MAPLE PROCESSING OF TOSYLATE-PULLULAN-TAILOR-MADE BIOMATERIAL THIN FILMS FOR DRUG DELIVERY SYSTEMS

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Abstract: We report the successful deposition of tosylate-pullulan by MAPLE using a KrF* excimer laser. The thin films are composed of starting materials preserving their initial chemical structure. We have demonstrated that MAPLE can provide an improved approach to growing high quality thin films of tosylate-pullulan, including an accurate thickness control highly required in targeted drug delivery. The fluence plays a key role in the optimization of the depositions of this polysaccharide. This new pullulan—tailor-made biomaterial with desirable functional groups are potentially used not only for new drug delivery systems but also as substrates for cell growth, and as agents in immunology testing.

Introduction

Rapid advances in the synthesis of nanoscale materials along with our ability to fabricate nano meter size structures could lead to significant breakthroughs in therapeutics and diagnostics.

In recent years, in association with progress and innovation in the field of pharmaceutical technology, there has been an increasing effort to develop controlled release dosage forms for many drugs [1]. Controlled release dosage forms have many advantages in safety and efficacy over immediate release drug products in that the frequency of dosing can be reduced, drug efficacy can be prolonged and the incidence and/or intensity of adverse effects can be decreased [2].

The development of new drugs often means the development of poorly soluble drugs. Such drugs commonly suffer from poor bioavailability when dosed orally, and require harsh solvents when prepared for parenteral use. In the last few years there has been resurgence in interest in pullulan (a natural water-soluble polysaccharide), particularly for higher-value health and pharmaceutical applications [3]. Various protocols have been adopted in order to perform the desired experiments, since pullulan, like most polysaccharides, has poor solubility in common organic solvents.

Research today is focused on developing new oral delivery systems to improve the delivery of small molecular-weight drugs, proteins, and gene therapies that cannot normally be taken orally. Possible solutions to the problem referenced above are developing proper drug formulations for improved absorption of insoluble compounds and macromolecules that allow for improved bioavailability and release rates, potentially reducing the amount of dose required and increasing safety through reduced side effects [4], and developing effective ways of manufacturing drug formulations as coatings (i.e., thin films) with controlled sizes, morphology, and surface properties to improve handling, dispersion, and absorption [5].

Thin polymer films can be deposited by a wide variety of techniques that range in their complexity and applicability [6]. The choice of which deposition technique to use depends upon the physicochemical properties of the polymer, the requirements for film quality and the substrate that is being coated. MAPLE processing [7] has been shown to provide a method of producing complete, or continuous, coated drug of high encapsulation efficiency while requiring minimal processing [8-10]. Basically a core drug is encapsulated with a thin layer of a coating material, such as a surfactant or a biodegradable polymer. The coating may be applied to slow the rate of release of an active component, improve the dispersion / flow properties, or to increase the absorption into the systemic circulation.

The MAPLE process also has several advantages over conventional techniques including: it is a fast

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process with run-times on the order of minute, a variety of coating materials can be used, making it possible thus it is possible to produce films from materials with proven biocompatibility, it is a dry, solventless technique that can be conducted under a sterile conditions, drug agglomeration / adhesion can be minimized by applying coatings that affect the bonding nature and electrostatic charge on the surface, formation of capsules by depositing coatings onto the drug surface will make it possible to control drug release kinetics by: (a) diffusion of the drug though a polymeric coating, (b) degradation of a biodegradable polymer coating on the drug, releasing the core drug material [11].

Getting the right amount of drug to the right tissue or organ and keeping it there is where most therapies fail. MAPLE process is directed towards producing new once-a-day delivery systems in therapeutic areas of unmet critical need.

We report the successful deposition of tosylate-pullulan derivatives by MAPLE. KrF* excimer laser source (λ= 248 nm) operated at repetition rate of 10 Hz has been used. This new pullulan—tailor-made biomaterial with desirable functional groups is potentially used not only for innovative drug delivery systems but also as potential linings for artificial organs, as substrates for cell growth, and as agents in immunology testing.

Materials and Methods

We used in our experiments a new derivate of pullulan: tosylate-pullulan synthesized by the Petru Poni Institute of Macromolecular Chemistry, Iasi, Romania, from pure pullulan made in this country (P-20 type) following a patented original method by the National Institute for Chemical-Pharmaceutical R&D, Bucharest, Romania [3]. Tosylate-pullulan was obtained by an esterification reaction of the hydroxyl groups with tosyl chloride in the presence of a basic medium. After preliminary tests this biopolymer is soluble in chloroform (melting point of 209 K). Also it is an appropriate solvent for the MAPLE experiments because of its good absorption at the 248 nm KrF* laser wavelength. The colloidal solution containing 2% tosylate-pullulan was carefully mixed and then frozen at 77 K.

The UV-MAPLE deposition setup has been described in great detail elsewhere [7]. Briefly the setup consists of an excimer laser source, directed into a vacuum chamber that encloses a substrate holder and a liquid nitrogen cooled target holder. After freezing, the target was rapidly mounted inside the deposition chamber and rotated at 0.25 Hz to avoid heating and possible piercing by the multipulse laser irradiation. Before deposition, the chamber was evacuated down to a residual presure within the range of (5-9) 10^{-4} Pa. Films were grown by MAPLE arrangement at the IP ASCR, Prague using a pulsed KrF* excimer laser with 248 nm, τ = 20 ns and operating at 10 Hz, rotated target holder -cooled to LN temperature). The laser radiation was focused by a fused silica lens placed outside chamber. The laser beam incident angle was 45º with respect to the perpendicular to the target surface. The depositions have been done on both side polished <111> Si. The target-substrate distance was 3 cm. The laser spot area was 20 mm^2. The number of subsequent pulses applied for a deposition of one film was (1,500 – 13,300). The laser fluence was set within the range of (90-500) mJ/cm^2 for tosylate-pullulan. The overall MAPLE deposition conditions are given in Table 1.

Table 1: MAPLE deposition conditions for tosylate-pullulan (N35-N39)

Sample AFM symbol	Fluence [mJ/cm^2]	Thickness [nm]	Deposition rate [Å/pulse]
N 35	500	3,871	25.8
N 36	300	2,152	7.17
N 37	200	839	4
N 38	100	96	0.19
N 39	90	224	0.16

The characterization of all MAPLE films was carried out by Atomic Force Microscopy (AFM), Raman spectroscopy and profilometry. AFM measurements were performed with a Quesant Atomic Force Microscope with a resolution of 500 cm^{-1} at a 1 Hz scan frequency. A Renishaw in Via spectrometer (Renishaw, U.K.) was used to collect Raman spectra. Samples were excited with a HeNe laser (632.8 nm) focused on the sample with a 100x microscope objective. A 10 s integration time was used, and the signal was summed 10 scans in the extended scan mode. After irradiation, the samples were visually inspected through the microscope, but no signs of laser damage to the sample were observed. Profilometry measurements were recorded with an Alphastep profilometer.

Results

As noticed in Table 1 in case of tosylate-pullulan thin films MAPLE depositions, we found threshold fluence values of 90 mJ/cm^2 (N39). Decreasing fluence at slightly beneath these values no deposition was observed.

AFM investigations

Figure 1 contains the AFM micrographs of tosylate-pullulan thin films deposited by MAPLE. The surface morphology hardly relies on the laser fluence and evolves from small globular structures to quite large porous compact regions. As previously discussed at the fluences values slightly below 90 mJ/cm^2 no deposition was detected.

At a fluence of 90 mJ/cm^2 we observe a randomized network consisted of small grain having an average diameter of 100nm (symbol N39). The surface seems to be under percolation limit (Table 1). At the fluence of

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100 mJ/cm^2 (symbol N38) a swelling process has started and a gel-network is noticed on the surface. At the fluence of 200 mJ/cm^2 we observe small globular structures uniformly distributed having an average diameter of 800 nm (symbol N37) indicating an intact structure. At the fluence of 300 mJ/cm^2 we noticed conformational modifications corresponding to different amorphous-crystalline forms that can be induced. In this case even an agglomeration tendency of globular structures indicating a degradation tendency (symbol N36) is observed. Further these agglomerated structures become compact but revealing a high porosity (with an approximate dimension of about 100 nm for 500 mJ/cm^2 (symbol N35) indicating an advanced degradation tendency.

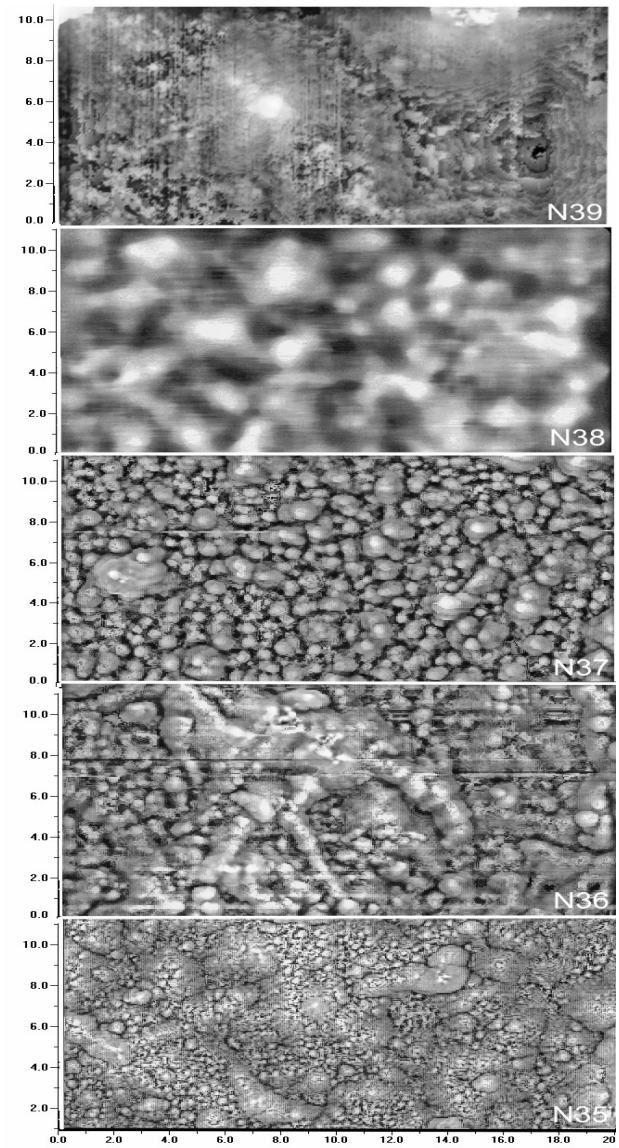

Figure 1: AFM micrographs MAPLE-deposited thin films of tosylate-pullulan at the fluence of 90 mJ/cm^2 (symbol N39), 100 mJ/cm^2 (symbol N38), 200 mJ/cm^2 (symbol N37), 300 mJ/cm^2 (symbol N36), and 500 mJ/cm^2 (symbol N35).

Raman spectroscopy

The Raman spectra of the tosylate-pullulan starting material (Ref) and thin films deposited by MAPLE at 90 mJ/cm^2 (N39), 100 mJ/cm^2 (N38) and 200 mJ/cm^2 (N37) are shown in Figure 2. Raman signature of the reference is typical to a partial organized material.. We assume that the biopolymer-solvent interaction is strong and a part of solvent molecules are still embedded in the material causing the aforementioned swelling process. During laser irradiation these swelled biopolymer macromolecules have been also transferred on the substrate. Nevertheless this confirms the globular structures presence noticed above at the fluence of 90 mJ/cm^2. The intense Raman spectra lines at ~ 1600 cm^{-1} and ~800 cm^{-1} show the solvent effect. At the fluence of 100 mJ/cm^2 (N38) when the surface is under percolation limit Raman spectra could not be suitably recorded. Also at this fluence just above to the fluence threshold value the solvent practically left but some of residual solvent molecules reacted to the biopolymer macromolecules forming O-H and S=O-Cl bonds. These bonds formed "bridges" between biopolymer domains generating a gel-network. As general remark Raman investigations are consistent with AFM images where the transformation from tosylate-pullulan at fluence of 90 mJ/cm^2 (N39) to residues at fluence of 500 mJ/cm^2 (N35) change the morphology from globular material to large clusters. Raman investigations revealed that spectra of samples deposited at fluence of 90 mJ/cm^2 (N39) and 200 mJ/cm^2 (N37), respectively, were analogous to the tosylate-pullulan reference spectra.

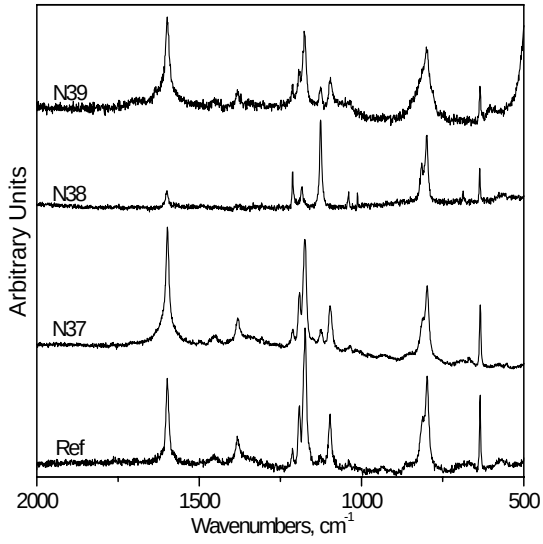

Figure 2: Raman spectra of tosylate-pullulan starting material (symbol Ref) and thin film deposited by MAPLE at the fluence of 90 mJ/cm^2 (symbol N39), 100 mJ/cm^2 (symbol N38), 200 mJ/cm^2 (symbol N37).

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Conclusions

We demonstrate in this work that MAPLE is suitable for producing tosylate-pullulan thin films with close resemblance to the starting structures. In case of tosylate-pullulan thin films MAPLE depositions, we found threshold fluence value of 90 mJ/cm^2. Raman spectroscopy investigations revealed that spectra of samples deposited at fluence of 90 mJ/cm^2 and 200 mJ/cm^2, respectively, were analogous to the tosylate-pullulan reference spectra. At the fluence at 100 mJ/cm^2 when the surface is under percolation limit Raman spectra could not be suitable recorded. AFM investigations showed in case of tosylate-pullulan that the surface morphology also hardly depends on the laser fluence and evolves from small globular structures uniformly distributed having an average diameter of 800 nm (200 mJ/cm^2) indicating an intact structure to quite large compact regions revealing a high porosity (with an approximate dimension of about 100 nm (500 mJ/cm^2). These points indicate an advanced degradation tendency. We conclude that MAPLE can provide an improved approach to growing high quality thin films of pullulan derivatives, including an accurate thickness control highly required in targeted drug delivery.

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