Chapter 25
Pulsed Laser Processing of Functionalized Polysaccharides for Controlled Release Drug Delivery Systems

Functionalized Polysaccharides Processed for Drug Delivery

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Abstract We report on the deposition of triacetate-pullulan polysaccharide thin films on drug pellets (diclofenac sodium) by matrix assisted pulsed laser evaporation method. The radiation generated by a pulsed excimer KrF* laser source ($\lambda = 248$ nm, $\tau = 20$ ns) operated at 2 Hz repetition rate was used for ice targets evaporation. The timed – controlled drug delivery was proved by spectroscopic *in vitro* studies and *in vivo* anti-inflammatory investigations on rabbits. We showed that the coating of drug pellets with triacetate-pullulan thin films resulted in the delayed delivery of the drug for up to 30 min.

Keywords Controlled drug release • Triacetate • MAPLE

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25.1 Introduction

Research in drug delivery and targeting systems has traditionally focused on maintaining the drug levels within a desired therapeutic range inside the affected regions, and to prevent side effects associated with over and under dosing [1–3]. However, to accomplish precise control of the drug, biodegradable coating multilayers are required and these prerequisites are generally incompatible with conventional wet chemical casting. We proposed the use of biopolymer (triacetate-pullulan) coating of active drug substance pellets (diclofenac sodium) to ensure the controlled drug delivery in the organism of test (rabbits) animals. To this purpose, we deposited triacetate-pullulan thin films onto the surface of active drug pellets by matrix-assisted pulsed laser evaporation (MAPLE) method using a KrF* excimer laser source ($\lambda = 248$ nm, $\tau = 20$ ns, $\nu = 2$ Hz). The novel part of our contribution consists in studying the biocompatibility and active substance release by dedicated *in-vitro* and *in-vivo* studies.

25.2 Experimental

25.2.1 Materials

We synthesized triacetate-pullulan, a derivative of pullulan (P-20 type), following a patented original method by the National Institute for Chemical-Pharmaceutical R&D, Bucharest, Romania [4]. Triacetate-pullulan with a substitution degree of about 2.9 was obtained by an esterification reaction of the hydroxyl groups with acetic anhydride in acetic acid medium and in the presence of sulphuric acid. This biopolymer is soluble in organic solvents as chloroform, methylene chloride, acetone, DMF and DMSO. In our study we used the chloroform (having a melting point of 209 K) as a solvent in MAPLE experiments because of its good absorptivity at the 248 nm (the KrF* laser wavelength) [5, 6]. As an active pharmaceutical agent, we selected the diclofenac sodium in tablets of 25 mg dose. This is a well-known non-steroidal anti-inflammatory drug.

25.2.2 Deposition Conditions

The colloidal solutions containing less than 2% triacetate-pullulan in chloroform were carefully mixed and then frozen at liquid nitrogen temperature. After freezing, the obtained ice targets were rapidly mounted inside the deposition chamber and rotated with 0.25 Hz to avoid overheating and possible piercing by multipulse laser evaporation. Prior to deposition, the chamber was evacuated down to a residual pressure of 13 Pa. Thin films of triacetate-pullulan were obtained with a KrF*

laser source generating pulses of 248 nm wavelength and 20 ns duration at a frequency repetition rate of 2 Hz. The laser radiation was focused by a fused silica lens placed outside the chamber. The incident angle of the laser beam was 45°. During deposition, the drug pellet substrate was kept at room temperature. The target–substrate distance was set at 4 cm. After preliminary tests and according to previous experience [7, 8] we used an incident laser fluence of 400 mJ/cm^2. The irradiation spot area was 11.5 mm^2. The number of pulses applied for the deposition of one film was 15,000.

For *in vitro* tests, few trials have been performed in view of establishing the dispersion/solubilization conditions of the active substance. The diclofenac sodium was solved in different dispersion/solubilization environments: (i) Physiologic serum (pH = 5.5), (ii) Basic physiologic serum with NaOH, (pH = 8.5 corresponding to small intestine), and (iii) Acidic physiologic serum with HCl, (pH = 3 corresponding to stomach). The used concentration was 25 mg active substance in 10 ml dispersion/solubilization environment. To measure *in vitro* time-release profiles from uncoated and coated (with MAPLE-deposited thin films) drug pellets we followed a specific protocol: two sets of uncoated and coated pellets were immersed in 50 ml basic physiologic serum (pH = 8.5) placed in two Erlenmeyer recipients. All experiments were performed at 37°C while recipients were stirred at every 30 min. UV/VIS spectra of the active substance in solution were recorded with a Secomam – Dathelie UV/VIS spectrophotometer taking the dispersion/solubilization environment as reference. In case of *in vivo* tests the pellets were administered (12 mg/kg) to two sets of *Chinchilla* rabbits. After 30 min, both rabbits' ears were moistened for 35 times with an inflammatory solution: pyridine (10 ml), distilled water (2.5 ml), volatile Oleum Sinapis and ethylic ether. The ear thickness was monitored every 30 min.

25.3 Results and Discussion

25.3.1 In Vitro Tests

The dispersion/solubilization conditions and corresponding response times of gastroresistant coating were given in Table 25.1. Following these data, the basic physiologic serum with NaOH, pH = 8.5 was further used due to the resonable response time of 30 min only. The diclofenac sodium concentration in the dispersion/solubilization environment was 0.05 mg/ml.

To monitor *in vitro* time release profile of the active drug the UV/VIS spectra were recorded (Fig. 25.1).

The spectrum in Fig. 25.1 exhibits two absorption maxima at 214 and 277 nm. *In vitro* time release profiles of the active drug were next obtained at these two characteristic wavelengths. The absorbance results at 214 and 277 nm for 3 h and 30 min recording time were represented in Figs. 25.2 and 25.3.

Table 25.1 Dispersion/solubilization conditions and response times

#	Dispersion/solubilization environment @ 37°C (25 Mg/10 Ml)	Dispersion/solubilization response time of gastroresistant coating (H)
1	Physiologic serum, pH = 5.5	6 – too slow
2	Basic physiologic serum with NaOH, pH = 8.5	0.5 – optimal conditions
3	Acidic physiologic serum with HCl, pH = 3	No solubility

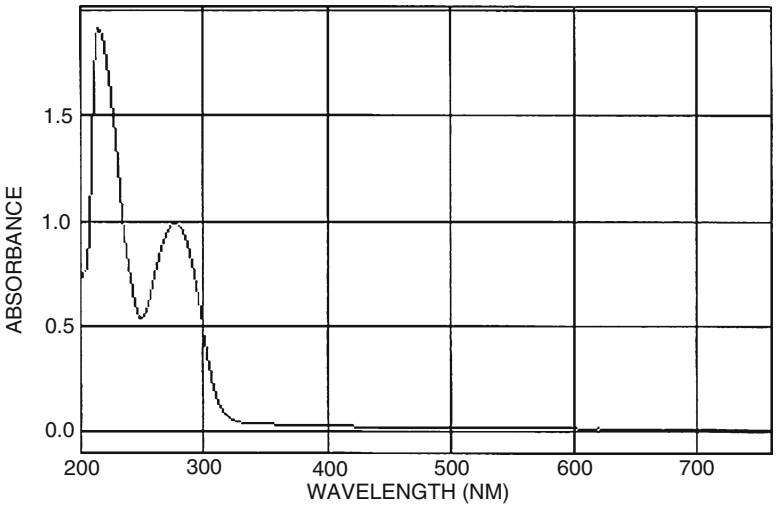

Fig. 25.1 Absorbance spectra of diclofenac sodium in physiologic serum w/NaOH

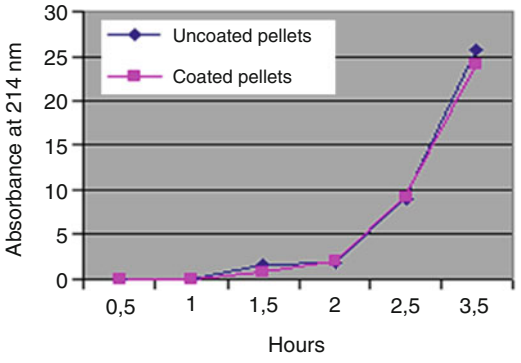

Fig. 25.2 Time release profile of uncoated and coated pellets at 214 nm

From Figs. 25.2 and 25.3, a retard of about 1 h was visible in case of both uncoated and coated pellets. In Fig. 25.2, a larger active substance delivery was noticed after 1.5 h for the uncoated pellets indicative for the existence of a controlled release effect. After 2 h, the delivery got again similar for the two pellets.

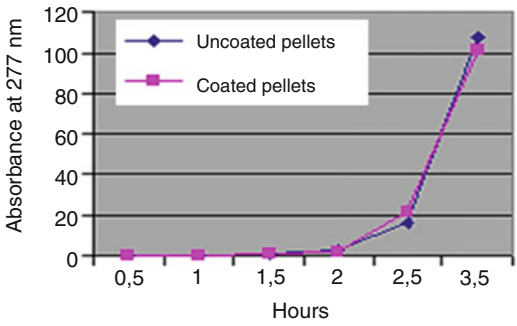

Fig. 25.3 Time release profile of uncoated and coated pellets at 277 nm

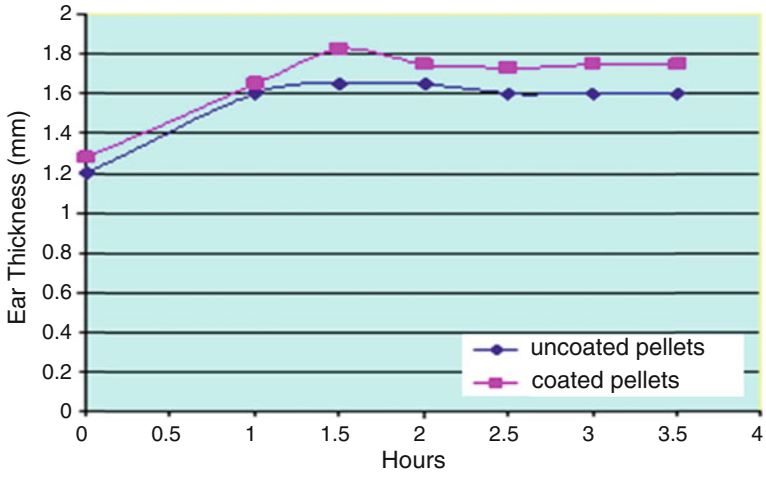

Fig. 25.4 Ear oedema thickness versus drug release time

25.3.2 In Vitro Tests

Ear oedema presence, consistence and vascular patterns were monitored after the inflammatory solution application. The total observation time was 3 h and 30 min. Initially the ear aspect was rather similar in both cases. Then the vascular pattern was accentuating reaching a maximum after 1–1.5 h from induced inflammation (1.5–2 h after drug administration).

From Fig. 25.4, it is observed that the coating of pellets does not impede enteric active drug dispersion and absorption. Nevertheless for coated pellets a slight (30 min.) retardation of inflammatory effect was noticed. Indeed, in the

case of uncoated pellets the maximum effect was reached after 30 min to the difference of coated pellets when the maximum effect was reached after 1 h from the inflammation installation. Results indicated that the triacetate-pullulan thin films coating induced a retard dispersion/absorption effect of the active drug action at the enteric level.

25.4 Conclusion

We showed that MAPLE can provide an improved approach to growing high quality triacetate-pullulan thin films with close resemblance to the starting composition and accurate thickness highly required in controlled release drug delivery systems. *In vitro* tests revealed that MAPLE-deposited thin films allowed proper dispersion/solubilization of active diclofenac sodium. Our spectroscopy studies performed at the diclofenac sodium absorption peak maxima at 214 and 277 nm evidenced a noticeable time release difference between uncoated and coated drug pellets. The coating with triacetate-pullulan thin films induced a retard effect (up to 30 min) of the drug release. This data were congruent with the results of the *in vivo* anti-inflammatory tests on rabbits after the dosage of the uncoated and coated drug pellets.

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