New preparation method of PLA-based biomaterials containing molecular iodine layer on their surface

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Abstract: Poly(lactic) acid (PLA) is biodegradable polymer that nowadays is being used as a material for various medical applications. However, it has several drawbacks like low biological activity and absence of radiopacity. Iodine and iodine-containing compounds are well-known for their antibacterial and radiopaque properties. In order to improve PLA-based biomaterials properties we developed new surface modification method based on "solvent/non-solvent" treatment with subsequent entrapment of iodine either from its vapour or solutions. It was shown that developed technique allows us to create an iodine layer on PLA-based materials surface with estimated amount of iodine from 10^{-9} to 10^{-8} g/cm^2. Stability of such layer was also investigated and it was shown that it is stable enough to be used for medical applications requiring long-term iodine release into water medium.

Key-words: poly(lactic) acid, iodine, surface modification, implants

1 Introduction

Poly(lactic) acid (PLA) is biodegradable polymer that nowadays is being used as a material for sutures [1], implants [2] and other medical devices [3]. Intensive application of PLA is connected with biodegradability [4], biocompatibility [5] and processibility [6] of this material. But also it has a number of disadvantages; the most important of them is low biological activity because of the lack of reactive groups. Another problem concerning the use of PLA as a material for implants production is absence of radiopaque properties that makes PLA-based implants tracking impossible.

Iodine and iodine-containing compounds are well-known for their antibacterial and radiopaque properties. Also they were used to impart these features to different polymers. Polymer-iodine complexes are called iodophores and have shown a great antibacterial activity owing to prolonged release of free iodine to environment. They could be used as bandages, tanks and hoses for storage and transportation of blood and plasma, as well as for water disinfection [7-8]. For example, nylon fibres modified with iodine have demonstrated good antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae [9-10]. Natural rubber which is widely used for biomedical applications like gloves, catheters, blood bags was also iodinated using iodine or its complex with polyvinyl pyrrolidone. Such materials had antimicrobial properties and were designed to evade infection [11]. Iodination of polystyrene derivatives was done for the same purpose and these materials were expected to have various applications in implantable clinical devices [12]. Another application of composite polymers containing iodine is radiotherapy and diagnostics using iodine isotopes 123, 125 or 131 that are introduced in various ways into the polymer [13].

Thus, iodine-containing PLA-based composites have a great potential to be used in a number of medical applications such as creating X-Ray visible biodegradable implants, implants containing radioactive iodine isotopes for diagnostics and radiotherapy, biomaterials with antibacterial properties, and others.

A few examples are existed for PLA modification with iodine. Its surface was modified by complex of iodine with another polymers [14]. Iodine was covalently bonded to PLA in solution by using classical organic chemistry approaches [8, 15]. However, due to fact that PLA has a lack of chemically active centres iodination by N-iodosuccinimide carried out in the presence of peroxides went difficultly and had provided an introduction of less than 0.5% chemically bound iodine [15].

Wide range of application areas of PLA and iodinated polymers and the absence of direct simple

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and reliable method of obtaining PLA-based materials doped with iodine make the developing of such technique an urgent and highly relevant task.
The aim of this research was to obtain PLA-based material with iodine in molecular form on the surface. Applied method was based on "solvent/non-solvent" treatment technique, which allows entrapment of different molecules from the environment without affecting bulk properties of the polymer [16-19]. It was realized with simple laboratory equipment and allowed us to create a iodine layer on PLA-based materials surface with estimated amount of iodine from 10^{-9} to 10^{-8} g/cm^2.

2 Materials and Methods

2.1 Preparation of PLA films

To produce PLA films, the solution of PLA PURASORB® PL65 (Purac, The Netherlands) in dichloromethane/chloroform mixture (40:60 v/v) (Punreac, Spain) with concentration of 1.7% was used. Time of homogenization was 12 h. Then 18 g of the solution were poured into a dry 100 mm petri dish and dried in solvent vapours for 72 h. PLA films with 30±1 μm thickness were obtained.

2.2 PLA films modification methods

PLA films were treated with "solvent/non-solvent" mixture and then exposed to an iodine-containing medium.

2.2.1 Determination of optimal conditions of iodine adsorption from vapours

PLA films were treated with "solvent/non-solvent" mixture (toluene/ethanol=3/7, v/v), that was selected previously in model experiment [16], for 10 min and then exposed into iodine vapours for 10, 30, and 60 min. Iodine vapours were produced at 70˚C in glass cylinder with smooth edges and 50 ml volume that was covered with the petri dish at the top. Excessive iodine was removed from the surface of the films with 96% ethanol.

2.2.2 Determination of optimal conditions of iodine entrapment from ethanol solution

PLA films were treated with "solvent/non-solvent" mixture (toluene/ethanol=3/7, v/v) for 10 min and then soaked in 0.1 M solution of iodine in ethanol or water/ethanol mixture (1/1, 3/7 or 7/3 v/v) during 16, 24, and 48 h. Excessive iodine was removed from the surface of the films with 96% ethanol.

2.3 Stability determination of iodine layer created on PLA-based materials

In order to evaluate the stability of created iodine layer modified PLA-based films were soaked in distilled water, 0.01 M phosphate buffer saline (PBS), 0.90% (w/v) solution of NaCl (NS), or stored under the atmosphere, or vacuum for 7 days. The amount of iodine released from the films (%) was determined on 1, 2, 3, 5 and 7 day of the experiment.

2.4 UV-spectroscopy

Amount of entrapped iodine was determined using UV-spectroscopy (Specord 250 Plus, Analytik Jena AG, Germany). UV-spectra of the obtained samples were recorded in absorbance mode, wavelength was from 200 to 600 nm. Amount of the adsorbed iodine was calculated using Beer–Lambert–Bouguer law at a two characteristic maximums of iodine (λ_{max1}=360 nm, ε_1=12000 l·cm^{-1}·mol^{-1}, λ_{max2}=440 nm, ε_2=5800 l·cm^{-1}·mol^{-1}). The following equation was used:

$$C = \frac{A \cdot V \cdot Mr}{\varepsilon \cdot l \cdot S} \qquad (1)$$

where C is amount of iodine on the surface (g/cm^2), A —absorbance, ε — attenuation coefficient (l·cm^{-1}·mol^{-1}), l — path length (cm), Mr – iodine molar mass (g/mol), V — volume of modified PLA film (cm^3), and S — surface area of modified PLA film (cm^2).
The average absorbance was calculated with five measurements for every sample.
The iodine release from the samples into the distilled water, PBS, NS, atmosphere and vacuum was quantified using following equation:

$$\omega = \frac{C_0 - C_i}{C_0} \cdot 100\% \qquad (2)$$

where ω is amount of iodine released from the films (%), C_0 – initial concentration of iodine on the films, C_i – current concentration of iodine on the films.

3 Results and Discussion

3.1 Optimal conditions for iodine adsorption

Qualitative and quantitative analysis of the adsorbed iodine amount was provided with UV-spectra of obtained samples.

3.1.1. Iodine adsorption from vapours

Determination of optimal conditions of iodine adsorption from vapours was carried out using toluene/ethanol=3/7 (v/v) as "solvent/non-solvent" system. In the UV-spectra two characteristic maximums at 300 nm and 477 nm were observed.

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There is a shift of these peaks in comparison with iodine solution UV-spectrum due to the solid state of the investigated samples. For quantification maximum at 477 nm was used. Comparison of the intensity of these peaks of the samples that were exposed to iodine vapours for 10, 30 and 60 min (Fig.1) showed that after 10 minutes the amount of adsorbed iodine didn't vary significantly.

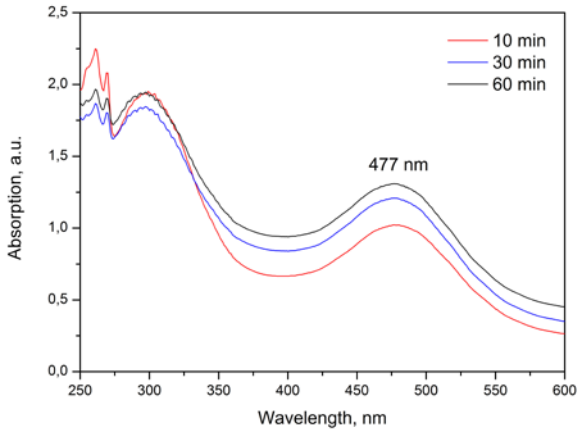

Figure 1. UV-spectra of PLA-based films with iodine adsorbed from vapours.

The quantity of iodine (g/cm^2) entrapped on PLA-based films surface from iodine vapours is presented in Table 1. The amounts of adsorbed iodine after 10 min treatment was $(1.31\pm0.12)\cdot10^{-8}$ g/cm^2. Thus, 10 min vapour treatment was chosen as optimal one and these conditions were used for future experiments.

Table 1. Amount of iodine after exposing toluene/ethanol treated PLA-based films for different time period in iodine vapours.

Time, min	**Amount of iodine, g/cm^2 (P=0.95)**
10	$(1.31\pm0.12)\cdot10^{-8}$
30	$(1,40\pm0.09)\cdot10^{-8}$
60	$(1,62\pm0.15)\cdot10^{-8}$

3.1.2 Iodine adsorption from its solutions

Determination of optimal conditions of iodine adsorption from its solutions was carried out using toluene/ethanol=3/7 (v/v) as "solvent/non-solvent" system as well. In the UV-spectra characteristic maximum at 450 nm was observed while maximum in the short wavelength region appeared blurred. For quantification maximum at 450 nm was used. It happened due to the solid state of the investigated samples.

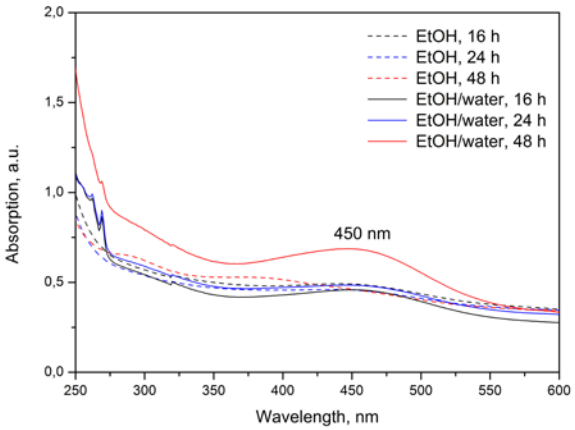

Figure 2. UV-spectra of PLA-based films with iodine adsorbed from the ethanol and water-ethanol solution (1/1 v/v) of iodine.

Comparison of intensity of the peaks at wavelength of 450 nm on the UV-spectra of the samples that were soaked in iodine solution in water/ethanol (1/1 v/v) mixture (Fig. 2) showed that the amount of adsorbed iodine increased with time. For the samples soaked in iodine solution in pure ethanol the amount of adsorbed iodine decreased with time. It could be explained by partial desorption of iodine from the samples to the solvent. The quantity of iodine (g/cm^2) entrapped on PLA-based films surface from iodine solutions is presented in Table 2. The greatest amount of adsorbed iodine was achieved from water-ethanol mixture after 48 h.

Table 2. Amount of iodine after exposing toluene/ethanol treated PLA-based films for different time period in iodine solutions.

Time, h	**Amount of iodine, g/cm^2 (P=0.95)**	
	Ethanol solution	**Water-ethanol mixture (1/1 v/v)**
16	$(6.50\pm0.13)\cdot10^{-9}$	$(6.40\pm0.11)\cdot10^{-9}$
24	$(6.07\pm0.10)\cdot10^{-9}$	$(6.06\pm0.09)\cdot10^{-9}$
48	$(3.46\pm0.11)\cdot10^{-9}$	$(9.07\pm0.10)\cdot10^{-9}$

In order to determine optimal water/ethanol ratio to be used for iodine solution preparation two additional systems were tested: water/ethanol=3/7 (v/v) and water/ethanol=7/3 (v/v). PLA-based films were treated with solvent/non-solvent mixture and then soaked in 0.1 M iodine solutions for 48 h as an optimal time chosen. The amount of entrapped iodine is presented in Table 3.

Table 3. Amount of iodine after exposing toluene/ethanol treated PLA-based films in iodine solutions with different composition.

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Composition of iodine solution	Amount of iodine, g/cm^2 (P=0.95)
Water/ethanol=3/7 (v/v)	$(1.03\pm0.11)\cdot10^{-8}$
Water/ethanol=1/1 (v/v)	$(9.07\pm0.10)\cdot10^{-9}$
Water/ethanol=7/3 (v/v)	$(9.23\pm0.13)\cdot10^{-9}$

Composition of iodine solution doesn't have a great impact on the amount of adsorbed iodine. Thus, water/ethanol=1/1 (v/v) was chosen as it contains enough ethanol for sufficient iodine solubility.

From the obtained data it is clear that entrapment of iodine from the vapour phase allows us to create materials containing higher amount of iodine on the surface. Both methods have their advantages and disadvantages. On the one hand, the use of iodine vapours provides opportunity to create one-side modified biomaterials (by using a mask). Moreover, there is no need to purify solvents from iodine after modification process. On the other hand, the use of iodine solutions demands less energy spending and prevents from breathing hazardous iodine vapours. Both technologies have perspective to be applied in future. Thus, following experimental conditions were recognised as optimal ones as they allow us to entrap greatest amount of iodine on PLA-based materials surface: treatment of PLA-based films with toluene/ethanol=3/7 (v/v) for 10 min and then exposing into iodine vapours for 10 min or soaking in 0.1 M solution of iodine in water/ethanol mixture=1/1 (v/v) during 48 h. Samples obtained by using these experimental procedures were investigated for iodine layer stability.

3.2 Determination of iodine layer stability on modified PLA-based materials

The dynamic of iodine release to the distilled water, PBS, NS, atmosphere, and vacuum from PLA-based materials modified in iodine vapours using optimal conditions is shown on Fig. 3. From the graph we can see that the most intensive release was observed from the modified samples stored in the atmosphere (32.12%). The amount of iodine released while soaking samples in water was 23.35% that is more than in PBS and NS. However, if one looks at the dynamics of the iodine release into the liquid mediums it can be noticed that after the 2nd day the amount of iodine released into the distilled water doesn't rise significantly.

The dynamic of iodine release to the distilled water, PBS, NS, atmosphere, and vacuum from PLA-based materials modified in iodine solution using optimal conditions described above is shown on Fig. 4. The iodine release dynamics has in general the same profile as for PLA-based materials modified in iodine vapours. The most intensive iodine release was observed from the modified samples stored in the atmosphere (34.31%).

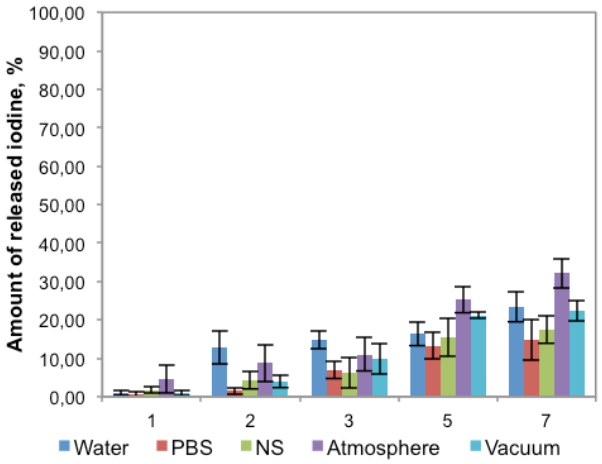

Figure 3. Iodine release into the distilled water, PBS, NS, atmosphere, and vacuum from PLA-based materials modified in iodine vapours (P=0.95).

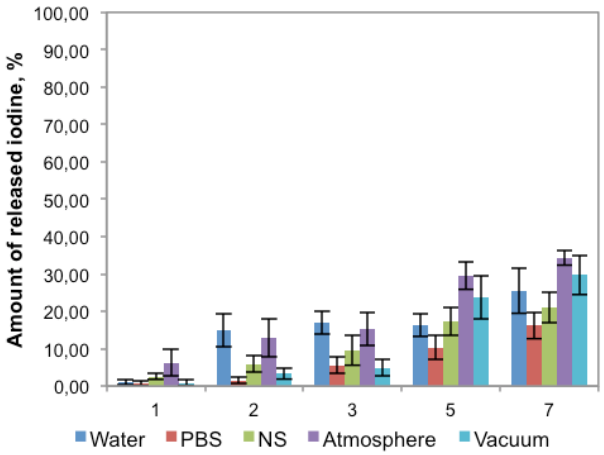

Figure 4. Iodine release into the distilled water, PBS, NS, atmosphere, and vacuum from PLA-based materials modified in iodine water-ethanol solution (P=0.95).

According to the data iodine layer on the modified samples is less stable in the atmosphere while in the liquid mediums more stability is observed. Thus, obtained modified PLA-based materials can be used for prolonged iodine delivering of iodine into the water mediums which is very important for radiotherapy and maintenance of long-term bactericidal activity. Moreover, we can propose the appropriate storage for such materials that can increase their shelf life: for example, they can be stored in distilled water.

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4 Conclusions

Method of preparation of PLA-based biomaterials containing molecular iodine layer on their surface was developed. It was shown that iodine could be entrapped both from its vapours and solutions. Optimal modification conditions were determined and the use of iodine vapours was shown as the most efficient method that allows us to create an iodine layer on PLA-based materials surface with estimated iodine amounts of 10^{-8} g/cm^2. Determination of iodine layer stability on modified PLA-based materials has demonstrated that this layer is non-stable in the atmosphere (32-34% of iodine amount loss). At the same time, modified samples are quite stable in water mediums. This makes them appropriate for the use in radiotherapy, and as a material for medical devices with long-term bactericidal activity. Also they can be applied as an X-Ray visible implants available for tracking.

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