Investigation of polyelectrolyte multilayers deposited on corona charged composite polylactic acid / poly(ε-caprolactone) substrates

I Bodurov, M Marudova*, A Viraneva, T Yovcheva, A Grigorov

Department of Physics, Faculty of Physics and Technology, University of Plovdiv “Paisii Hilendarski, 24 Tzar Asen Str., 4000 Plovdiv, Bulgaria
E-mail: marudova@uni-plovdiv.net

Abstract. In the present study chitosan and casein polyelectrolyte multilayers (PEMs) deposited on composite polylactic acid (PDLA) / poly(ε-caprolactone) (PEC) substrates were investigated. The substrate’s morphology was investigated using polarized light microscopy and their degree of crystallization was studied by the application of differential scanning calorimetry. The substrates obtained were charged in a corona discharge system, consisting of a corona electrode (needle), a grounded plate electrode, and a metal grid placed between them. Positive or negative 5 kV voltage was applied to the corona electrode. 1 kV voltage of the same polarity as that of the corona electrode was applied to the grid. The dependences of the normalized surface potentials on the storage times of positively and negatively charged substrates were studied. Layer-by-layer (LbL) technique was used for multilayer deposition on the substrates. PEMs with different number of layers (4 or 8) were obtained. A model drug Benzydamine hydrochloride was loaded in the casein layers in order to evaluate the effect of the kind of substrate on the drug immobilization and release. A study of the drug release kinetics in saline buffer was carried out and the amount of the released drug was calculated spectrophotometrically. It was shown that the experimental results fit to the Peppas model with a very good level of correlation and the model parameters differ depending on the used substrate.

1. Introduction
Biodegradable polymer films, comprised of different materials such as poly-lactic acid (PLA), poly (ε-caprolactone) (PCL) and poly ethylene terephthalate (PET), are a popular choice in the field of biomedicine [1, 2]. In the search of novel drug delivery techniques many researchers have investigated the physical and chemical properties of these materials in order to better understand and implement them in the form of biodegradable bases for multilayer films. One of the drawbacks of pure polymer films comes from their modification requirements. The use of corona discharge has been demonstrated to improve the biocompatibility of PLA and PCL materials [3, 4], however the fine tuning of their properties can require more intensive methods.

Polymer blending of two different kinds of biopolymers (for example PLA and PCL) in different ratios has been demonstrated to improve the film properties, when compared to pure films [5, 6, 7]. In addition small variations of the blend composition can lead to significant changes of the mechanical properties of the films [7]. In his 2012 research [8] N. Noroozi and his colleagues show that PLA/PCL blends are immiscible at all compositions, with clear boundaries between the different phases.
Additionally the introduction of small quantities of PCL is shown to increase the crystallinity of the PLA component of the blend.

The implementation of corona discharge can further improve the properties of the polymer blends as it is often implemented for the creation of multilayers on pure polymer films for drug delivery [9]. In fact the difference in the charge retention capabilities of the two components of PLA/PCL blends, as well as the formation of island like patterns of the different components, can be highly beneficial for charge retention [10]. This, combined with the improvements in the mechanical properties of the blends, can lead to the creation of improved biodegradable multilayer films for controlled drug delivery.

In this paper we aim to investigate the impact of different substrate ratios on the release of benzydamine hydrochloride from casein/chitosan multilayers, when compared to pure polymer films.

2. Materials and methods

2.1. Materials
Poly(ε-caprolactone) was purchased from Lactel Absorbable Polymers (USA). Poly(D-lactic acid), sodium caseinate (casein sodium salt from bovine milk), chitosan (high molecular mass, degree of deacetylation > 75%), and benzydamine hydrochloride were delivered from Sigma-Aldrich and were used without further purification. All other used chemicals were with analytical grade.

2.2. Methods

2.2.1. Substrate preparation. The substrates were prepared by casting a mixture of two chloroform solutions of 1% w/v PDLA and 1% w/v PCL at the desired ratios (25/75, 50/50 and 75/25). These solutions were dried at room temperature until the complete evaporation of the solvent. Two films of pure PDLA and PCL with the same polymer concentration were also created. The resulting films were kept in a dessicator at room temperature and relative humidity (RH) of 54%.

2.2.2. Corona charging of the substrates
The substrates obtained were charged in a corona discharge, in order to achieve positive or negative electric charge on their surface. The charging in a corona discharge was carried out by means of a conventional corona triode system, consisting of a corona electrode (needle), a grounded plate electrode and a grid placed between them. The substrates were placed on the grounded plate electrode and were charged for 1 minute at room conditions. Positive or negative 5 kV voltage was applied to the corona electrode. 1 kV voltage of the same polarity as that of the corona electrode was applied to the grid. After charging, the initial surface potential of the samples $V_0$ was measured. The electrets surface potential was measured by the vibrating electrode method with compensation, by which the estimated error was better than 5%.

2.2.3. Layer-by-layer deposition. The Casein/chitosan polyelectrolyte multilayers (PEMs) were formulated by alternative dipping of the precharged substrate into 1% casein and 1% benzydamine hydrochloride with pH 8 and 1% chitosan solutions with pH 5.0. The ionic strength for both solutions was 100 mM. The deposition always started with the polyelectrolyte, charged oppositely to the substrate. Each deposition step was followed by rinsing in distilled water. The casein/chitosan PEMs consisted of 4 or 8 layers.

2.2.4. Polarizing microscopy. The morphology of the obtained substrates was examined by means of polarizing microscope Leica Microsystems, model DM4 (Germany).

2.2.5. Differential scanning calorimetry (DSC). The crystallinity of the substrates was determined by DSC 204F1 Phoenix (Netzsch Gerätebau GmbH, Germany) instrument. An indium standard
(T_m=156.6°C, ΔH_m=28.5 J/g) was used for the heat flow and the temperature calibration. All of the samples were hermetically sealed in aluminum sample pans and an empty pan, identical to the sample one, was used as reference. The measurements were conducted under argon atmosphere in the temperature range from 20°C to 250°C at a heating rate of 10°C/min.

2.2.6. Benzydamine hydrochloride drug content. Benzydamine hydrochloride loaded films (3 samples of each type) were placed into 20 mL phosphate buffer saline (pH 7.4) and stirred continuously for 72 h on a magnetic stirrer. Then, the samples were sonicated for 5 minutes and filtered using Chromafil® syringe filter (0.45 mm). The amount of BH was determined using UV/Vis spectrophotometer monitoring the band at wavelength 306 nm. The drug concentration was calculated from a standard calibration curve of BH in phosphate buffer saline (pH 7.4).

2.2.7. Evaluation of the drug-release kinetics and mathematical modeling of the process. The dissolution study was performed using the stirred beaker method. Each BH loaded film (2×2 cm, 5 samples of each type) was put into a beaker containing 20 mL dissolution media (phosphate buffer saline, pH 7.4). The temperature was maintained at 37±1°C and the rotation speed was kept at 50 rpm throughout the experiment. 3 mL of the saline solution, where the PEMs were immersed, were collected at a specific time interval. The liquid was filtered (Chromafil® syringe filter, 0.45 mm), and then analyzed spectrophotometrically (the band at 306 nm). The amount of 3 mL of saline solution was added to the rest of the test solution. In order to determine the drug release kinetics of the PEM samples, Korsmeyer-Peppas kinetic model was utilized.

3. Results and discussion

3.1. Electret properties – time storage influence
Time dependences of the normalized surface potential for positively and negatively charged PDLA, PEC and PDLA:PEC electrets were studied for 360 minutes. The surface potential was measured once of 10 minutes for the first 60 minutes when the charge was rapidly decaying. After this period, steady state values of the normalized surface potential were established for all investigated electrets. Time dependences of the normalized surface potential for all investigated samples are presented in figure 1.

![Figure 1](image-url) Normalized surface potential time dependences for PDLA, PEC and PDLA:PEC films at both types of corona charging: a) positive and b) negative.

Each point in the figure is a mean value from 6 samples. The calculated standard deviation was better than 5 % from the mean value with confidence level 95 %.

The experimental results presented in figure 1 show that:
The values of the normalized surface potential for samples, charged in a positive corona, are higher than those for samples, charged in a negative corona, independently of the material type. Charging in a corona discharge leads to formation of different ions on electret surface. In case of a positive corona the dominating ions are $H^+(H_2O)n$ and in case of a negative corona - $CO_3^−$. Those ions are bound in traps of various depths and are released from them depending on the surrounding conditions.

For all investigated samples the normalized surface potential values are decaying exponentially for the first 60 minutes. After this the rate of decay decreases and is practically stabilized within 360 minutes. Electrets’ surface potential depends on the amount of trapped charges in different surface states of the samples. In the initial period of time the surface potential rapidly decreases because of release of the weakly captured charges from the shallow energy states. Then the surface potential stabilizes to a steady state value caused by the tightly captured charges in the deep energy traps.

The values of the normalized surface potential are highest for the PEC electrets independently of the corona polarity. Probably this is due to the degree of crystallinity measured by DSC methods (see figure 2).

3.2. Phase state of composite poly(lactic acid)/poly(ε-caprolactone) films

The crystal state of the substrates was determined by DSC (Figure 2). The melting enthalpy of PDLA corresponds to degree of crystallinity 9% and that of PEC – to degree of crystallinity 59%. The melting of the composite film PDLA:PEC 50:50 is carried out at two temperatures – 67.3°C and 151.0°C, which are the characteristic melting temperature of PEC and PDLA. Based on this result it can be assumed that PDLA and PEC are immiscible at molecular level and formed heterogeneous areas. Similar results are confirmed by other authors [8].

![DSC thermograms PDLA, PEC and composite substrates](image)

**Figure 2.** DSC thermograms PDLA, PEC and composite substrates

The degree of crystallinity of the composite film is 68%, which is higher than the crystallinity of the one-component films.

3.3. Morphology of composite poly(lactic acid)/poly(ε-caprolactone) films
It is important to study the morphology of the substrate, since it largely determines the mode and the type of the deposited thereon thin polyelectrolyte layers. The surface morphology of the PDLA, PEC and PDLA/PEC films is presented in figure 3.

The PDLA film is characterized by flat surface without visible crystal units. During the fabrication of the poly \((\varepsilon\text{-caprolactone})\) substrate from solution, the long time for evaporation of the solvent facilitates the preparation of large (size of about 200 \(\mu\)m) spherulites. Spherulites are composed of well observed lamellae. The PDLA:PEC 50:50 morphology is characterized with small crystal unit, which do not form spherulites.

The presence of non-heterogeneous areas in the structure of the PEC and PDLA:PEC films determines the better ability for capturing of electrical charges, which explains the greater stability of these electrets.

### 3.4. Benzydamine hydrochloride loading capacity

The amount of the BH, which was loaded to one PEM unit (2 x 2 cm) built-up on substrates is presented in table 1.

| Sample          | Positive corona | Negative corona |
|-----------------|-----------------|-----------------|
|                 | 4 layers        | 8 layers        | 4 layers        | 8 layers        |
| PDLA            | 457.6572        | 593.6974        | 1004.33         | 1320.204        |
| PDLA:PEC 50:50  | 396.1382        | 483.9637        | 536.8884        | 625.7096        |
| PEC             | 944.0957        | 1023.681        | 2004.398        | 2476.466        |

The presented results show that:
- The loaded amount of BH is higher in the PEMs consisting of 8 layers.
- The loading amount of BH is higher in the PEMs, built-up on negatively charged substrates. A possible reason could be the higher initial surface potential values of negatively charged substrates.
- The biggest amount of uploaded drug is observed in PEMs assembled on PEC substrates. This correspond to the highest values of the normalized surface potential of the PEC films.

### 3.5. Benzydamine hydrochloride release behavior

The BH release from PEMs assembled at different substrates is presented in figure 4. The cumulative BH release was around 30 – 50% during the first hour and 60 – 80% during the first 6 hours. These results demonstrate that there is no burst release of surface adhered drugs. Therefore, the BH is tightly
bound inside the polyelectrolyte layers. The release is faster from PEMs built-up on negatively charged substrates, probably because these PEMs finish with BH loaded casein layer. In this case the diffusion path is shorter and BH leaves the structure at an increased rate.

For PEMs assembled on positively charged substrates, the slowest rate of release is from PDLA substrates. Thus, they represent delayed release systems. In the case of negatively charged substrates, the slowest release is from PDLA:PEC composite substrates.

![Graphs showing BH release from PEMs with 4 and 8 layers, assembled on positively charged (a and c) and negatively charged (b and d) substrates.](image)

**Figure 4.** BH release from PEMs with 4 and 8 layers, assembled on positively charged (a and c) and negatively charged (b and d) substrates

The obtained results demonstrate that the BH release profile could be controlled by varying the composite of the substrate and its surface charge. Based on the drug release results, the release kinetics dependences were fitted to Korsmeyer-Peppas model [11]. The correlation coefficients were 0.99. The exponent n in the Korsmeyer-Peppas model was used to characterize the release mechanism of BH. For all investigated samples it was less than 0.5, demonstrating Fickian diffusion-controlled release mechanism.

4. Conclusion
The BH release from chitosan/casein PEMs, assembled on different substrates, was examined in this study. It was shown that the steady state values of the normalized surface potential for PDLA electrets are the lowest compared to others investigated films independently of the corona polarity. This may be due to the lowest value of the degree of crystallinity. The highest BH loading capacity was observed for PEMs assembled on negatively charged PEC substrates, which can be contributed to their high surface potential and the ability to bind bigger amount of polyelectrolytes. In general, the BH loading and release profile could be controlled by changing the polarity and the substrates and the PDLA:PEC ratio.
Acknowledgments
This work was supported by the Bulgarian National Scientific Fund, Project No KP-06-N38/3.

References
[1] Xiao L., Wang B., Yang G. and Gauthier M 2012 Poly(Lactic Acid)-Based Biomaterials: Synthesis, Modification and Applications (Biomedical Science, Engineering and Technology) ed D N Ghista (Rijeka: IntechOpen) pp 247 - 282
[2] Lendlein A., Rehahn M., Buchmeiser M R., Haag R 2010 Macromol. Rapid. Commun. 31 1487 - 1491
[3] Nandhakumar S., Dhanaraju M D., Sundar V D., Heera B 2017 Bulletin of Faculty of Pharmacy 55(2) 249 - 258
[4] Chaiwong C., Rachanapun P., Wongchaiya P., Auras R. Boonyawan D 2010 Surf. & Coatings Technol. 204 2933 - 2939
[5] Zhu B., Bai T., Wang P., Wang Y., Liu C., Shen C 2020 Int. J. of Biol. Macromolecules 153 1272 - 1280
[6] Broz M E., VanderHart D L., Washburn N R 2003 Biomaterials 24 4181 - 4190
[7] Yeh J T., Yeh J., Wu C., Tsou C., Chai W., Chow J., Huang C., Chen K. and Wu C 2009 Polymer-Plastics Technol. Eng. 48 571 - 578
[8] Noroozi N., Schafer L., Hatzikiriakos S 2012 Polymer Eng. Sci. 2348 – 2359
[9] Yovcheva T., Viraneva A., Marinova A., Sotirov S., Exner G., Bodurov I., Marudova M., Pilicheva B., Uzunova Y. and Vlaeva I 2018 IEEE Transactions on Dielectrics and Electrical Insulation 25 766-771
[10] Karmazova P. and Mekishev G 1992 EPL 19 481
[11] Bataglioli R A., Taketa T B., Neto J R., Lopes L. M., Costa C A. R. and Beppu M M 2019 Thin Solid Films 685 312-320