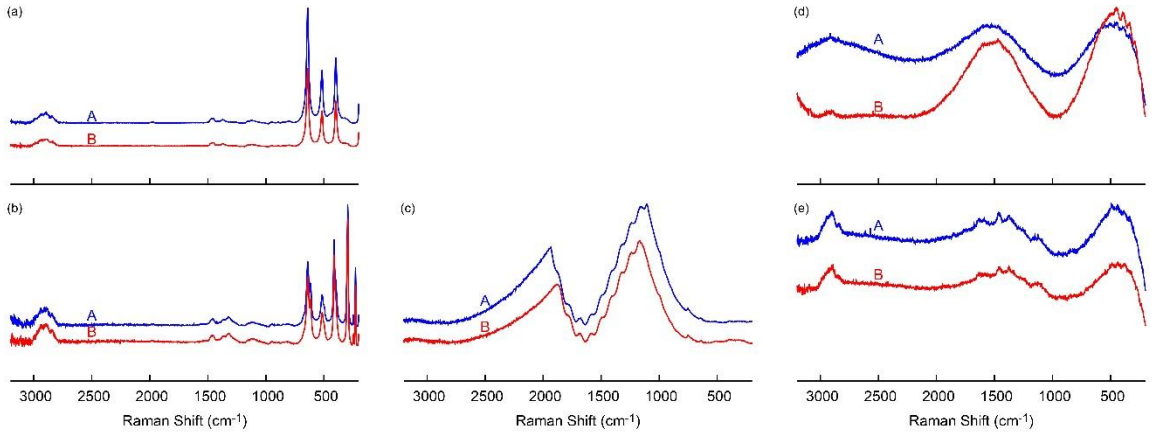

Fig. 12. Raman spectra of a raw film and a coating treated with ArF laser. (a): SPW-W, (b): SPW-R, (c): SPW-G, (d): SNC-P, (e): SNC-G (A: original, B: ArF laser).

TG investigation was performed on each sample type. It can be seen (Fig. 13) that the TG curves of the ablated films run together with the curves of the original, untreated film.

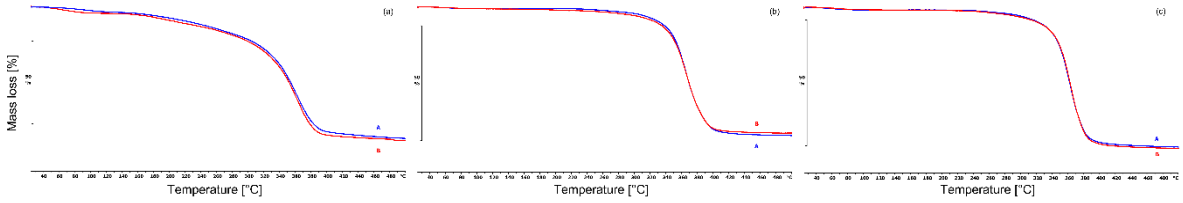

Fig. 13. TG curves of a raw Sepifilm coating, and the films treated with ArF laser. (a): SNC-P, (b): SPW-W, (c): SPW-R (A: original, B: ArF laser treated).

Based on the measurement results, it can be stated that laser ablation differentiates the ingredients of the coatings, which can affect drug identification. However, the ArF laser could be a suitable tool for marking coatings for pharmaceutical companies, but for limited type of coatings, that do not contain TiO_2.

For further studies, a laser with suitable parameters for TiO_2 removal was needed.

4.5.4. KrF laser

Since many coatings contain TiO_2, the use of ArF lasers would be greatly limited. To solve this problem, the higher wavelength (248 nm) KrF laser was chosen, as previous studies have shown that the excimer laser is safe and the ablation threshold of TiO_2 is below 248 nm according to the literature.

In this case, API containing tablets with 2 layers of coating were tested. Whole QR codes were applied on their surface. TiO_2 particles were completely removed during laser ablation and did not interfere with decoding.

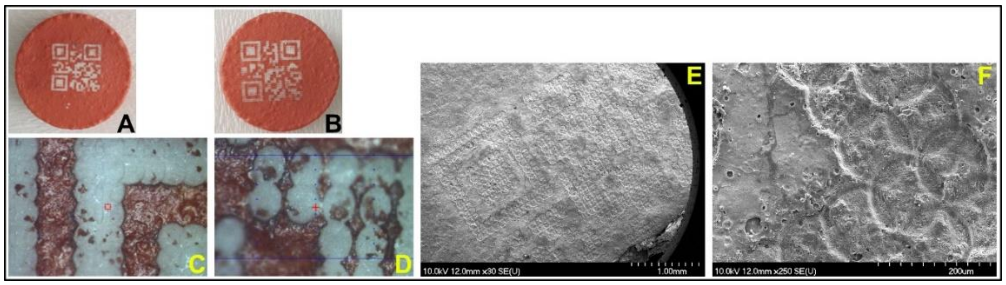

Fig. 14. KrF laser-encoded tablet. A: 4x4 mm QR code, B: 5x5 mm QR code, C: microscopic image of the 4x4 mm code, D: microscopic image of the 5x5 mm code, E: SEM image (30x magnification 30x), F: SEM image (magnification 250×)

In Fig. 14D it is clearly visible that the QR code is made of overlapping dots. It was possible to generate a readable code from oval-shaped holes, too, even when laser irradiation was not perfect, as the code has error correction capability that can restore the missing data. Care must be taken not to punch through the E-L30 D55 layer. The depth of ablation can be controlled by changing the number of the pulses on the sample place or the fluence, which allows the accurate setting of the penetration depth to the coating layer. It took 1.5 and 2 hours to create such a code by the KrF laser, depending on whether there was an overlap between the holes or not. Fewer shots mean faster but still effective marking. The right ratio needs to be found.

As shown in the SEM images (Fig. 14EF), the ablated surface of the sample has no large damage. Only a physical change is observed in the structure as a result of the removal of the coating by the KrF laser, and no obvious sign of chemical change is detected.

To determine if there was a laser-induced change in the API, Raman measurements were performed on the fracture surface of the tablets, too. Sample analyses were made at 10 points directly below the lasered coating surface and at 10 points in the core of the lasered tablets, 10 spectra were averaged at each point. The mean of these spectra was compared with the average of 10 spectra taken from the core of an untreated tablet and with the spectrum of Ibu. These spectra were normalized to peak 1604 cm^{-1} of the Ibu spectrum and are shown in Fig. 15.

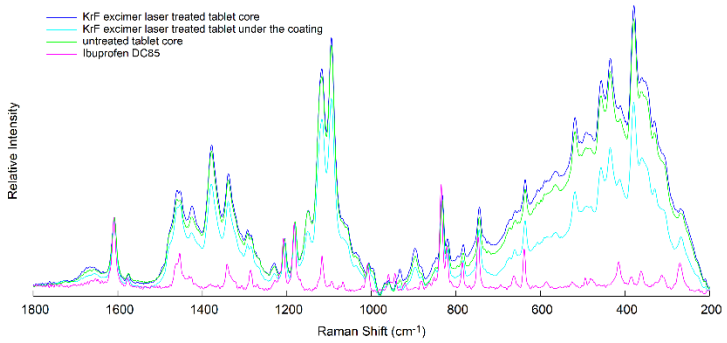

Fig. 15. Averaged and normalised (to peak Ibu 1604 cm-^{1}) spectra taken from the KrF laser treated and non-treated places, spectra of untreated tablet core and Ibu spectrum.

There was no significant difference between the spectra taken from the KrF laser treated area and the spectra taken from the non-lasered area. The most characteristic peaks of Ibu are present in all the spectra, with no slip visible. The observed peak intensities can be attributed to the relative inhomogeneity of the materials in the tablet, depending on how rich or poor Ibu was in the studied region.

It can be stated that no chemical structural change was observed after the labelling.

4.5.5. Ti:sapphire femtosecond laser

Finally, a near-infrared (800 nm), short-pulse femto laser was tested. The assumption was if the pulse is ultrashort, the heat effect is insignificant, and no or just little chemical or thermal damage occurs during the removal of the material. As it works at a high wavelength, it has to be suitable for the elimination of TiO_2.

It took about 10 minutes to ablate the QR code shown in Fig. 16. An important factor in the marking speed is the laser repetition rate, and for the femto laser this is high at 200 Hz, which dramatically shortened the marking process compared to the ArF laser, where it took 1.5-2 hours.

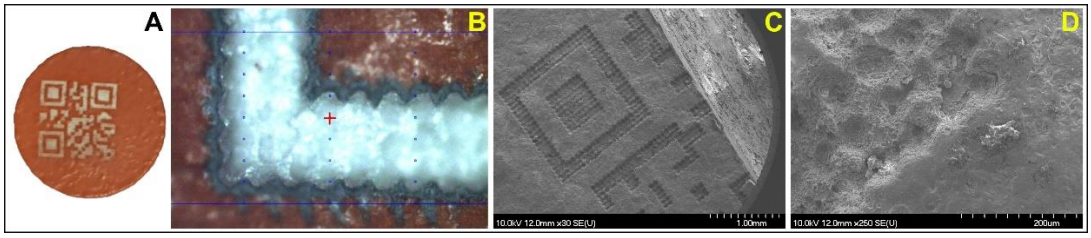

Fig. 16. Tablet encoded by femto laser. A: Visible to the naked eye, B: microscopic picture, C: SEM image at a magnification of 30x, D: SEM image at a magnification of 250x.

Fig. 16CD shows a SEM image with a part of the QR code at different magnifications. The dots which compose the QR code are clearly visible, and only a physical change can be detected on the surface. The femto laser neighter cause any significant damage to the coating structure during the treatment.

During the disintegration study, 6 tablets did not disintegrate in acid within two hours, according to the European Pharmacopoeia, only after transfer to the artificial intestinal fluid, thus proving that the first coating, resistant to gastric acid, was not damaged during the procedure.

Although reducing the ablation time to 10 minutes is considered as a great result, but it is still a long time to produce tablets on an industrial scale. Nevertheless, it was considered important to measure the dissolution parameters of lasered tablets, as a continuation of the experiment to confirm that it is possible to mark tablets with functional coating without damaging them during the procedure. Fig. 17 shows that of the 4 tablets tested, three remained intact in the gastric juice for 120 minutes. For tablets A, C and D, the amount of dissolved active ingredient was 0.15%, 2.12% and 0.92%, respectively, while for tablet B, the amount of dissolved active ingredient was 35.2%.

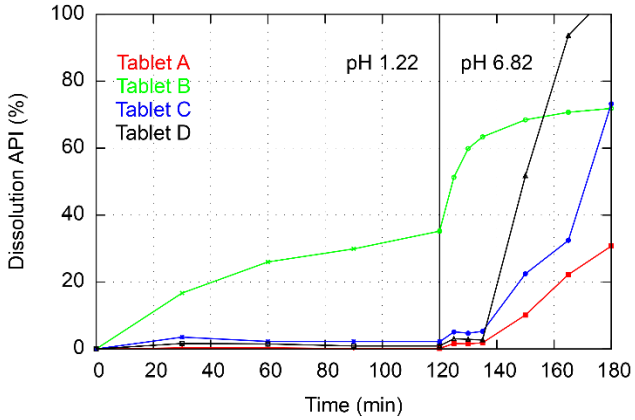

Fig. 17. Drug dissolution curves of the 4 coated and lasered tablets.

This may be due to the fact that the experimental tablets were coated together with placebo tablets to save time and material, and it is possible that they were not properly mixed during coating due to their different geometries. The coating thickness of the tablets may also vary for identical tablets, as observed by M. Wolfgang et al. in their coating studies, where it varied from 56.3 µm to 86.9 µm. The literature also confirms that the shape of the tablet directly influences intra-tablet coating uniformity.

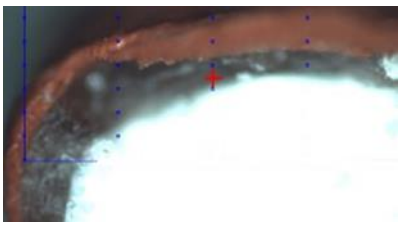

Fig. 18. Uneven thickness of the double coated tablet's coatings

The film layers of a halved, dual-coated tablet are shown in the micrograph in Fig. 18, which shows that the coating thickness varies over a wide range within a tablet. There is seen the difference in the thickness of the coating on the top and on the side of the medicine. In the

present study, it can be assumed that, for the reasons indicated above, the functional coating of tablet B was thinner and was damaged during marking. Further dissolution profile of the tablets in phosphate buffered saline solution is shown also in Fig. 17. It can be concluded that during the one-hour in vitro studies, the tablets acted in accordance with pharmacopoeial standards, and the disintegration and dissolution process started. It is seen that the dissolution of Tablet B started earlier than that of the others, and the final concentration was lower. The explanation for this phenomenon is that the dissolution of Tablet B had already started in the gastric medium.

Fig. 19 display the tablet surface and the cross-section of femto laser treated tablets, respectively. In Fig. 19(a)B E-L30 D55 is seen in the laser treated areas, while in Fig. 19(a)C the warm colours show the API Ibu, which suggests that the coating thickness was not consistently even, and the laser might have reached the API, or it penetrated into the coating in this case as well.

In Fig. 19(b)A the arrow points to the missing SPW-R coating. The profiling shows that the API is mostly in the tablet core (Fig. 19(b)C), but in the same picture, the area of the inner coating is green, which means that the API partly migrated from the tablet core to the E-L30 D55 film. According to the literature, such migration during the coating process can happen if the coating is aqueous based.

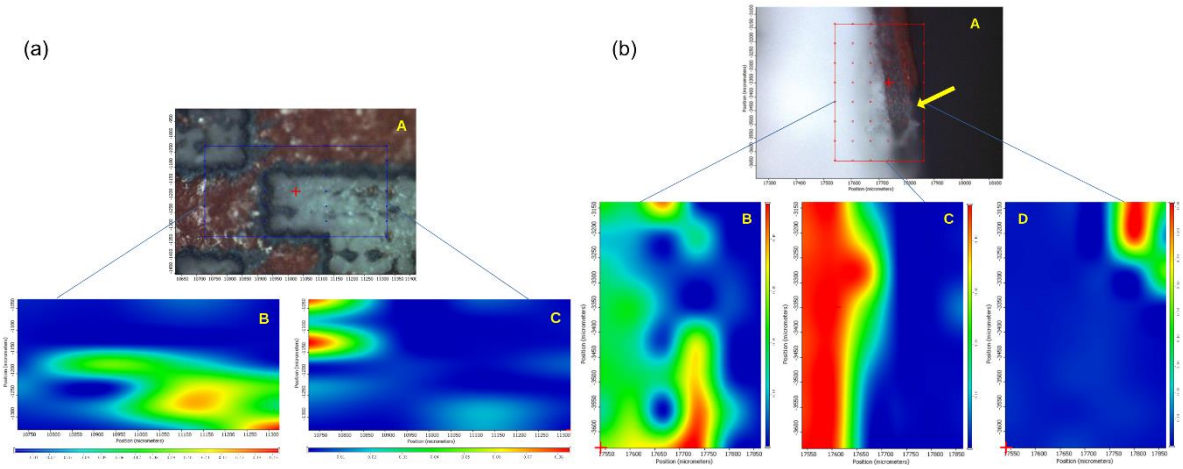

Fig. 19. (a): Surface of the tablet treated by femto laser. A: Microscopic picture of the lasered tablet, B: chemical map profiled to E-L30-D55, C: chemical map profiled to Ibu. (b) Cross-section surface of the femto laser treated tablet. A: Microscopic picture of the halved tablet, the arrow pointing to the missing SPW-R coating, BCD: chemical maps of the tablet surface profiled to: B: E-L30 D55, C: Ibu, D: SPW-R.

Raman measurements were also performed on the fracture surface of femto laser-treated tablets to determine if there was a change in the active ingredient. The spectra are shown in Fig. 20.

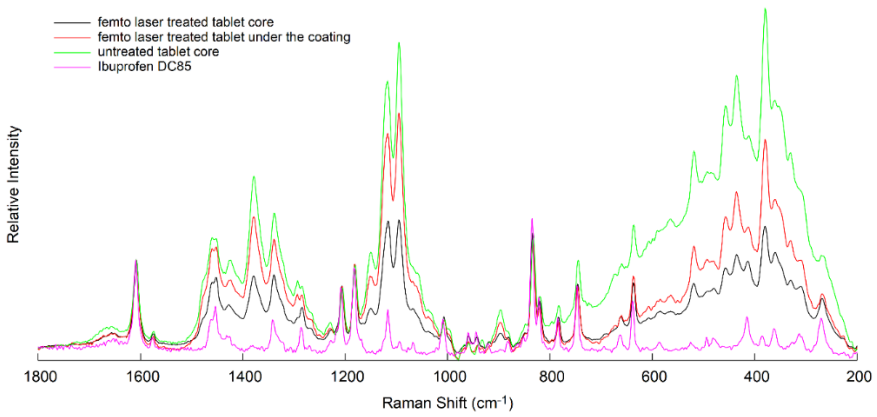

Fig. 20. Averaged and normalised (to Ibu peak 1604 cm^{-1}) spectra taken from the femto lasered and non-lasered places, spectra of untreated tablet core and Ibu spectrum.

Similar to the KrF laser results, there was no significant difference between the spectra from the laser treated region and untreated areas. Here again, the different peak intensities can be attributed to the relative inhomogeneity of the tablet material, depending on the Ibu richness of the area investigated. It can be concluded that no chemical structural change occurred

5. SUMMARY

The aim of the experiment was to develop a new, efficient way of a unique marking method to combat drug counterfeiting. For five different lasers, investigations were made to find out how the drug changes in response to the laser beam.

- Tablets were selected for coding as they are widely used, and are physicochemically stable.
- Laser was chosen as a coding tool as it is a non-contact method, thus minimising the problems of contact contamination.
- In selecting lasers, the goal was to compare different types of instruments during ablation and to obtain a general overview of their effects on the drug. As the study progressed, it was found out that certain lasers had a detrimental effect on the sample, so it was necessary to look for devices that would not cause changes in the material.
- When laser coding is applied, it should be considered that the tablet has to be coated. At least one coloured layer should be applied to the drug, even if it already has a functional coating on, as the 2D code is created by ablating the coating.
- It is clear from the preliminary experiments that great care must be taken when choosing coating materials to ensure that they are compatible with the laser of choice, for example, that the laser is able to remove all components of the coating, such as TiO_2, that the remained white particles do not interfere with 2D code recognition.
- The choice of the optimal instrument and its parameterisation is essential for coding.

6. CONCLUSION AND PRACTICAL USEFULNESS

The results presented in this thesis provide useful information for laser drug coding. The present research led to the following findings:

- A kutatómunka során három potenciális lézerrel, 2 excimer lézerrel (ArF és KrF) és femto lézerrel valósult meg az ablálás az anyag minőségi változása nélkül a lézeres jelölés során.
- The aimed anti-counterfeit coding technology was accomplished with three potential lasers, excimer laser (ArF and KrF) and femto laser, which did not cause a qualitative change in the material during laser marking.
- It was found that the ArF laser requires higher fluence, or wavelength, to be able to exceed the ablation threshold of TiO_2, so the usability of this type of laser is limited to TiO_2 -free coatings.
- The higher repetition rate of the femto laser allows faster and more efficient coding, which has key importance in mass production.
- Further results show that due to the high performance in the femtosecond region, the wavelength is no longer the critical parameter as it is for a nanosecond or longer pulses. Thermal effects are negligibly low in the fs region even at high peak powers. The thermal effects of laser ablation can be avoided by reducing the wavelength or the impulse length based on the current study.
- The excimer laser is a laser for laboratory use, while femto lasers are commonly used in the industry. It is known that the efficient use of this technology requires further development in speed. Other near-infrared pulsed lasers that operate at multi-kHz versions (with a repetition frequency of multi ten-kHz and also MHz) could potentially further shorten ablation time. Those devices could even be used for line speed marking in pharmaceutical companies.
- Various coating materials were tested, Eudraguard® control, SEPIFILM™ NATurally COLoured coatings agents, Sepifilm™ PW coating systems, Eudragit L30 D55®. Experience showed that the ability to laser the coatings is determined not only by the type of coating, but also by the quality and parameters of the laser.

New findings/practical relevance of the work

On the basis of the study, a novel, non-contact, functionally advanced marking technology was developed by using the lasers mentioned in this study, highlighting the femto laser as a potential solution for pharmaceutical companies that would like to have additional protection against drug counterfeiters or to label personalized medicines.

It should be noted that the method needs further development and scaling-up to enable this technology to serve the high volumes of industrial production.

LIST OF ORIGINAL PUBLICATIONS

1) **Ludasi K.**, Oláh I., ifj. Regdon G., Gyógyszerhamisítás elleni védelem, modern gyógyszerazonosítási technológiák alkalmazása – I. rész, Gyógyszerészet, 2018, 62, 80-87

2) **Ludasi K.**, Oláh I., ifj. Regdon G., Gyógyszerhamisítás elleni védelem, modern gyógyszerazonosítási technológiák alkalmazása – II. rész, Gyógyszerészet, 2018, 62, 140-147

3) **K. Ludasi**, T. Sovány, O. Laczkovich, B. Hopp, T. Smausz, G. Regdon Jr., Unique laser coding technology to fight falsified medicines, European Journal of Pharmaceutical Sciences, 2018, 123, 1-9, **Q1 IF: 3.532**

4) **K. Ludasi**, T. Sovány, O. Laczkovich, B. Hopp, T. Smausz, G. Regdon Jr., Comparison of conventionally and naturally coloured coatings marked by laser technology for unique 2D coding of pharmaceuticals, International Journal of Pharmaceutics, 2019, 570, 118665, **Q1 IF: 4.845**

5) **K. Ludasi**, O. Jójárt-Laczkovich, T. Sovány, B. Hopp, T. Smausz, A. Andrásik, T. Gera, Z. Kovács, G. Regdon Jr., Anti-counterfeiting protection, personalized medicines − development of 2D identification methods using laser technology, International Journal of Pharmaceutics, 2021, 605, 120793, **Q1 IF:5,875(2020)**

PRESENTATIONS RELATED TO THE THESIS

1) **K. Ludasi,** T. Sovány, O. Laczkovich, B. Hopp, T. Smausz, G. Regdon jr.: Unique laser coding technology to fight falsified medicines, 7th BBBB International Conference on Pharmaceutical Sciences, Balatonfüred, Hungary, 5-7 October 2017 (Poster presentation)

2) **K. Ludasi**, O. Laczkovich, T. Sovány, B. Hopp, T. Smausz, G. Regdon jr.: Fighting Against Falsified Pharmaceuticals by 2D Laser Coding Technology in Case of Using Naturally Colored Polymer Film Coating, 12th Central European Symposium on Pharmaceutical Technology and Regulatory Affairs, Szeged, Hungary, 20-22 September 2018 (Poster presentation)

3) **K. Ludasi**: Lézeres technológiával a gyógyszerhamisítás ellen, SZTE Orvos- és Gyógyszerésztudományok doktori iskolák II. Ph.D. Szimpóziuma, Szeged, Hungary, 30 November 2018.

4) **K. Ludasi**, G. Regdon Jr.: Anti-counterfeiting protection, development of modern drug identification technologies using laser technology, I. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Hungary, 31 January 2019

5) **K. Ludasi**, G. Regdon jr.: Development of QR coded tablets for anti-counterfeiting of drugs by laser technology, II. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Hungary, 23-24 January 2020

6) **K. Ludasi**, G. Regdon jr.: Gyógyszerhamisítás elleni védelem, modern gyógyszerazonosítási technológiák alkalmazása, „Hét Csillagos Gyógyszerész", Kötelező Továbbképző Tanfolyam, SZTE, Szeged, 19. November 2016, 27. May 2017, 18. November 2017, 26. May 2018, updated

7) **K. Ludasi**, O. Laczkovich, T. Sovány, B. Hopp, T. Smausz, G. Regdon jr.: Védelem a gyógyszerhamisítás ellen, bevont tabletták egyedi kódolása lézerrel, II. Fiatal Technológusok Fóruma, MGYT Gyógyszertechnológiai Szakosztály, Budapest, Hungary, 10. April 2019

8) **K. Ludasi**: Medical training Course for resident physicians at Neurological Clinic , Szeged, Gyógyszerhamisítás, 03. September 2019

K. Ludasi, A gyógyszerhamisítás aktuális kérdései, „Hét Csillagos Gyógyszerész", Kötelező Továbbképző Tanfolyam, Szeged, Hungary, 20.-22. November 2020, 30. May 2021. (Online presentation)