

Surface modification. Two types of surface modification (physical and chemical) were used for this study. A standard system of a corona discharge was utilized for the physical modification. This system consists of a corona electrode (needle), a grounded plate electrode and a grid placed between the two. All substrates were charged for 1 minute at 5 kV of negative voltage, 1 kV of the same polarity being supplied to the grid. The chemical modification was carried out in two steps. First the surface of the substrates was hydrolyzed in a 0.3 M solution of aqueous sodium hydroxide (NaOH) at 45° C for 15 minutes. The samples were then washed in dilute HCL (0.1 N) and deionized water three times to remove any residual NaOH. Finally, the hydrolyzed samples were submerged in a methanol solution, containing 75 mg of EDAC for 4 hours. This was done to activate and condense the free carboxyl (-COOH) groups, created during the hydrolysis. After activation all samples were rinsed three times in methanol to remove any residual EDAC. Four different modification combinations were investigated in this study – two of them being pure modifications (only chemical or purely physical) and two combinations of both modifications (chemical followed by physical and physical followed by chemical). After modifications the surface potential of all of the studied samples was measured with the use of the vibrating electrode method with compensation, like the one described in [7].

Multilayers creation. The creation of the multilayers on the modified surface of the substrates was carried out with the use of a layer-by-layer (LbL) deposition technique in a MSM SLEE automatic carousel stainer. The multilayers were formed by consecutive dipping in two acetate buffer (pH 5) solutions – one containing 0.1 % chitosan and another containing 0.05 % xanthan and 0,05 % locust bean gum (LBG). The enzyme -galactosidase, used in this study, was included in the xanthan solution. Each dipping step lasted 15 minutes and was followed by an acetate buffer (pH 5) wash for 5 minutes to remove any residual solution before the next dipping step. This procedure was repeated until the creation of 8 layers on the surface of the modified substrates.

Water contact angle measurement. All water contact angle measurements were performed under standard conditions (at room temperature and normal air pressure). Five measurements were performed for each type of modification on different places of the surface of the multilayer films. The average of those five results was used for determination of the hydrophobicity of the

modified samples. Tiny droplets of 2 μ l were used in order to reduce the effect of surface roughness on the water contact angle. The drops were deposited on the surface with the use of a precise 10 μ l microsyringe (Innovative Labor System GmbH, Germany). Contact angles were obtained by using the tangent of the drop profile from pictures captured with an USB microscope. Image processing was performed using public domain ImageJ software (ImageJ v1.51k software).

Determination of enzyme activity. The enzymatic activity of the immobilized enzyme was determined with the use of the ONPG method. This method utilizes the reaction between the immobilized enzyme -galactosidase and O-nitrophenyl- -D-galactopyranoside (ONPG). The multilayer samples were submerged in a mixture of 1500 μ l of ONPG solution (ionic strength 2.0 mM) and 900 μ l of deionized water for 60 minutes at 37 °C. At the 30th and 60th minute 800 μ l were taken from the reaction liquid and 4 ml of sodium carbonate solution (with ionic strength 1 M) was added to stop the reaction. Through hydrolysis the enzyme reacts with the ONPG solution and produces two byproducts - -D-galactose and o-nitrophenol. The amount of reaction is then determined by measuring the amount of o-nitrophenol (colored yellow) produced during the reaction by measuring the absorption of the reacted liquid at 405 nm with the use of a spectrophotometer. This test was repeated several times at 24 hour increments to determine the amount of immobilized enzyme after repeated use.

RESULTS AND DISCUSSION

Time storage influence on the electrets surface potential decay

Dependences of the normalized surface potential on the storage time for negatively charged PDLA substrates (PDLA NCD), PDLA substrates chemically modified with EDAC (PDLA EDAC) and substrates modified with combinations of a negative corona and EDAC (PDLA NCD EDAC or PDLA EDAC NCD) were studied for 360 minutes. The surface potential was measured once of 5 minutes for the first 60 minutes when the charge was rapidly decaying. After this period, steady state values of the surface potential at a time of 360 minutes were established for all investigated samples. 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 %.

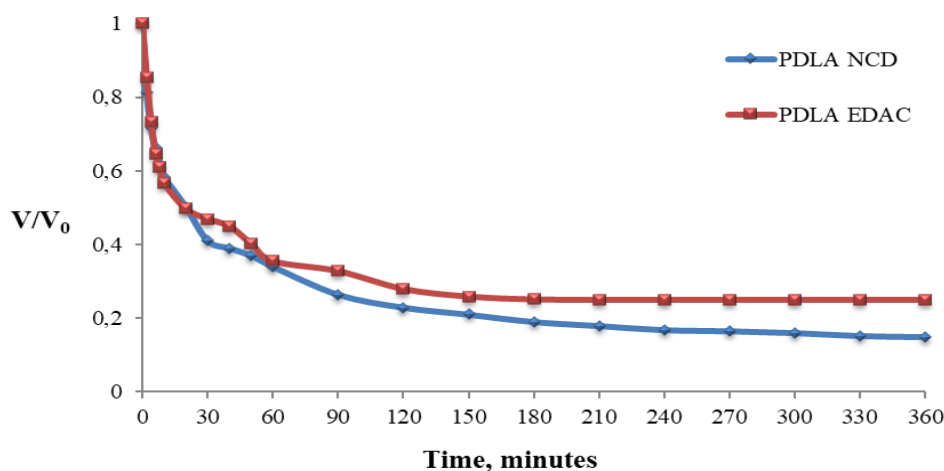


Figure 1. Normalized surface potential time dependences for modified PDLA substrates

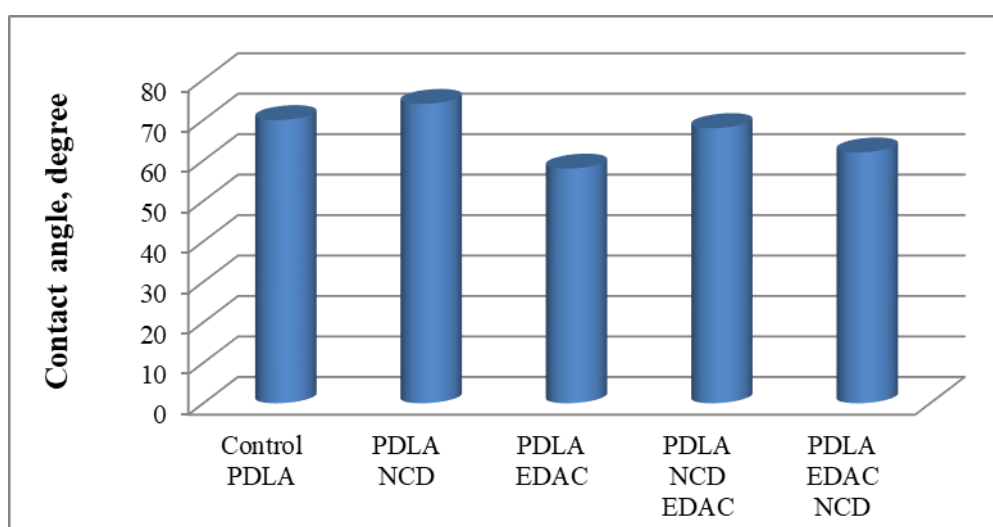


Figure 2. Water contact angles for different PDLA modification combinations

It was found that for the substrates obtained by combination of chemical and physical modification, the surface potential decreases very rapidly and time dependences cannot be constructed. Therefore, only time dependences of the normalized surface potential for negatively charged PDLA substrates and PDLA substrates chemically modified with EDAC were obtained (Fig. 1).

The experimental data presented in Figure 1 demonstrate that:

✓ There is an initial fast decay of the normalized surface potential that occurs in the first 60 minutes after the initial charging. After this initial period the rate of charge decay slows down and the remaining surface charge decreases at a slower rate and then becomes practically stable after at the 360th minute. The initial rapid decrease in the surface charge density can be explained with the release of weakly captured charges from the shallow energy traps. The steady state value is achieved due to the remaining charge consisting of mostly tightly captured charges in the deep energy traps.

The exponential decay of the electrets charge is consistent with the one measured by Sessler [8].

✓ The values for the physically modified PDLA samples (PDLA NCD) are lower than those for the chemically modified samples (PDLA EDAC). This can be caused by the difference in the type of dominant ions on the surface. The different types of dominant ions can be bounded in traps of varying depths and then released depending on the surrounding conditions.

Water contact angle measurements

Surface wettability of the film coatings is directly governed by their chemical composition. Thus, it is of importance to measure contact angles in order to calculate the values of surface free energy. The surface contact angle for all samples was measured and their surface energy was determined. The results for surface contact angles are presented in Figure 2.

The surface of PDLA is hydrophobic and when water was used as test liquid and PDLA as target

surface, high values of water contact angle were expected because water is hydrophilic in nature whereas PDLA surface is hydrophobic in nature. If by chemical reaction PDLA surface was modified and polar groups are inserted on the surface, then a decrease of values of contact angle is expected. This decrease will be different for different polar functional groups.

Our research shows that the negative corona loading increases the value of the contact angle for PDLA samples. For PDLA modified with EDAC, lower contact angle values were observed compared to those loaded in corona discharge. The contact angle values of the two modification combinations are between those of PDLA NCD and PDLA EDAC. The results obtained show that the values of free surface energy of the PDLA EDAC are the highest comparing with values of other investigated samples (Figure 3).

A higher surface energy will cause good wetting and has respectively a lower contact angle. Therefore, we can conclude that the modification with the test substances in this article leads to better wettability of their surface.

Enzyme activity

The activity of the immobilized enzyme was used to determine the effect of the different modification combinations. The amount of enzyme activity was measured at the 30th and 60th minute of the reaction on four consecutive days and one

further measurement was performed after 20 days to determine the long-term retention of activity.

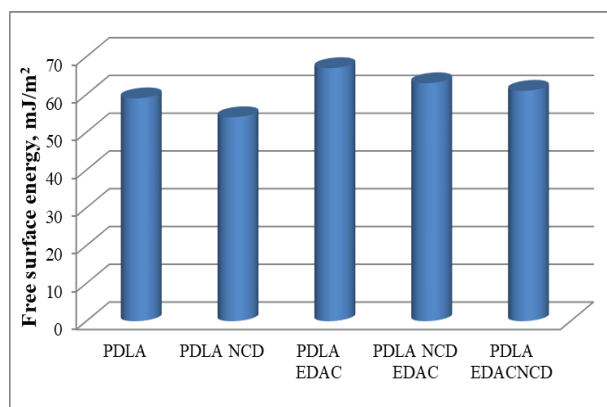


Figure 3. Free surface energy for different PDLA modification combinations.

After the end of the activity test all samples were removed from the reactive fluid and left to dry in normal atmospheric conditions and the assay was repeated with fresh reactive solution on the following day. Three samples of each type of modification were tested and the average values of the activity were calculated. The amounts of enzyme activity for each type of modification at the 30th and 60th minute of the reactions are displayed on Figures 4 and 5, respectively.

Table 1 presents the average values of the measured activity for all modification types for the entire duration of the enzymatic assay measurements.

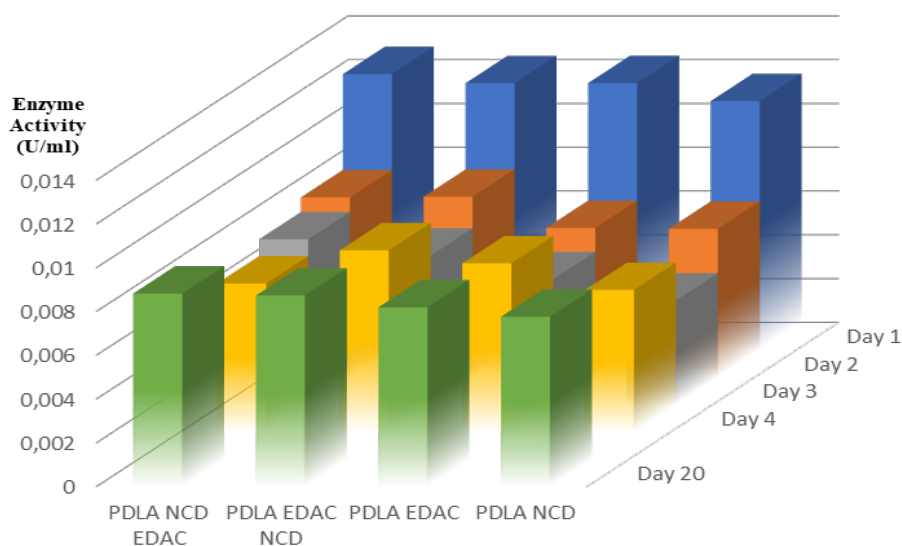


Figure 4. Enzyme activity of immobilized β -galactosidase at the 30th minute of the assay*

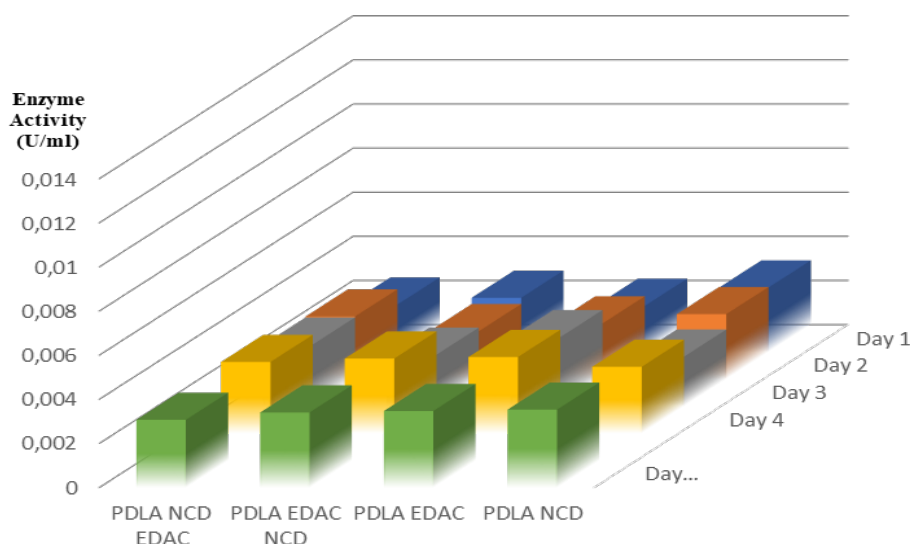


Figure 5. Enzyme activity of immobilized β -galactosidase at the 60th minute of the assay

Table 1. Average enzyme activity of immobilized β -galactosidase

Enzyme (U/ml)		PDLA NCD	PDLA EDAC	PDLA EDAC NCD	PDLA NCD EDAC
Day 1	30 min	0.0111 0.0004	0.0122 0.0003	0.0122 0.0004	0.0126 0.0006
	60 min	0.0029 0.0002	0.0022 0.0001	0.0024 0.0001	0.0021 0.0001
Day 2	30 min	0.0067 0.0003	0.0068 0.0002	0.0084 0.0003	0.0081 0.0002
	60 min	0.0028 0.0001	0.0025 0.0001	0.0022 0.0001	0.0030 0.0002
Day 3	30 min	0.0065 0.0001	0.0067 0.0003	0.0068 0.0005	0.0074 0.0003
	60 min	0.0025 0.0001	0.0031 0.0002	0.0026 0.0001	0.0028 0.0001
Day 4	30 min	0.0065 0.0002	0.0077 0.0004	0.0078 0.0004	0.0068 0.0003
	60 min	0.0030 0.0002	0.0034 0.0002	0.0034 0.0002	0.0032 0.0001
Day 20	30 min	0.0077 0.0004	0.0082 0.0003	0.0075 0.0002	0.0076 0.0004
	60 min	0.0034 0.0002	0.0032 0.0001	0.0030 0.0002	0.0031 0.0001

The results displayed in Figures 4 and 5 and Table 1 show that the chemical modification with EDAC improves the level of retention of the enzyme activity in all cases, with both combinations of the two modification types providing higher values than any of the single

modifications. The data also demonstrate that the level of activity decreases by around 40 % after the first day and remains stable for prolonged periods of time (up to 20 days).

The increase in enzymatic activity of the chemically modified samples can be explained with the higher amount of reactive side chains created on

the surface of the polymer substrate during hydrolysis when compared to the surface degradation from the plasma treatment. These reactive carboxyl chains are then further activated by the EDAC treatment which changes the type of crosslinking between the surface of the polymer and the two polysaccharides used in the creation of the multilayers. As both the physical and chemical modification increase the amount of side chains on the surface, their combinations display the expected higher level of activity when compared with single modifications.

The results obtained in this study demonstrate the suitability of both chemical and physical modification methods for the improvement of the properties of PDLA substrates. The collected data also show that any of the two investigated types and combinations of modifications can produce multilayer films that can maintain a stable level of enzyme activity for a prolonged period of time.

CONCLUSION

The results obtained in this paper demonstrate that both the physical and chemical modifications of the surface of a PDLA substrate can improve its properties and assist in immobilization of bioactive materials on its surface through the creation of multilayer structures. The collected data also indicate that combinations of different modification techniques are superior to any single modification, as utilizing a second modification method can mitigate some of the drawbacks of the first modification. These results can assist in widening of the field of potential applications of different biopolymers. The results of this study can assist the

development of different biomedical products and applications. The level of control, provided by the combination of different types of surface modifications can lead to the creation of two different biomedical applications: the development of drug carrying biodegradable multilayers with controlled drug release rate and the development of multiuse biodegradable sensors.

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