BENZYDAMINE HYDROCHLORIDE IMMOBILIZATION IN MULTILAYER STRUCTURES BASED ON LYOPHILIZED COMPOSITE POLYLACTIC ACID / POLY(E-CAPROLACTONE) SUBSTRATES

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ABSTRACT

In the present paper the polyelectrolyte multilayers (PEMs) deposited on lyophilized composite polylactic acid / poly(ε-caprolactone) substrates were investigated. The substrates were charged under positive corona discharge for 1 min with 5 kV voltage applied to the corona electrode and 1 kV voltage of the same polarity applied to the grid. The dependences on the time of storage of the normalized surface potential of the charged substrates (positive) were investigated. Solutions of 1 % casein in phosphate buffer (pH 8) and 0.1 % chitosan in acetate buffer (pH 5) with added benzydamine hydrochloride were prepared. The creation of the multilayers was carried out by utilizing the technique Layer-by-Layer (LbL). The first built-up layer always possesses an electric charge opposite to that of the substrate. The loading efficiency and drug release kinetics of the chosen model drug were carried out spectrophotometrically.

Keywords: corona discharge, porous composite films, multilayer structures, drug delivery.

INTRODUCTION

Application of biodegradable materials in different medical fields has demonstrated the potential of these materials when compared to other more conventional materials. Polymers such as polylactic acid and poly-(ε caprolactone) are being increasingly utilized in many different applications from tissue engineering to drug delivery [1 - 3]. Different modification methods such as surface corona discharge treatment [4 - 6] can further improve biocompatibility and assist in the creation of multilayer structures on the surface of the charged polymer films. Different bioactive materials can be incorporated within those multilayers, thus further increasing the number of biomedical applications [7, 8]. Freeze drying (or lyophilization) on the other hand provides an easy and controllable method for the creation of porous structures of the chosen polymers, which increases the active surface of the material and improves the incorporation of different bioactive materials [9 - 11]. In our study we investigate the impact of different ratios of the two chosen polymers on the drug loading and delivery properties of lyophilized composite films.

EXPERIMENTAL

Materials

All of the materials used in the study were of analytical grade. Polymer (Poly (D-lactic acid) (PDLA)), model drug (benzydamine hydrochloride (BH)), and two polyelectrolytes (casein sodium salt from bovine milk and chitosan with high molecular mass and degree of deacetylation greater than 75 %) were purchased from Sigma Aldrich. All of these materials were used as delivered. The rest of the used materials were of analytical grade.

Methods

Substrates preparation

A mixture of the two polymers (PEC and PDLA) was utilized for the creation of the substrates. Several mixtures (in 1,4-dioxane) with concentration of 10 % w/v and different mass ratios of polymers (25/75, 50/50 and 75/25) were deposited in glass dishes and placed at -16°C until frozen solid. Pure mixtures of both polymers were additionally prepared in the same conditions. All samples were freeze dried for 72 hours at -50°C and 10 Pa until the solvent has completely evaporated. The created films were placed in an exicator for 1 day at room temperature and 54 % relative humidity (RH). The following multilayer deposition was carried out on the surfaces of these samples.

Corona treatment of the lyophilized substrates

Corona discharge was utilized for the modification of the surface of all of the used samples. A conventional system (called triode) was utilized for this step. All of the prepared samples were positioned on a plate electrode that was grounded. Then positive voltage of 5 kV was applied to the corona electrode, while the grid received voltage of 1 kV with the same polarity. All charging was done for 60 s. The surface potential of the charged films right after charging (V_0) was determined with the use of a method called vibrating electrode with compensation. The error of the measurements was determined to be lower than 5 % [12].

Layer-by-layer deposition of casein/chitosan multilayers

Several different solutions were needed to be prepared for the assembly of the polyelectrolyte multilayers:

- Solution of casein in phosphate buffer with concentration of 1 % at pH 8 and ionic strength of 100 mM.
- Solution of chitosan and drug (BH) in acetate buffer with concentration of 0.1 % polyelectrolyte and 1 % drug at pH 8 and ionic strength of 100 mM.

The layer creation was carried out layer by layer with the previously charged sample being dipped subsequently in each polyelectrolyte with a washing step between each layer. The first deposited layer always has to be with a charge opposite to the one of the dipped samples. Programable slide stainer (MSM Carousel Slide Stainer, SLEE medical GmbH, Germany) was utilized for this process. The dipping process followed a predetermined programme with 900 s in the polyelectrolyte and 300 s washing step. This was repeated for the creation of 8 layers [12].

Differential scanning calorimetry (DSC)

DSC 204F1 Phoenix (Netzsch Gerätebau GmbH, Germany) apparatus was utilized for the determination of the phase states of the studied substrates. An indium standard (T_m = 156.6°C, ΔH_m = 28.5 J g^{-1}) was used for the calibration of the instrument for both temperature and heat flow. All film samples were placed in hermetically sealed pans, made from aluminium, for measurements with an empty pan used for reference. All measurements were performed in argon atmosphere at ranges of 20 to 250°C at a rate of heating of 10°C min^{-1}.

Netzsch Proteus - Thermal Analysis software was used for the measurements of the melting temperature (T_m) and the melting enthalpy (ΔH_m) of the samples.

Based on the determined melting enthalpy of the PEC samples, the percentage of crystallinity (χ_{PEC}) was calculated according to Eq. 1:

$$\chi_{PEC} = \frac{\Delta H_m}{\Delta H_m^0 \cdot \omega_{PEC}} \cdot 100 \quad (1)$$

where χ_{PEC} is the percentage of crystallinity of PEC; ΔH_m is the specific melting enthalpy [J g^{-1}]; ΔH_m^0 is the melting enthalpy of 100 % crystalline polymer ($\Delta H_m^0 = 139.3\ \mathrm{J\,g^{-1}}$ for PEC [13]) and ω_{PEC} is the mass fraction of PEC.

The PDLA crystallinity (χ_{PDLA}) was calculated from the melting enthalpy and cold crystallization enthalpy according to Eq. 2:

$$\chi_{PDLA} = \frac{(\Delta H_m - \Delta H_{cc})}{\Delta H_m^0 \cdot \omega_{PDLA}} \cdot 100 \quad (2)$$

where χ_{PDLA} is the percentage of crystallinity of PDLA; ΔH_m and ΔH_{cc} are the melting enthalpy and cold crystallization enthalpy of PDLA [J g^{-1}]; ΔH_m^0 is the melting enthalpy of 100 % crystalline polymer ($\Delta H_m^0 = 106.0\ \mathrm{J\,g^{-1}}$ for PDLA [14]) and ω_{PDLA} is the mass fraction of PDLA.

Benzydamine hydrochloride (BH) drug content

The determination of the amount of loaded drug

within the samples was carried out by placing the prepared samples in a set volume of phosphate buffer saline (20 mL at pH of 7.4) and kept on a magnetic stirrer for 3 days at standard body temperature and 50 rpm. The resulting solutions were sonicated for 300 sec using by UP100H - Compact Ultrasonic Laboratory Device (Germany), and were subsequently filtered in a standard 0.45 μm syringe filter from ChromafilVR. The quantity of released drug was measured with the use of a spectrophotometer at the UV/Vis range (Metertech SP8001, Metertech Inc., Nangang, Taipei, Taiwan) at a set wavelength of 306 nm. A standard calibration curve for BH was used to determine the concentration of the incorporated drug in the same buffer as the test [12].

Benzydamine hydrochloride (BH) drug release

The determination of the drug release kinetics of the prepared samples was performed with the use of a standard method using stirred beakers. Precut samples with set dimensions (2 × 2.5 cm) were placed in pre-prepared beakers, containing standard dissolution medium (same as the one used for the determination of the drug content) with a set volume of 20 mL at standard body temperature and 50 rpm stirring speed. A set volume (3 mL) was extracted from the fluid and replaced with fresh solution at set time intervals. The taken fluid samples were ran through a filter and their drug content was measured spectrophotometrically at 306 nm with the same device as described in the previous section. All measurements were performed three times and the results were averaged and their standard deviations were determined. Mathematical modelling was utilized for the analysis of the release kinetic curves [12].

RESULTS AND DISCUSSION

Time Storage influence on the electrets surface potential decay

The dependences on time of the normalized surface potential of all studied samples were measured for 6 h. For the first half hour measurements were taken every 5 min due to the rapid decrease of potential, after which measurements were taken less frequently, due to the decrease of the rate of decay, and the measurement was continued until the determination of the steady state values of each sample. All of the collected data from this part of the experiment is presented in Fig. 1.

The data of the Fig.1 represents the averaged value of 5 samples with standard deviation determined to be less than 5 % from the mean with 95 % confidence level.

From the displayed experimental results, it can be determined that the rate of decay of the normalized surface potential for the first hour of the study is exponential, after which point the rate decreases and

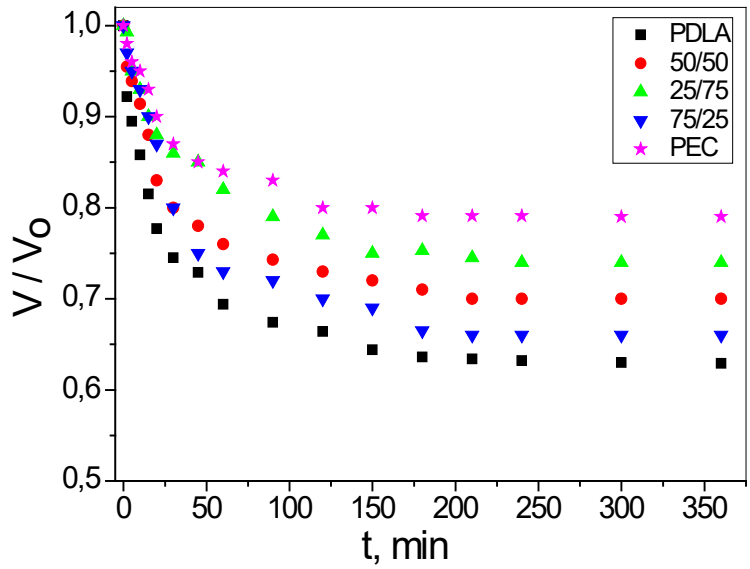


Fig. 1. Time dependences of the normalized surface potential for PDLA, PEC and PDLA/PEC substrates charged in a positive corona.

stabilizes before the 6th hour. These results demonstrate the existence of different surface states that are localized on the surface of the sample and that contain entrapped charges within them. The initial exponential decrease can be accredited to the release of weakly captured charges from any shallow energy states. After this period the potential becomes stable at a set value, which can be due to the remaining tightly entrapped charges in deeper traps. Similar tendencies have been observed in [15]. The results demonstrate that electrets created from PEC possess the highest values, when compared to all other types. This can be explained with the differing crystallinity degrees that were determined with the use of the DSC method (Fig. 2 and Table 1).

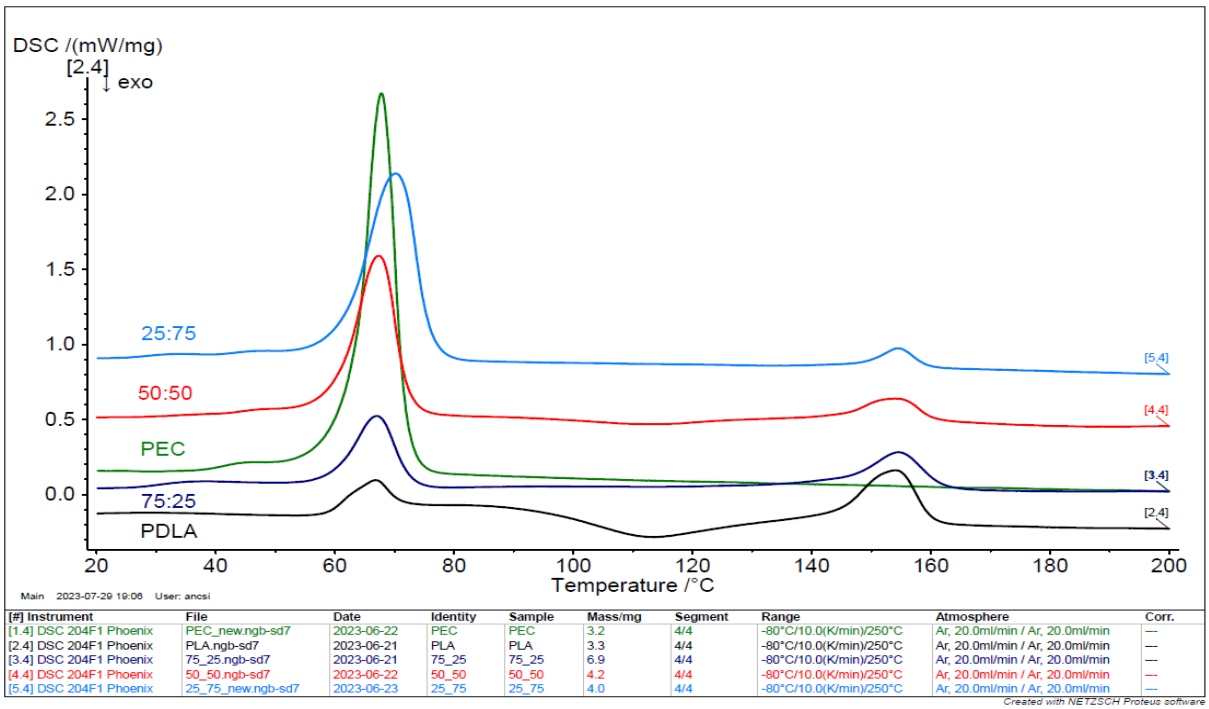
Phase state of polylactic acid / poly(ε-caprolactone) films

Phase state of the PDLA, PEC and PDLA/PEC films were studied with differential scanning calorimetry (DSC) measurements. DSC analysis was performed to determine the degrees of crystallinity of the investigated samples. The obtained heating curves are shown in Fig. 2.

The thermal transitions, melting temperature (T_m), melting enthalpy (ΔH_m), enthalpy of cold crystallization (ΔH_{cc}), and degrees of crystallinity for the compounds and for the entire films are listed in Table 1.

According to the results shown in Fig. 2, all samples have distinct melting transitions. The glass transition temperature of PEC ($T_g \approx 63$°C) was not detected probably because of the very high degree of crystallinity (Table 1). The value of the glass transition temperature of poly-D-lactic acid (PDLA) is in agreement with the literature at 61.2°C [16] which can be distinguished only in neat samples of the polymer due to its overlap with the melting transition temperature of the other polymer PEC (T_m) at around 67°C. Melting transitions in the mixed polymer samples were not influenced by their composition, which can be indicative of the immiscibility of the two polymers at the molecular level. Other authors report similar results [17]. The cold crystallization condition of poly-D-lactic acid can be distinguished for two samples (neat PDLA and 50/50).

From the results presented in Table 1, it can be seen that the addition of PDLA to PEC cusses an increase in the degree of crystallinity. The addition of PEC to PDLA was a similar but much more pronounced effect on increasing the degree of crystallinity. Therefore, an



[#] Instrument	File	Date	Identity	Sample	Mass/mg	Segment	Range	Atmosphere	Corr.
[1.4] DSC 204F1 Phoenix	PEC_new.ngb-sd7	2023-06-22	PEC	PEC	3.2	4/4	-80°C/10.0(K/min)/250°C	Ar, 20.0ml/min / Ar, 20.0ml/min	---
[2.4] DSC 204F1 Phoenix	PLA.ngb-sd7	2023-06-21	PLA	PLA	3.3	4/4	-80°C/10.0(K/min)/250°C	Ar, 20.0ml/min / Ar, 20.0ml/min	---
[3.4] DSC 204F1 Phoenix	75_25.ngb-sd7	2023-06-21	75_25	75_25	6.9	4/4	-80°C/10.0(K/min)/250°C	Ar, 20.0ml/min / Ar, 20.0ml/min	---
[4.4] DSC 204F1 Phoenix	50_50.ngb-sd7	2023-06-22	50_50	50_50	4.2	4/4	-80°C/10.0(K/min)/250°C	Ar, 20.0ml/min / Ar, 20.0ml/min	---
[5.4] DSC 204F1 Phoenix	25_75_new.ngb-sd7	2023-06-23	25_75	25_75	4.0	4/4	-80°C/10.0(K/min)/250°C	Ar, 20.0ml/min / Ar, 20.0ml/min	---

Fig. 2. DCS thermograms for PDLA, PEC and PDLA/PEC films during heating.

Table 1. DSC data for the thermal transitions and degree of crystallinity of PDLA/PEC films.

Sample	PEC			PDLA				Total crystallinity,%
	T_m, °C	H_m, J g^{-1}	c_{PEC}, %	T_m, °C	H_{cc}, J g^{-1}	H_m, J g^{-1}	c_{PDLA}, %	
PEC	67.8	110.3	79.2	-	-	-	-	79.2
25/75	70.3	84.8	81.1	154.7	-	5.7	21.6	66.2
50/50	67.4	57.6	82.7	154.8	4.8	13.4	16.3	49.5
75/25	67.0	31.3	89.9	154.8	-	16.9	21.2	38.4
PDLA	-	-	-	154.2	20.9	23.8	2.7	2.7

Table 2. Amount of BH loaded to one PEMs unit built-up on different substrates.

Sample	PEC	25/75	50/50	75/25	PDLA
Loading efficiency, μg	2526 ± 50	1132 ± 31	499 ± 60	621 ± 22	588 ± 20

assumption could be done, that the inhomogeneities and the presence of two phases in the mixtures induce nucleation effect and contribute to an increase in the degree of crystallinity. This hypothesis is in agreement with previously reported results on the nucleation of PDLA in the presence of PEC [18].

The total crystallinity of the films decreases when the content of PDLA increases (see Table 1). This result can explain the more stable electret behavior of the samples containing a larger amount of PEC.

Benzydamine hydrochloride (BH) loading efficiency

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

The data shows that the loading efficiency of the PEMs strongly depends on the substrate composition. The amount of loaded BH varies between (499 ± 60) μg to (2526 ± 50) μg, and increases when PEC dominates in the substrate. It is the highest in PEMs assembled on PEC substrates. This result corresponds to the highest values of the normalized surface potential of the PEC films and hence probably the most stable structure of the PEMs.

The BH loading in chitosan/casein PEMs deposited on composite polylactic acid / poly(ε-caprolactone) substrates without being lyophilized was already reported by our group [19]. In both studies, the tendency to increase the amount of incorporated BH in the structures built on PEC substrates is preserved. In the case of the lyophilized substrates, the loaded BH increased significantly, and in the lyophilized PEC substrates, it was 4 times more than in the non-lyophilized. Probably this result is due to the porous morphology of the lyophilized films, which are able to retain a larger number of polyelectrolytes and the drug included in them [20].

Benzydamine hydrochloride (BH) release

Fig. 3 represents the values of the study of the release kinetics of the incorporated in the PEMs drug (BH) for all investigated polymer ratios. The data shows that for the first hour between 65 to 82 % of the drug is being released, with the value increasing to 82 to 95 % during the initial 6 h.

These results demonstrate the presence of a burst effect, which is probably due to the porous structure of the films and bigger diffusion coefficient in comparison with dense without porous structures [19]. The release is faster from PEMs built-up on PDLA substrates, when 90 % of the drug leaves the structure during the firsts three hours. The slowest release is observed from PEMs built-up on PEC substrates. Probably because of the higher surface charge density of the PEC the multilayers are tightly attached to the substrate and are characterized with denser structure.

The Weibull model (as shown in Eq. 3) was used for

Table 3. Weibull model parameters.

Sample	PEC	25/75	50/50	75/25	PDLA
a	11.5 ± 0.8	11.1 ± 1.3	16.7 ± 1.3	30.2 ± 1.5	53.5 ± 3.2
b	0.31 ± 0.01	0.20 ± 0.01	0.36 ± 0.01	0.46 ± 0.02	0.35 ± 0.02
R^2	0.996	0.969	0.979	0.988	0.990

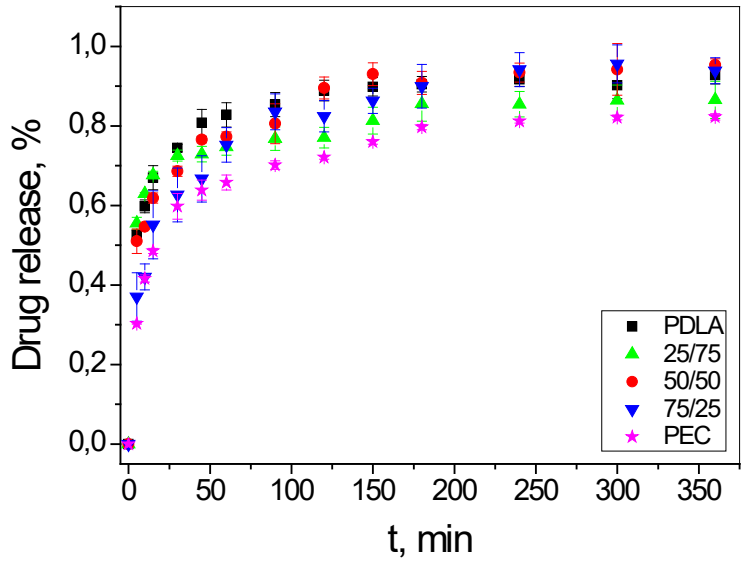


Fig. 3. BH release from PEMs assembled on positively charged PDLA/PEC substrates.

the mathematical processing of the gathered results in order to better characterize the release rates [21]:

$$M = M_0\left[1 - exp\left(-\frac{(t-T)^b}{a}\right)\right] \quad (3)$$

where M is the amount of dissolved drug as a function of time; M_0 - the total amount of released drug; t - time; T - lag time caused by dissolution process; a - scale parameter of the time dependence, and b -shape of the dissolution curve progression.

All of the model parameters for the drug release measurements are represented in Table 3.

The minimum value for the a parameter is calculated for PDLA/PEC = 25/75, which is interpreted as the fastest release. The slowest release (maximal a parameter) is observed from the PEMs deposited on PDLA substrates. The lowest value of the b parameter for the PDLA/PEC = 25/75 samples demonstrates the steepest increase of the BH release.

CONCLUSIONS

The results of this paper investigate the release of a model drug (BH) from PEMs, created on the surface of freeze-dried (lyophilized) composite polymer substrates, created from PDLA and PEC at different mass ratios. The data demonstrates that PEC possesses the highest steady state values of the normalized surface potential when compared to all other samples, which can be attributed to the high crystallinity degree of the polymer. PEC substrates, possessing positive charge, demonstrate the highest loading efficiency, which can be contributed to their higher polyelectrolyte binding capabilities and high surface potential. The loading and release profiles of the drug BH can be controlled by a variation in the ratio of the two polymers.

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