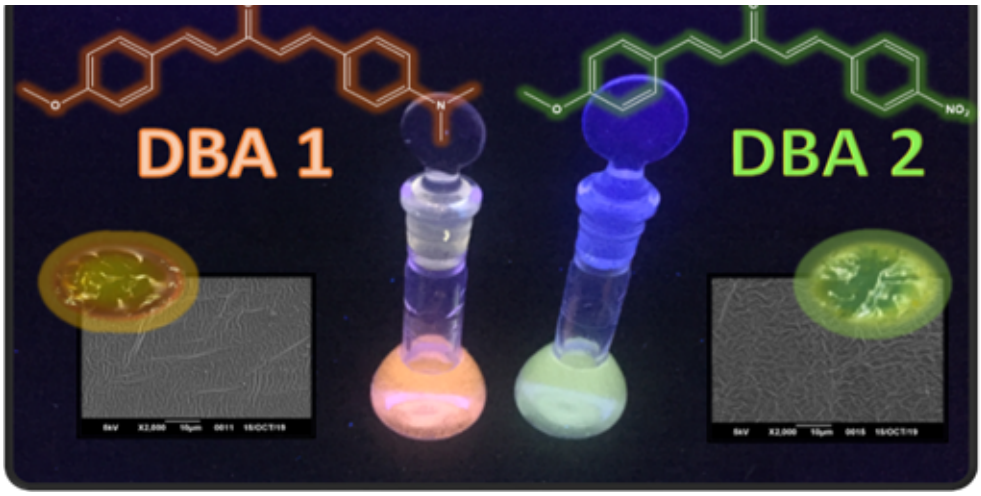
IJAMB
INTERNATIONAL JOURNAL OF ADVANCES IN MEDICAL BIOTECHNOLOGY

Pla membranes loaded with dba analogs. Controlled release study during hydrolitic degradation

GJ. Alcántara Blanco[1*]; N. Urdaneta[2]; MA. Sabino[1]
**Corresponding author: e-mail address: gabalcant@gmail.com*

Abstract: In this research two dibenzylideneacetone (DBA) analogs compounds: (1E,4E)-1-(4-(dimethylamino)phenyl)-5-(4-methoxyphenyl) penta-1,4-dien-3-one (DBA-1) and (1E,4E)-1-(4-methoxyphenyl)-5-(4-nitrophenyl)penta-1,4-dien-3-one (DBA-2) were loaded in poly(lactic acid) (PLA) membranes. These DBA analogs can be applied in the development of controlled drug release systems. PLA membranes were elaborated by solvent casting. It was found that these fluorescent compounds can cause a small percentage of hemolysis in human red blood cells in the concentration range of 200-500 µg/mL. Therefore, they can be considered non-toxic at these concentrations. Hydrolytic degradation of PLA membranes loaded with DBA analogs was studied at a temperature of 37 °C under acid, neutral, and basic pH conditions for a maximum time of six weeks. This hydrolysis was monitored by measuring the loss of mass of the membranes, changes in pH environments, variations in the molecular weight of the PLA matrix, and changes in surface morphology observed through Scanning the Electron Microscopy (SEM) technique. The amount of DBA analog released during the degradation time was determined by UV-visible spectrophotometry, and so, the release profile. Employing SEM, it was observed that the membranes presented a major degradation under basic pH conditions, with a higher percentage of release in an acid medium for both DBA analogs studied.

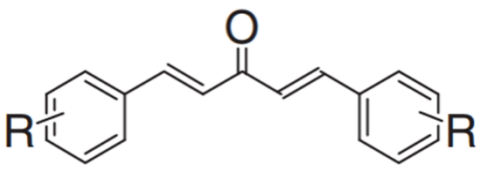
Keywords: Dibenzylideneacetone. PLA membranes. Control release. Poly(lactic acid). Hydrolytic degradation



Introduction

One of the natural compounds that have been studied extensively in recent years is curcumin and its analog compounds: chalcone and dibenzylideneacetone.[1-6] These compounds exhibit different biological properties and have generated interest in the scientific community, especially in the dibenzylideneacetone analogs. Dibenzylideneacetone (generally abbreviated as DBA) is a curcumin analog which chemical structure (see Fig. 1) consists of an α, β-unsaturated ketone with two aromatic rings interconnected by a bridge conjugated to a carbonyl group in the center. Several DBA analogs have interesting pharmacological properties such as antioxidants,[7,8] anticancer, [9-11] anti-inflammatory [12] and antiparasitic.[13-16] Also, they have been used as an active ingredient in sunscreens[8,17] due to their high extinction coefficient in the ultraviolet region, and recently it has been evaluated that they exhibit non-linear optical effects (NLO)[18] being applied in areas such as photodynamic therapy (PDT),[19] in two-photon excitation microscopy (TPEF/2PEF),[20] electrical switches, [21] in 3D microfabrication [22] and optical limitation.[23]

Figure 1. The general structure of Dibenzylideneacetone (DBA)



Despite the great potential and novel properties that curcu-

[1]Departamento de Química. Universidad Simón Bolívar. Caracas, Venezuela.

Received 08 january 2021; Accepted 21 Februaryt 2021; Available online 1 March 2021.
DOI: https://doi.org/ 10.52466/ijamb.v4i1.73

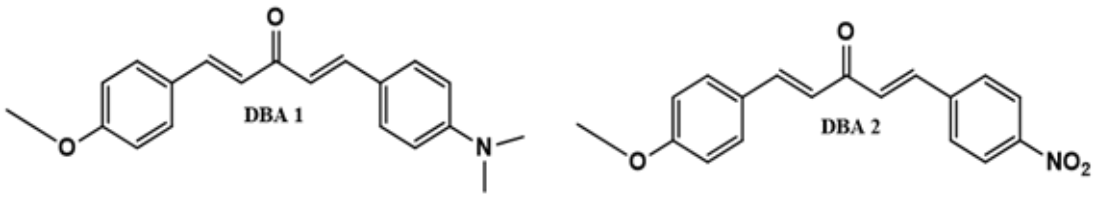
min analogs possess in the medicinal area, some of their derivatives and analog compounds are still poor in blood–brain barrier (BBB) penetration and are unstable *in vivo*.[24,25] These problems can be overcome with the use of liposomes, micelles, phospholipid complexes, membranes, and nanoparticles which are promising novel formulations. They provide longer periods of circulation, better permeability, and resistance to metabolic processes of derivative and analog compounds of curcumin.[26] An effective way to overcome this critical problem is to use drug delivery systems that deliver drugs or bioactive agents at the desired time and site of action. Potential advantages of enhanced drug delivery include: (1) continued maintenance of drug levels in a therapeutically desirable treatment; (2) reduction of harmful side effects due to targeted delivery to a particular cell or tissue type; (3) potentially decreased amount of drug needed; (4) decrease in the number of doses and possibly less invasive doses, which improves patient compliance with the prescribed drug regimen; and (5) facilitation of drug delivery for pharmaceuticals with short half–lives *in vivo* (e.g., peptides and proteins).[27] The ideal drug delivery system should have the same release rate without changing in time.[28] Delivery systems can be developed from a variety of organic and inorganic compounds such as polymers, lipids (liposomes, microemulsions), amphiphilic molecules, dendrimers, nanoparticles of zinc oxide, and titanium oxide,[29–30] membranes and scaffolds obtained by electrospinning.[31]

In the field of drug delivery, biodegradable polymeric nanostructures play an essential role due to their beneficial properties such as a high biocompatible with cells and tissues, high stability, that they do not cause thrombosis, do not activate neutrophils, and these structures are biodegradable.[32,33] But sometimes the encapsulation process could be inefficient and also the quantification of the delivery way could be inexact;[34] on the other hand, loading active compounds in macroscopic polymeric matrices as membranes can help evaluate their release profile in a more detailed way. Today, the term "membrane" covers a variety of materials and structures with very different properties. For example, membranes are used to produce potable water by reverse osmosis seawater desalination at bearable costs. Membranes are key components in medical devices and the separation of molecular mixtures in the chemical industry, biotechnology, pharmacology, and food processing.[35] Membrane science has become an interdisciplinary affair with contributions from physical chemistry, materials and life sciences, and process engineering.

In this case, the fabrication of membranes represents a simple method, which presents a high loading capacity, does not alter the loaded active compound, and allows a more detailed controlled release study because these structures are macroscopic and robust. Hence, their weight variations and viscosimetric molecular weight can be determined easily.

Taking into account the previous information, in this research, these two DBA analogs DBA–1 and DBA–2 (Fig. 2), were loaded in membranes using a poly–lactic acid (PLA) as a matrix. Previous reports indicate that these compounds have antimalarial properties [16] and have high quantum yields,[36] which indicate that these compounds are fluorescent. This property represents a great advantage because it allowed the monitoring of the molecules in the release process. Also, future applications would allow their monitoring within the patient's body using biosensors,[37] chemosensors,[38] or Single Molecule Detection (SMD).[39] In this way, the efficacy of the treatment can

Figure 2. The chemical structures of dibenzylideneacetone DBA 1 and DBA 2 analogs.



be evaluated, as well as its possible uses in biomedical, biotechnological, and pharmacological applications.

Experimental

Reagents

The analog compounds of DBA 1 and DBA 2 were previously prepared in the Laboratory of Organic Synthesis Laboratory.[36] Poly (lactic acid) (L/D) from HIMEDIA was used as the polymer matrix, and chloroform from Fisher Scientific (HPLC grade) was used as the common solvent for both PLA and DBA analogs. For the study of the different pH conditions of the hydrolytic degradation, the following reagents were used: sodium acetate trihydrate from Merck Millipore (99%), acetic acid from Sigma–Aldrich (99.8%), monosodium phosphate from J.T. Baker (98%), disodium phosphate from Riedel–de Haën (99.5%), ammonium chloride from Baker analyzed (99.7%) and ammonia from Merck Millipore (25%). A lactic solution from Ringer IPS from Venezuela for the dissolution of samples *in vitro* hemocompatibility tests and Triton X–100 from Merck used as a positive control in these tests.

Apparatus

For the study of hydrolytic degradation, a Boekel Scientific brand thermostatic bath was used. An Adventurer OHAUS analytical balance was used to measure the mass in the membranes before and after the degradation process; an Oakton pH11 Meter digital pH meter model 35614–80 was used to prepare buffer solutions and monitor the pH changes in the degradative medium of the polymeric matrices. Changes in the morphology of PLA membranes during hydrolytic degradation were observed through a JEOL JSM6390 scanning electron microscope. A Ubbelohde–Schott–Geräte capillary viscometer, capillary #I (capillary diameter: 0.63 mm), type 501 10 / I was used to obtaining the data to calculate the molecular weight of neat PLA and to determine the drop in molecular weight of the PLA membranes loaded and unloaded. An Agilent 8453 UV spectrophotometer with a diode array was used to determine the amount of DBA released during the degradation process.

Hemocompatibility test of DBA analogs

The hemolysis test was performed according to ISO 10993–4 (15) standards[40,41] in previously sterilized 1.5 mL microcentrifuge tubes. The positive control was a 1% v/v solution of Triton X–100; the negative control was a Ringer's lactic solution with

1% DMSO; For DBA analogs samples, solutions of 200 and 500 µg/mL were prepared with 1% v/v DMSO and Ringer's solution in 5mL volumetric flasks.

Human blood donated with prior consent was collected in a sterile environment in 5 mL BD Vacutainer® tubes which contained lithium/sodium heparin as an anticoagulant. The blood sample was transferred to a 15 mL falcon™ tube and centrifuged at 2000 rpm for 10 min. After the blood components had been separated, several washes were carried out with the Ringer solution to obtain a 100% suspension of erythrocytes. Subsequently, a 2.5% erythrocyte suspension was prepared, from which 800 µL was taken and mixed with 200 µL of each DBA in microcentrifuge tubes. These tubes were incubated for an hour at 37 ° C. After this time, each tube was centrifuged at 1500 rpm for 5 min, and the absorbance of the supernatant at 540 nm was measured. The percentage of hemolysis was determined employing the following equation (1):

$$\%Hemolysis = \frac{Sample\ absorbance - negative\ control\ absorbance}{positive\ control\ absorbance - negative\ control\ absorbance} \times 100\% \quad (1)$$

PLA membranes loaded and unloaded with DBA 1 and DBA 2 analogs

5% w/v solutions of PLA in chloroform were prepared in 25 mL volumetric flasks. Aliquots of 3 mL of this solution were taken and added to circular glass molds into a laboratory fume hood. The solvent was slowly evaporated at room temperature. After approximately 12 hours, the formation of transparent membranes was observed, and then they were carefully removed. Loaded PLA/DBA membranes elaboration was similar to PLA membranes. However, in these loaded membranes, PLA and DBA compounds were dissolved separately with chloroform. Later each one was added in the same flask until brought to volume 25 mL with the necessary solvent. The ratios used for loading were 10 and 20 µg of DBA per mg of PLA. Likewise, the solvent was allowed to evaporate, observing the formation of membranes loaded with DBA analogs. The fluorescent character of the membranes was also evidenced using ultraviolet light.

Hydrolytic degradation of PLA membranes

The hydrolytic degradation of the membranes was studied in function of time: two, four, and six weeks. This task was carried out by preparing three 0.2 M buffer solutions. An acid pH 4.85 buffer solution (sodium acetate trihydrate/acetic acid), a neutral pH 7.54 buffer solution (monosodium phosphate/monosodium phosphate), and a basic pH 9.20 buffer solution (ammonium chloride/ammonia). Each membranes samples were then placed into separate test tubes containing the respective buffer solution. The weight of all membranes was registered at zero time (before degradation). After, the test tube was introduced into a thermostatic bath at a temperature of 37 ° C for six weeks.

Experimental evidence of hydrolytic degradation of membranes

pH changes

Once the degradation time had elapsed, the remaining pieces of membranes were carefully separated from the buffer solutions. The pH of these remaining solutions was measured and stored to determine the amount of DBA released during the degradation process.

Loss of membrane weight

Pieces of membranes separated from the buffer solutions after the degradation weeks were completely dried at 37 ° C in an oven for 24 hours. Then, they were weighed on an analytical balance (w ± 0.0001) g.

Viscometric molecular weight measurement

The average molecular weight (Mv) of PLA and PLA/DBA membranes was determined by capillary viscometry technique. First, diluted PLA/$CHCl_3$ solutions were prepared from a 1% w/v stock solution. Second, the flow time of each polymer solution was measured using a digital stopwatch. With this procedure, the intrinsic viscosity [η] is obtained, and it is possible to determine the viscosimetric molecular weight (Mv) applying the Mark–Houwink–Sakurada equation ($\eta = K \times M v^{a}$). [42] The viscometric constants for the PLA / $CHCl_3$ case study at 25 ° C, are: a = 0.777 and K = 0.0131 (Rojas, et al., 2014).

Morphological changes of PLA membranes observed by SEM

The surface morphology of each membrane was observed before and after hydrolytic degradation using Scanning Electron Microscopy (SEM) JEOL JSM6390; the applied voltage was set to 15–20 kV. A coating with a thin layer of gold was necessary for the SEM analysis of the samples, which was carried out with a metallization sputter–coater Blazers–SCD–03.

Determination of the released quantity of DBA analogs

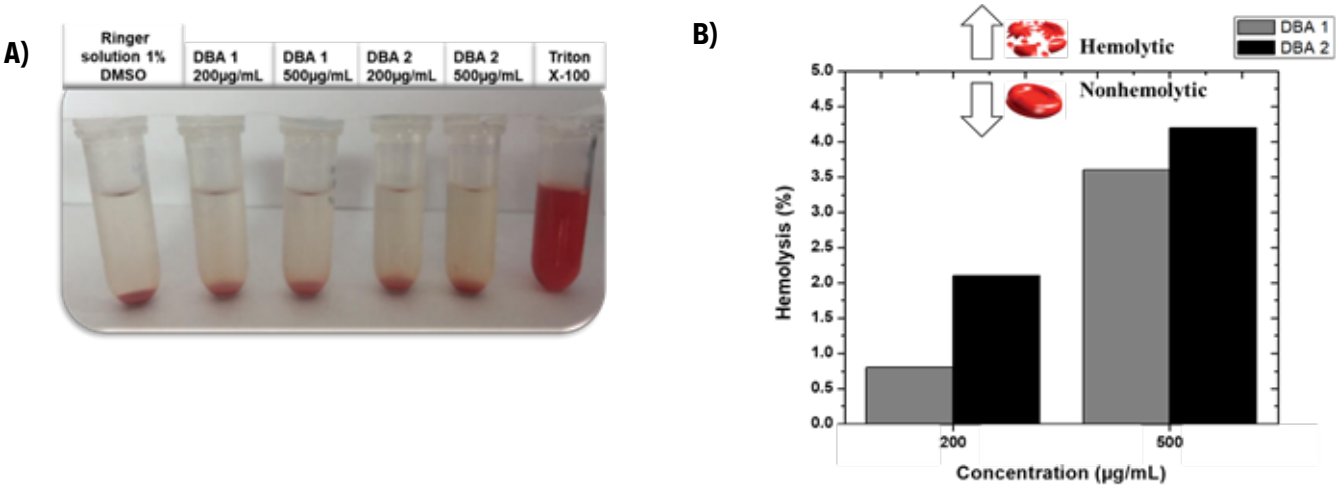
Once the hydrolytic degradation study was over, the aqueous buffer solutions stored were subjected to liquid–liquid extraction with $CHCl_3$. The organic phase was dried over anhydrous magnesium sulfate and then filtered. Next, this organic phase was brought to a known volume, and the absorbance was measured in a UV–Visible spectrophotometer to finally determine the amount of DBA released at the different degradation times.

Results and discussion

Hemocompatibility study of DBA 1 and DBA 2

The scientific literature report that PLA is a biodegradable polymer that does not generate adverse effects on the cells and tissues. [43] But when loading a compound within a polymeric matrix of PLA, in this case, DBA analogs, it is essential to study the interactions of these compounds with the membrane of red blood cells (RBC) since they can cause lysis of these cells. Besides, they can negatively affect the safety and biocompatibility of the structures loaded with this compound. [44] Hemoglobin release was used to quantify the hemocompatible properties of DBA analogs. The way to evaluate this was through UV/visible spectrophotometry. Fig. 3a shows the qualitative hemolysis of the samples of DBA 1 and DBA 2 at 200 and 500 µg/mL, along with the positive and negative control. It was qualitatively observed that these samples were very similar to those of the negative control, presenting little lysis compared to the positive control. The hemolysis percentages of each DBA analog at the two concentrations studied were obtained employing equation 1. According to ISO 10993–4 (15) standards, samples with a hemolysis percentage of less than 5% are considered non–toxic. Samples with a percentage of hemolysis between 2–5% are classified as mildly hemolytic, while those with a percentage of hemolysis less than 2% are classified as non–hemolytic. [40] Fig. 3b) shows that both DBA analogs turn out to be non–hemolytic and mildly hemolytic at the concentrations studied.

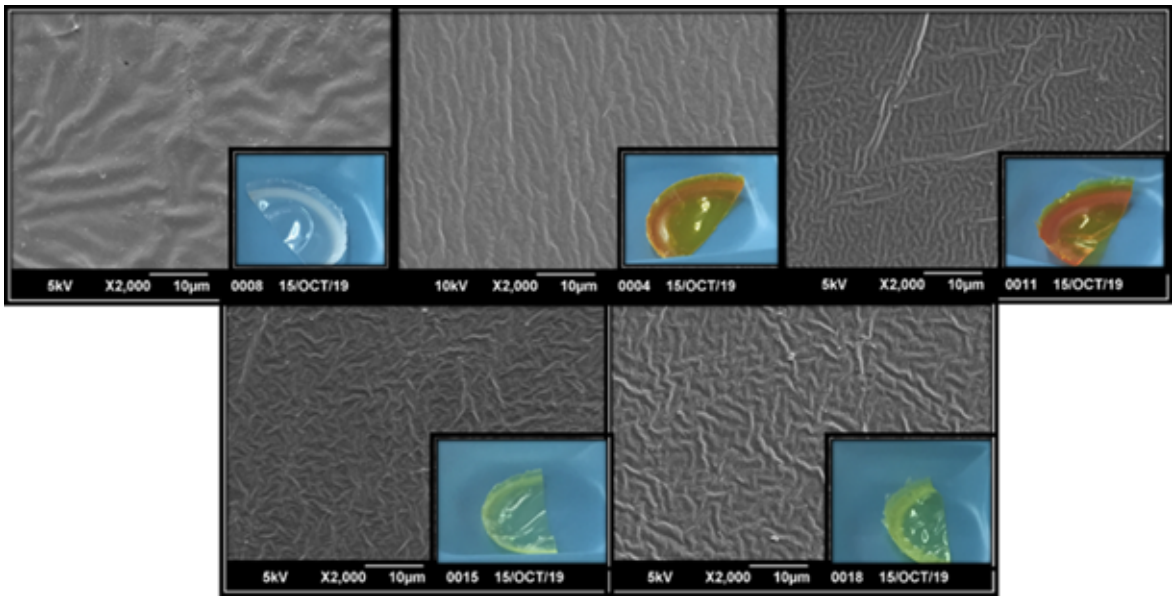
Figure 3. a) Qualitative assay of the hemocompatibility test of DBA analogs with the positive control and the negative control after incubation and the centrifugation process; b) graphic representation of the percentage of hemolysis obtained in human erythrocytes after incubation for 45 minutes at 37°C with the DBA analogs at different concentrations.



Obtaining unloaded PLA membranes and loaded PLA membranes with DBA analogs

The surface of membranes obtained by solvent casting can be seen in Fig. 4, where the SEM micrographs of both membranes that are not loaded and those loaded with DBA 1 and DBA 2, in ratios of 10 and 20 µg of DBA per mg of PLA, are shown. The loaded membranes have the characteristic color of the corresponding DBA. The membranes obtained have a certain flexibility and are translucent, which is conferred to the properties of amorphous PLA.[45] Morphologically, they all have a rough surface, although macroscopically, they look smooth. This characteristic could be due to solvent evaporation. This morphology is similar to the PLA membranes obtained in other work.[46]

Figure 4. SEM micrographs of the surface of PLA membranes: 1) unloaded, 2) loaded with 10 µg of DBA 1, 3) loaded with 20 µg of DBA 1, 4) loaded with 10 µg of DBA 2 and 5) loaded with 20 µg of DBA 2.



Study of hydrolytic degradation of membranes

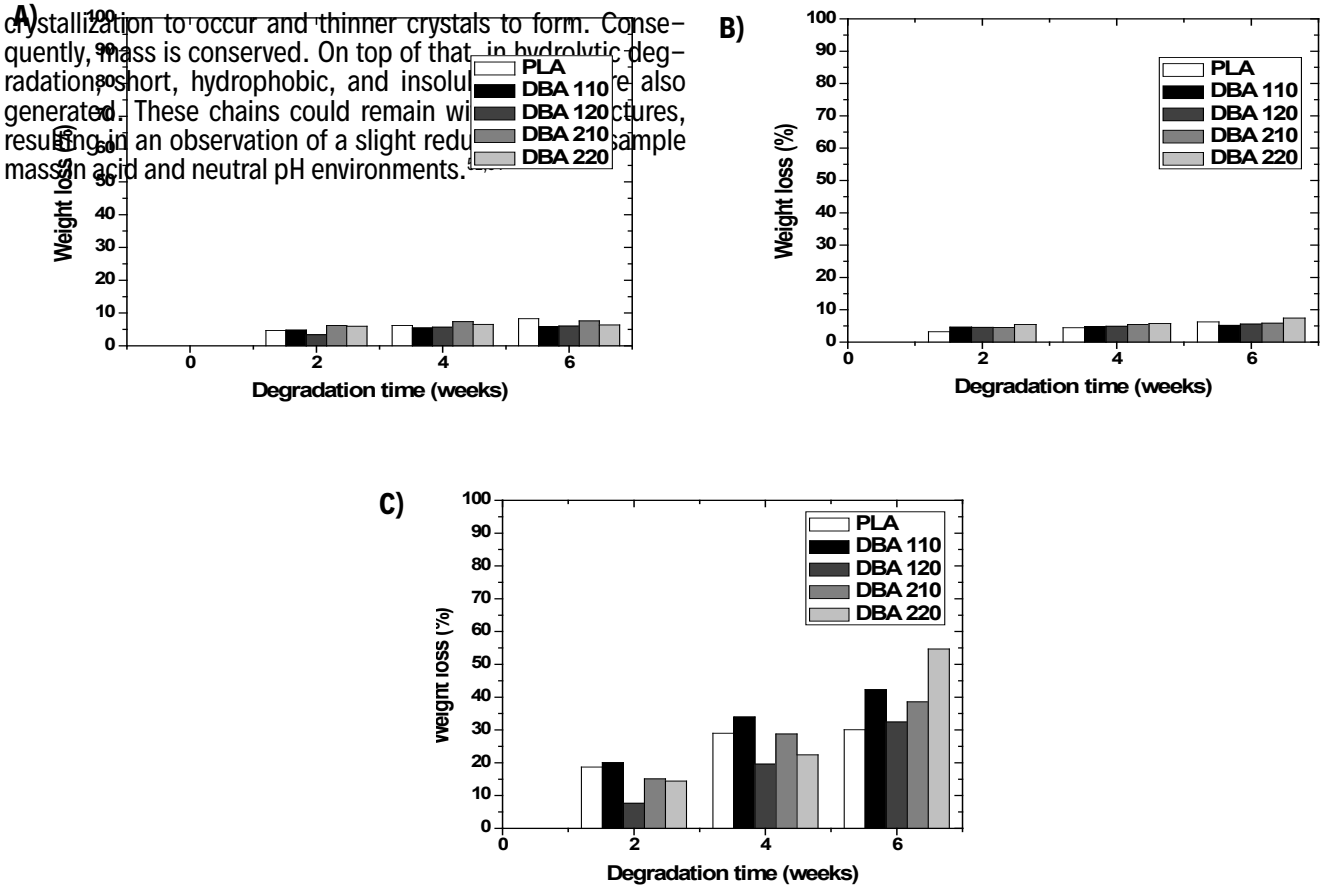
Under hydrolytic degradation, PLA is degraded by hydrolysis of the ester bond in oligomers.[47] This hydrolytic degradation of the ester groups can follow different routes: acid–catalyzed, base–catalyzed, and uncatalyzed.[48] Furthermore, the carboxylic end groups of the polymer chain act catalytically to affect the hydrolytic degradation of PLA in an autocatalytic and maintenance process.[49] This hydrolytic degradation proceeds in a heterogeneous way: it is faster within the molecular structure than on the surface.[50] Hydrolytic degradation is governed by four basic parameters: the rate constant, the amount of water absorbed, the diffusion coefficient of chain fragments within the polymer, and the solubility of degradation products.[51] It has been reported in the literature that the hydrolytic degradation process can be divided into three stages: (1) initial hydration or water absorption of the materials; (2) gradual decrease in molecular weight without weight loss; and (3) weight loss through the formation and dissolution of water–soluble oligomers.[52] The results obtained from the loss of mass, pH of the medium, variation of the viscosimetric molecular weight, and morphological changes during the hydrolytic degradation of membranes are shown below.

Loss of membrane mass

The loss of mass of the PLA membranes is an indication that significant amounts of soluble low molecular weight oligomers are removed from the polymer matrix, and they can diffuse into the hydrolysis medium. Fig. 5 shows the percentage of mass loss as a function of time in each of the unloaded and loaded membranes in the different degradative mediums. For better comprehension of Fig. 5 and the following ones, unloaded membranes were called PLA, the loaded ones with **DBA 1** in proportions of 10 and 20 µg of DBA per mg of PLA were called DBA 110 and DBA 120 respectively, and for those loaded with DBA 2 they were called DBA 210 and DBA 220. It is observed that membranes loaded with DBA analogs are the ones that lose the highest amount of mass in contrast to the unloaded ones. This observation could indicate the possible release of the loaded compound from the polymeric matrix. The membrane loaded with DBA 220 in the basic medium presented the highest mass loss. Membranes subjected to a degradative medium of acid and neutral pH lose a small amount of mass. It can be inferred that in basic medium occurs the highest release of the loaded compound. In this sense, in basic medium, it is observed that there is a faster degradative process.

However, a lower loss of mass does not mean that a degradative process is not taking place. In amorphous regions, random hydrolytic cleavage of ester bonds generates polymer chains with lower molecular weight[53] allowing cleavage–induced crystallization to occur and thinner crystals to form. Consequently, mass is conserved. On top of that, in hydrolytic degradation, short, hydrophobic, and insoluble chains are also generated. These chains could remain within the structures, resulting in an observation of a slight reduction of sample mass in acid and neutral pH environments.[54]

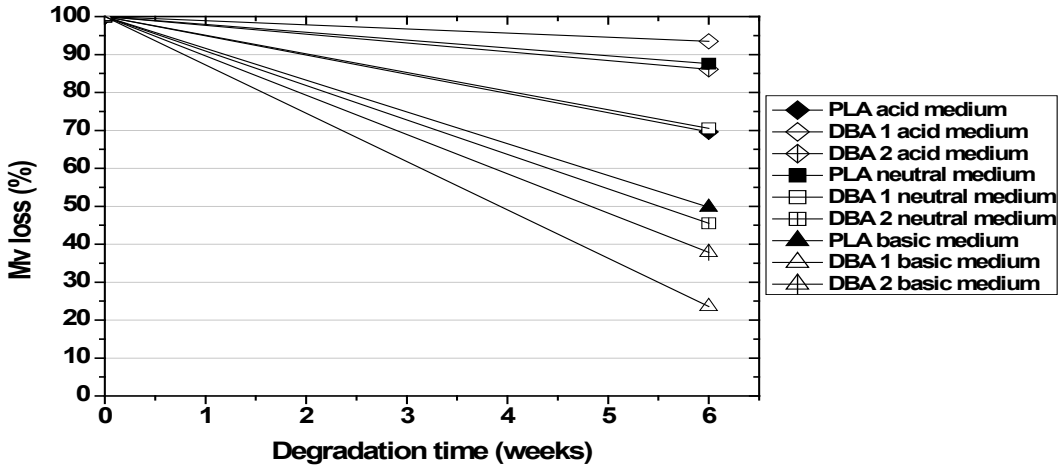
Figure 5. Loss of mass of the unloaded PLA membranes and loaded PLA membranes with DBA analogs as a function of degradation time at 37°C in different buffer solutions: A) pH 4.03, B) pH 7.54 and C) pH 9.2.



Variation of the Molecular Weight of PLA in the membranes as a function of the hydrolysis conditions

Another way to visualize the hydrolytic degradation process is by measuring the changes in molecular weight. During the hydrolytic degradation, the diffusion of the small molecular weight oligomers formed by the cleavage of the polymer chain causes a decrease in the molecular weight of the polymer matrix. There is a decrease in the viscosimetric molecular weight (Mv) in all membranes (Fig. 6). The higher loss of molecular weight is evidenced in the basic medium. This loss is represented by triangle symbols in figure 6, reaching losses of between 50–80% of Mv. This result agrees with the loss of mass of the membranes for this same medium, and it is observed that for acid and neutral medium, the loss of molecular weight is less, barely reaching around 30% loss in Mv.

Figure 6. Decrease in molecular weight of unloaded PLA membranes and loaded PLA membranes with DBA analogs in the different mediums as a function of degradation time at 37 ° C.



These results show that the loss of molecular weight is lower in loaded membranes with DBA analogs. A possible explanation for this result is to consider that the protons present in the acid medium can interact with the loaded compound and delay the hydrolysis of the polymeric matrix. Therefore, causing a lower mass loss and a slight drop in the Mv of the membranes, thus being a slower hydrolytic process.

Variation of the pH in the hydrolysis medium

The variation of the pH of the hydrolysis medium during the degradation process was another way of monitoring the hydrolytic degradation of the PLA membranes. The values obtained during the degradation period are presented in Fig. 7. The pH value changes markedly towards lower values for the basic degradative medium, unlike the acidic and neutral medium, which remains relatively constant. The decreases in pH during the hydrolytic degradation of PLA can occur for two reasons. First off, the degradation tends to increase the number of ends of the carboxylic acid chain, which autocatalyze the hydrolysis of the ester group. Second, only oligomers soluble in the environment aqueous medium are those that diffuse from the polymeric matrix. Before the degradation completion, the soluble oligomers closest to the surface can be leached out, while those in the core of the matrix remain trapped. As the last fraction begins to degrade into lactic acid, it will lead to the reduction of the pH in the core.[55] The effect of pH on the hydrolytic degradation of PLA has been studied in some works.[56–59] Even though the basic medium used in this work is not of such a high pH (9.2), it is observed that there is concordance with these previous investigations since, in these basic conditions, a decrease in pH over time is observed. Hence, it can be inferred that the degradation of PLA is faster in this medium than the others studied. A linear pH drop in the basic medium is observed for the unloaded PLA membrane, while for membranes loaded with DBA analogs in the same medium there is also a pH decay, but it is not entirely linear.

Physical and morphological evidence of hydrolytic degradation of membranes

The membranes showed physical changes after six weeks of hydrolytic degradation (Fig. 8). Among these changes, the loss of flexibility is observed, and they acquire a more accentuated coloration. These membranes turn out to be opaque and fragile after the degradation process. In the basic medium, the membranes were entirely fragmented. In contrast, in the other mediums, they presented less fragility, which indicates that the degradation of PLA is greater in a basic medium as evidenced above by Mv, weight loss, and pH.

The hydrolytic degradation leads to cleavage of the polymeric chains of PLA. This process generates a decrease in the elastic properties of the polymer. In other words, a reduction in its Young's modulus (E),[50] a characteristic parameter of each material, and it is a mechanical parameter to indicate the physical stability of these membranes. In this regard, there is a significant decrease in the elastic properties of the membranes in the basic medium, thus being easy to fracture. Opacity, which is a consequence of degradation, has been attributed to several phenomena: 1) light scattering due to the presence of water or

Figure 7. Change of pH in the degradation medium of PLA membranes unloaded and loaded as a function of degradation time at 37 ° C.

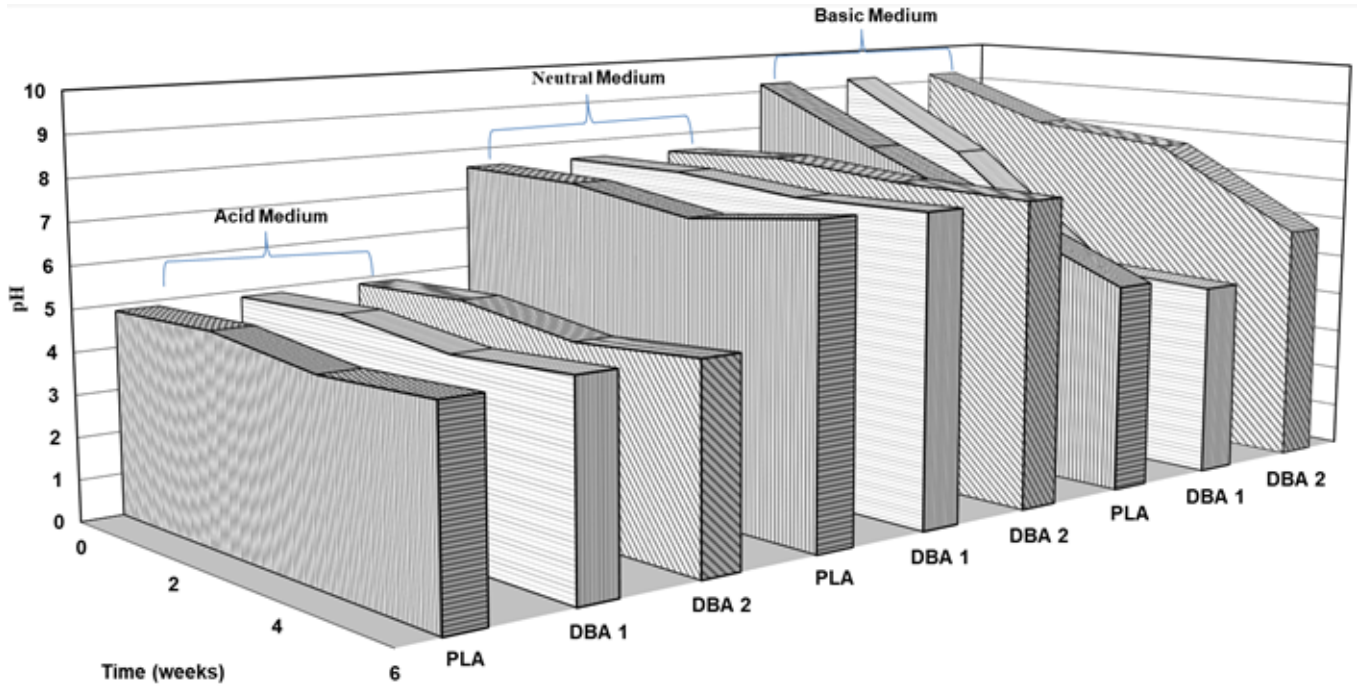
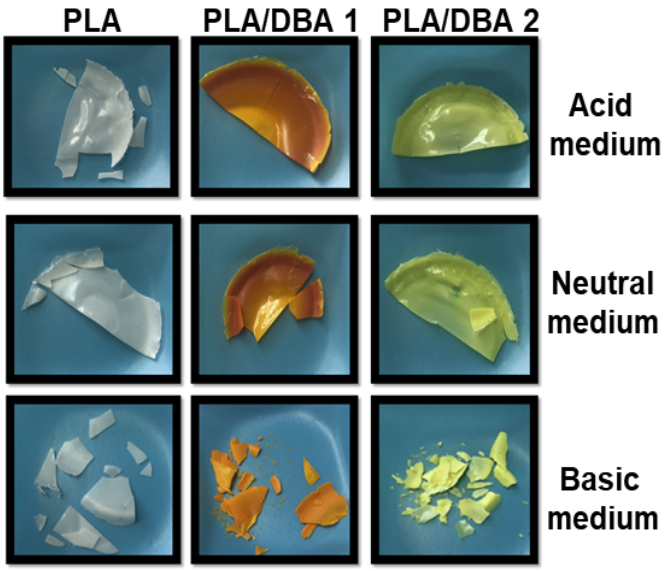


Figure 8. Physical evidence of hydrolytic degradation of unloaded PLA membranes and loaded PLA membranes with DBA analogs in different degradative media for six weeks.



the presence of degradation products formed during the hydrolytic process, 2) due to the formation of holes in the volume of the sample during degradation or 3) due to the evolution in crystallinity of the polymeric matrix.[60,61] The hydrolytic degradation of the polyester chains takes place at a higher rate in the amorphous areas of the matrix. It is expected that this phenomenon increases the relative crystallinity of the sample, which can result in a greater opacity of the material.[62–64]

On the other hand, it is primordial to observe the degraded membranes at a microscopic level. That way, the morphological changes in the membranes under the different degradation mediums can be distinguished (Fig. 9). It was observed that membranes in the acid medium presented a slight erosion on the surface, and in some cases, such as the unloaded PLA membranes, a small crack was observed. In general, they retain similarity with the morphology seen in the non-degraded membranes, such as the different fibrillar patterns on the surface and some roughness. Despite a noticeable change in morphology in the acid medium was not observed, these membranes were sensitive to the electron beam of the electron microscope. This phenomenon is an indication that there was a degradative process in the polymeric matrix. It can be noticed in the membranes subjected to neutral pH, some small cracks and marks on their surface. Moreover, these membranes turned out to be more photosensitive than those degraded in an acid medium. In the basic medium, more forceful

cracks are observed on the surface of the membranes, and in the loaded with DBA 2, erosion on the surface is noticeably visible compared to the other mediums studied, again indicating that the hydrolytic degradation of PLA at basic pH is the fastest.

These SEM micrographs in the basic medium corroborate the fact that the membranes subjected to this medium considerably decrease the elastic properties of PLA to such an extent that they are easily fragmented. In general, the hydrolytic degradation of PLA polymer matrices can proceed through two different mechanisms: (i) surface or heterogeneous erosion and (ii) bulk or homogeneous erosion. [65] In the case of surface erosion, polymeric degradation is faster than the diffusion of water into the polymeric matrix. In this way, degradation occurs mainly in the outermost layers of the polymer, thus reducing its dimensions and releasing the loaded compound from the outermost layer. Consequently, erosion only affects the surface and not the internal parts of the polymeric matrix, thus being a heterogeneous process.

In contrast, homogeneous erosion occurs when the dif-

Figure 9. SEM photomicrographs of the morphology of unloaded PLA membranes and loaded PLA membranes with DBA analogs degraded in acid, neutral, and basic mediums for six weeks at 37 ° C.

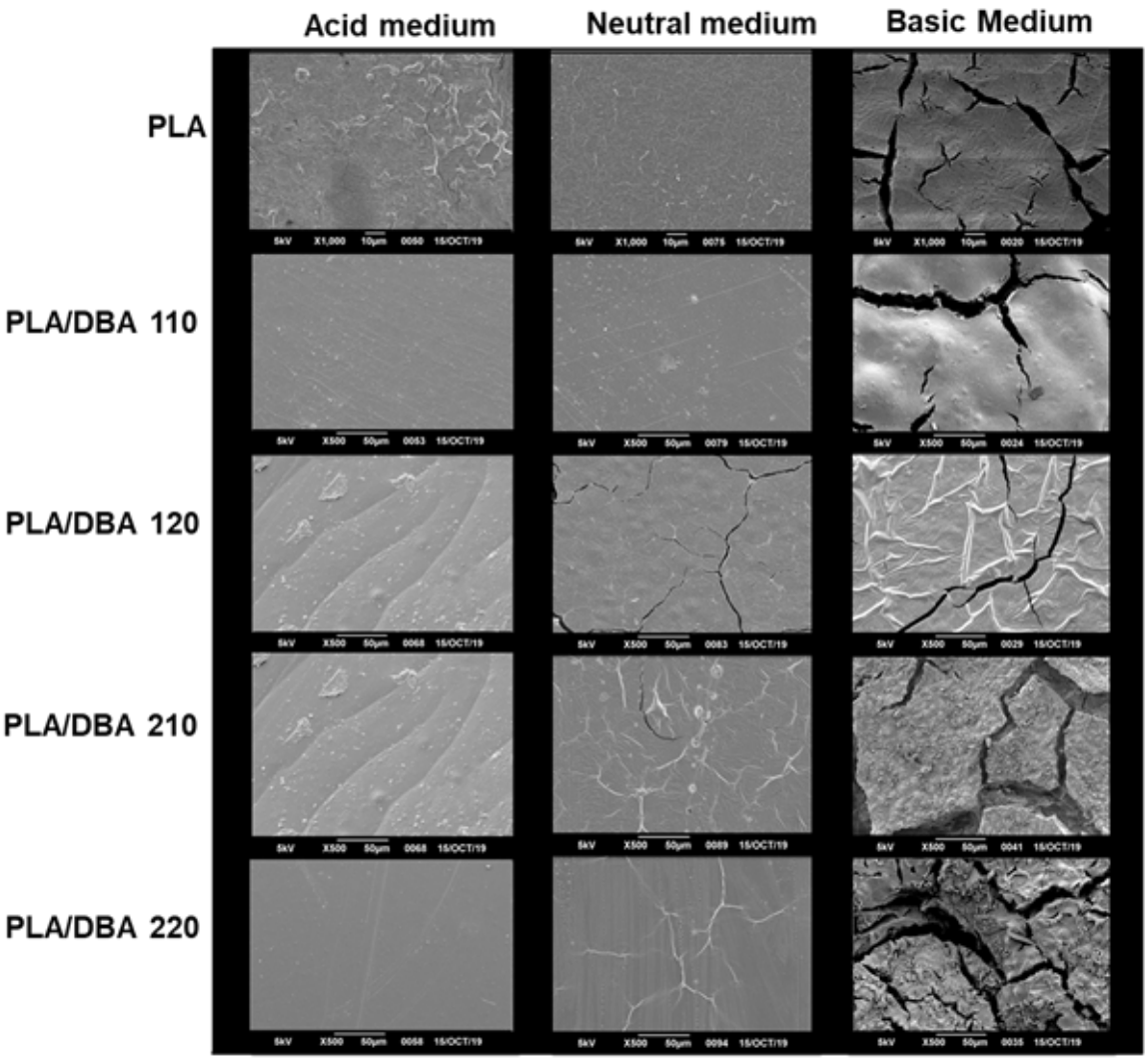
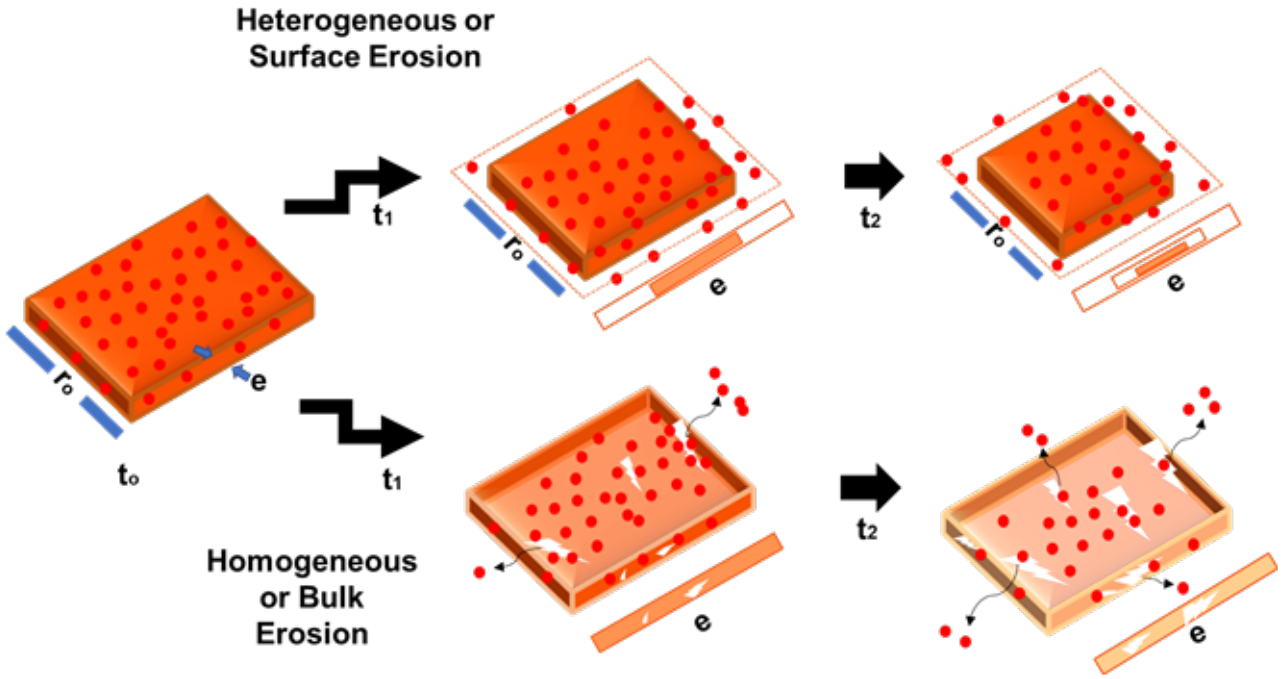


Figure 10. Schematic illustration of the principles of heterogeneous or surface erosion of polymer membranes; and homogeneous or bulk erosion into the polymer matrix. From an initial time (t_0) to a final time (t_2) during hydrolysis.



fusion of water is faster than the degradation of the polymer. Consequently, there is rapid hydration of the system that causes splits in the polymeric chains, thus being a homogeneous process,[66] and the compound is being released through the pores and/or cracks formed in the polymer matrix. These mechanisms are illustrated in Fig. 10. Seeing that there are cracks on the surface and surface size reduction in the membranes, especially for those degraded in a basic pH, it can be pointed out that their hydrolytic degradation could occur due to the heterogeneous erosion mechanism.

Release of DBA analogs present in the membranes

After analyzing the membrane degradation mechanism, it is predominant to study the release of the loaded compounds. There are many factors that influence the kinetics of the release of the loaded compound: 1) diffusion of the water within the polymeric matrix, 2) the solubility of the compound, 3) the polymeric degradation, the origin of aqueous pores, 4) diffusion of the compound and / or degradation products (oligomers) of the polymer within the polymer matrix, 5) changes in pH within the pores of the polymer matrix due to degradation products, 6) diffusion of the compound and / or products degradation through the pores, 7) diffusion of hydrogen ions or hydroxide ions from the release medium to the polymeric structure altering the internal pH of the system, 8) autocatalytic effects during polymeric degradation, 9) osmotic effects, 10) swelling of the polymer, 11) convection processes, 12) adsorption and desorption processes, 13) polymer type and viscosity grade, 14) temperature, 15) buffer concentration and 16) presence of additives.[66–72]

The degradation of PLA is critical for drug delivery behavior in controlled release systems based on this polymer. Homogeneous erosion and heterogeneous erosion combined with the autocatalytic effect of carboxyl groups probably disturbs a uniform release rate, specifically, the zero-order release state, which is desired when designing previous systems.[73] Furthermore, the diffusion of the drug through the polymeric matrix is a mechanism that contributes to the complexity of the phenomenon. This last mechanism is influenced by the swelling characteristics of the polymeric matrix. Matrix–drug interactions are another critical parameter influencing drug delivery systems. Chemical interactions between entrapped compounds and the biodegradable polymer can have a strong effect on polymer degradation and drug release.[74]

The release of DBA analogs in PLA membranes is shown through the release profiles in the different media and amounts studied (see figure 11). It can be seen that the highest release of both DBA analogs occurs in an acid medium in the proportions of 10 µg of DBA per mg of PLA and that after two weeks, a maximum amount of compound is released, which then decays over time. In the fourth week, an increase in the release of DBA 120 and DBA 210 is observed in the basic medium. In addition, an increase in the release of DBA 210 in the neutral medium and a decrease of DBA 110, DBA 120, and DBA 220 release in the neutral medium.

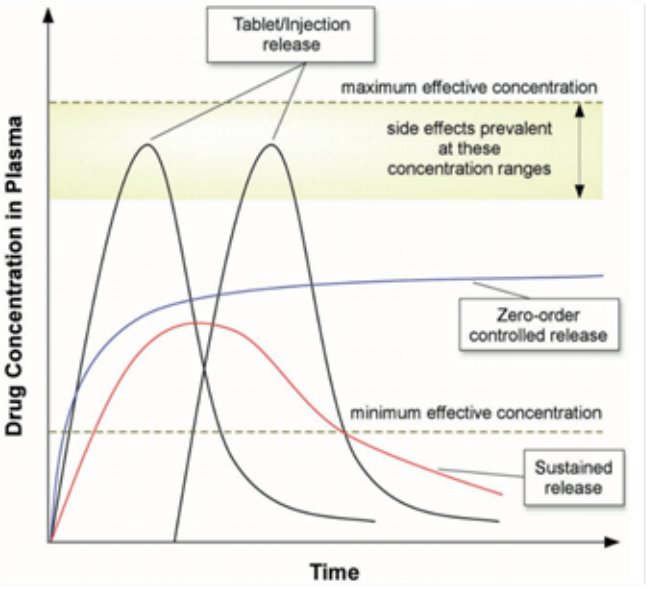
Membranes with a higher amount of load presented a lower release of the DBA than those containing a lower amount of DBA. According to the analysis and observation of SEM micrographs, it can be inferred that membrane degradation is favored by heterogeneous erosion. Within these systems, drug release is due to the degradation/erosion of the polymer surface, [75] thus generating a slower release of the loaded compound in the membranes. Another factor that may be involved in this observation is the possible interactions between the DBA and PLA. These interactions can cause a slow release of DBA.

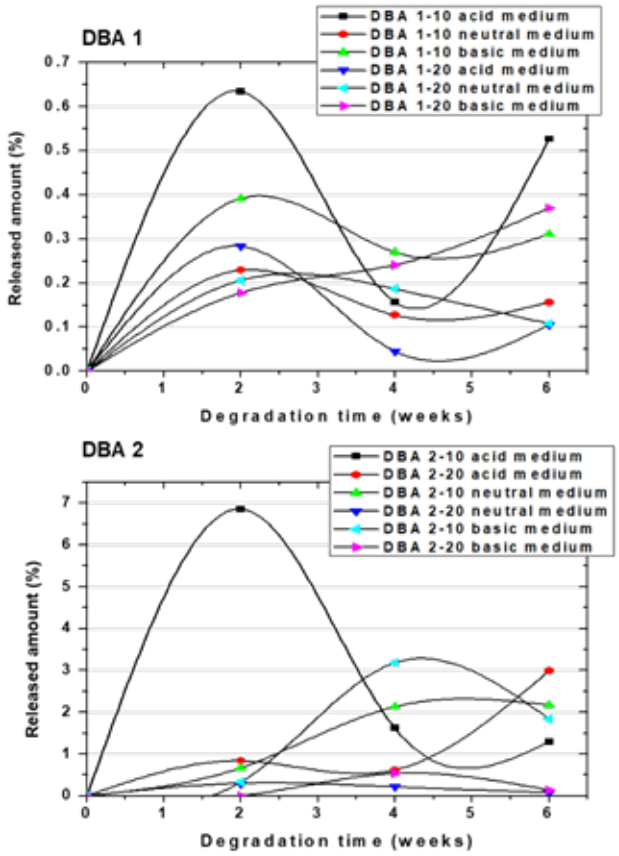
DBA analogs are hydrophobic compounds (two conjugated aromatic rings and alkenes) that could restrict the water absorption in the polymeric matrix, thus reducing the degradation rate and favoring the heterogeneous erosion mechanism. It is important to mention that membranes were not completely degraded. This leads to infer that, at longer study times, a higher degradation of the polymeric matrices and a higher DBA analogs release could be observed. Another interesting observation is that in the acid medium the release of

both DBA analogs is higher than the other mediums. Although the degradation in the acid medium occurs slowly compared to the neutral and basic medium. This pattern has been evidenced by the moderate loss of both weight and viscosimetric molecular weight of the membranes, the pH variations of the degrading medium, and the few morphological changes observed by SEM on their surfaces in the acid medium. This could indicate that protons present in acid pH interact with the DBA. Therefore, there is a decrease in the degradative process and an increase in the release of the loaded compound.

In Figure 11, the characteristic controlled release profiles

Figure 11. Comparison of release profiles of conventional formulations, ideal release systems, and sustained–release with the release profiles obtained from DBA analogs in polymeric in PLA/DBA membranes.[28]





in conventional formulations, in ideal release systems, and sustained release, are compared with the release profiles obtained for the membranes in the different degradation mediums studied. It is observed that the profile more similar to the ideal controlled release system profile is the DBA 210 membrane in the neutral medium, being an interesting result since pH (7.54) is quite close to the pH of the human blood. The release profiles of DBA 110 and DBA 210 show a similarity to the conventional formulations profiles in the time studied. The basic medium profiles for the DBA 110 and DBA 210 tend to be sustained release profiles. For membranes with a higher load of DBA 1, the release profile in acid and neutral mediums is similar to the sustained–release profile. On the other hand, for membranes with a higher load of DBA 2, their release during the study time was so low that not characteristic behavior regarding its release is observed.

Conclusions

Qualitative and quantitative hemocompatibility tests demonstrated that free DBA analogs do not present hemolysis at concentrations between 200–500 µg/mL. Therefore, they can be considered non–toxic at these concentrations. Hence, the PLA/DBA membranes can be considered non–toxic. In this way, it is safe to load this compound in PLA–based polymeric matrices in this concentration range and use it as a controlled release system. The studies of the hydrolytic degradation of the membranes under the different conditions confirmed the type of degradation process reported in the literature. Also, they confirmed that the pH of the medium is an important parameter that influences the rate of degradation. Although the basic medium induces a greater physical disintegration of the membranes, it was obtained that the highest percentage of release was observed in an acid medium for both DBA analogs. According to all results, the structures studied may have applications in the development of controlled drug release systems or load/ release of other biomolecules used in some biotechnological applications.

Acknowledgments

The authors thank the laboratories of Organic Synthesis, Instrumental Analysis, and the Scanning Electron Microscopy laboratory at Simón Bolívar University (USB) for their collaboration with materials and equipment.

References

[1] Zhou D, Xie D, He F, Song B, Hu D. Antiviral properties and interaction of novel chalcone derivatives containing a purine and benzenesulfonamide moiety. Bioorganic &

Medicinal Chemistry Letters. 2018;**28(11)**:2091–2097.

[2] Zhang W, Bai H, Han L, Zhang H, Xu B, Cui J et al. Synthesis and biological evaluation of curcumin derivatives modified with α–amino boronic acid as proteasome inhibitors. Bioorganic & Medicinal Chemistry Letters. 2018;**28(14)**:2459–2464.

[3] Nakamae I, Morimoto T, Shima H, Shionyu M, Fujiki H, Yoneda–Kato N et al. Curcumin Derivatives Verify the Essentiality of ROS Upregulation in Tumor Suppression. Molecules. 2019;**24(22)**:4067.

[4] Mbese Z, Khwaza V, Aderibigbe B. Curcumin and Its Derivatives as Potential Therapeutic Agents in Prostate, Colon and Breast Cancers. Molecules. 2019;**24(23)**:4386.

[5] Kazantzis K, Koutsonikoli K, Mavroidi B, Zachariadis M, Alexiou P, Pelecanou M et al. Curcumin derivatives as photosensitizers in photodynamic therapy: photophysical properties and in vitro studies with prostate cancer cells. Photochemical & Photobiological Sciences. 2020;**19(2)**:193–206.

[6] de Paula J, Bakoshi A, Lazarin–Bidóia D, Ud Din Z, Rodrigues–Filho E, Ueda–Nakamura T et al. Antiproliferative activity of the dibenzylideneacetone derivate (E)–3–ethyl–4–(4–nitrophenyl)but 3–en–2–one in Trypanosoma cruzi. Acta Tropica. 2020; **211**:105653.

[7] Naseer S, Lone S, Lone J, Khuroo M, Bhat K. LC–MS guided isolation, quantification and antioxidant evaluation of bioactive principles from Epimedium elatum. Journal of Chromatography B. 2015;**989**:62–70.

[8] Harizal H, Hidayanto A, Sari N. SYNTHESIS AND PRELIMINARY EVALUATION OF 3,3'–DIHYDROXY–4,4'–DIMETHOXYDIBENZYLIDENEACETONE AS SUNSCREEN AND ANTIOXIDANT ACTIVE COMPOUND. JURNAL KIMIA MULAWARMAN. 2018;**16(1)**:48.

[9] Prasad S, Yadav V, Ravindran J, Aggarwal B. ROS and CHOP Are Critical for Dibenzylideneacetone to Sensitize Tumor Cells to TRAIL through Induction of Death Receptors and Downregulation of Cell Survival Proteins. Cancer Research. 2010;**71(2)**:538–549.

[10] Yu H, Shin J, Nam J, Kang B, Cho S. Apoptotic effect of dibenzylideneacetone on oral cancer cells via modulation of specificity protein 1 and Bax. Oral Diseases. 2013;**19(8)**:767–774.

[11] Lee H, Choi E, Jung J, You M, Kim L, Cho S. Inhibition of specificity protein 1 by dibenzylideneacetone, a curcumin analogue, induces apoptosis in mucoepidermoid carcinomas and tumor xenografts through Bim and truncated Bid. Oral Oncology. 2014;**50(3)**:189–195.

[12] Liang G, Liu Z, Wang Z, Zhang Y, Xiao B, Fang Q et al. Discovery and evaluation of asymmetrical monocarbonyl analogs of curcumin as anti–inflammatory agents. Drug Design, Development and Therapy. 2014; 373.

[13] Lazarin–Bidóia D, Desoti V, Martins S, Ribeiro F, Ud Din Z, Rodrigues–Filho E et al. Dibenzylideneacetones Are Potent Trypanocidal Compounds That Affect the Trypanosoma cruzi Redox System. Antimicrobial Agents and Chemotherapy. 2015;**60(2)**:890–903.

[14] Yusuf A, Sada I, Hassan Y, Olomola T, Adeyemi C, Ajibade S. Synthesis, Antimalarial Activity, and Docking Studies of Monocarbonyl Analogues of Curcumin. Ovidius University Annals of Chemistry. 2018;**29(2**):92–96.

[15] Carapina da Silva C, Pacheco B, das Neves R, Dié Alves M, Sena–Lopes Â, Moura S et al. Antiparasitic activity of synthetic curcumin monocarbonyl analogues against Trichomonas vaginalis. Biomedicine & Pharmacotherapy. 2019;**111**:367–377.

[16] Spencer L, Peña–Quintero A, Canudas N, Bujosa I, Urdaneta N. Antimalarial effect of two photo–excitable compounds in a murine model with Plasmodium berghei (Haemosporida: Plasmodiidae). Revista de Biología Tropical. 2018;**66(2)**:880.

[17] Handayani S. Synthesis and activity test of two asymmetric dibenzalacetones as potential sunscreen material. Chemical. Biological And Environmental Engineering. 2009;119–122.

[18] Santos F, Abegão L, Fonseca R, Alcântara A, Mendonça C, Valle M et al. Bromo–and chloro–derivatives of dibenzylideneacetone: Experimental and theoretical study of the first molecular hyperpolarizability and two–photon absorption. Journal of Photochemistry and Photobiology A: Chemistry. 2019;**369**:70–76.

[19] Garcia G, Hammerer F, Poyer F, Achelle S, Teulade–Fichou M, Maillard P. Carbohydrate–conjugated porphyrin dimers: Synthesis and photobiological evaluation for a potential application in one–photon and two–photon photodynamic therapy. Bioorganic & Medicinal Chemistry. 2013;**21(1)**:153–165.

[20] Liu Y, Kong M, Zhang Q, Zhang Z, Zhou H, Zhang S et al. A series of triphenylamine–based two–photon absorbing materials with AIE property for biological imaging. J Mater Chem B. 2014;**2(33)**:5430–5440.

[21] Castet F, Rodriguez V, Pozzo J, Ducasse L, Plaquet A, Champagne B. Design and Characterization of Molecular Nonlinear Optical Switches. Accounts of Chemical Research. 2013;**46(11)**:2656–2665.

[22] Xing J, Zheng M, Duan X. Two–photon polymerization microfabrication of hydrogels: an advanced 3D printing technology for tissue engineering and drug delivery.

Chemical Society Reviews. 2015;**44(15)**:5031–5039.

[23] Ekbote A, Patil P, Maidur S, Chia T, Quah C. Structural, third–order optical nonlinearities and figures of merit of (E)–1–(3–substituted phenyl)–3–(4–fluorophenyl) prop–2–en–1–one under CW regime: New chalcone derivatives for optical limiting applications. Dyes and Pigments. 2017;**139**:720–729.

[24] Cui M, Ono M, Kimura H, Liu B, Saji H. Synthesis and Structure–Affinity Relationships of Novel Dibenzylideneacetone Derivatives as Probes for β–Amyloid Plaques. Journal of Medicinal Chemistry. 2011;**54(7)**:2225–2240.

[25] Ryu E, Choe Y, Lee K, Choi Y, Kim B. Curcumin and Dehydrozingerone Derivatives: Synthesis, Radiolabeling, and Evaluation for β–Amyloid Plaque Imaging†. Journal of Medicinal Chemistry. 2006;**49(20)**:6111–6119.

[26] Anand P, Kunnumakkara A, Newman R, Aggarwal B. Bioavailability of Curcumin: Problems and Promises. Molecular Pharmaceutics. 2007;**4(6)**:807–818.

[27] Langer, R. Drug delivery and targeting. Nature. 1998;**392**:5–10.

[28] Fenton O, Olafson K, Pillai P, Mitchell M, Langer R. Advances in Biomaterials for Drug Delivery. Advanced Materials. 2018;**30(29)**:1705328.

[29] Ochekpe N, Olorunfemi P, Ngwuluka N. Nanotechnology and Drug Delivery Part 1: Background and Applications. Tropical Journal of Pharmaceutical Research. 2009;**8(3)**.

[30] Adhikari S, Pant H, Mousa H, Lee J, Kim H, Park C et al. Synthesis of high porous electrospun hollow TiO2 nanofibers for bone tissue engineering application. Journal of Industrial and Engineering Chemistry. 2016;**35:75**–82.

[31] Sabino M, Loiaza M, Dernowsek J, Rezende R, Silva J. Techniques for manufacturing polymer scaffolds with potential applications in tissue engineering. Revista Latinoamericana de Metalurgia y Materiales. 2017;**37(2)**:1–27.

[32] Rao J, Geckeler K. Polymer nanoparticles: Preparation techniques and size–control parameters. Progress in Polymer Science. 2011;**36(7)**:887–913.

[33] Danhier F, Ansorena E, Silva J, Coco R, Le Breton A, Préat V. PLGA–based nanoparticles: An overview of biomedical applications. Journal of Controlled Release. 2012;**161(2)**:505–522.

[34] Kost J, Langer R. Responsive polymeric delivery systems. Advanced Drug Delivery Reviews. 2012; **64**:327–341.

[35] Ishihara K, Muramoto N, Shinohara I. Controlled release of organic substances using polymer membrane with responsive function for amino compounds. Journal of Applied Polymer Science. 1984;**29(1)**:211–217.

[36] Núñez J, Urdaneta N, Echevarría L, Alamo D. Síntesis y caracterización espectroscópica de dibencilidenacetonas y 3–bencilidentiocroman–4–onas. Av. cien. ing. 2012;**3(3)**:11–18.

[37] Nawrot W, Drzozga K, Baluta S, Cabaj J, Malecha K. A Fluorescent Biosensors for Detection Vital Body Fluids' Agents. Sensors. 2018;**18(8)**:2357.

[38] Dubonosov A, Bren V. Fluorogenic Polyfunctional Coumarin–Based Chemosensors for Multianalyte Detection. Fluorescence Methods for Investigation of Living Cells and Microorganisms. 2020.

[39] Fu Y, Zhang J, Lakowicz J. Highly Efficient Detection of Single Fluorophores in Blood Serum Samples with High Autofluorescence. Photochemistry and Photobiology. 2009;***85(3)***:646–651.

[40] Elahi M, Guan G, Wang L, King M. Improved hemocompatibility of silk fibroin fabric using layer–by–layer polyelectrolyte deposition and heparin immobilization. Journal of Applied Polymer Science. 2014;**131(18)**:n/a–n/a.

[41] Gallardo M, Barbosa R, Fook M, Vinicius L, Sabino, M. Synthesis and characterization of a novel biomaterial based on chitosan modified with amino acids. Matéria (Rio de Janeiro). 2019;**24(3)**, e12397.

[42] Lim L, Auras R, Rubino M. Processing technologies for poly(lactic acid). Progress in Polymer Science. 2008;**33(8)**:820–852.

[43] Anderson J, Shive M. Biodegradation and biocompatibility of PLA and PLGA microspheres. Advanced Drug Delivery Reviews. 1997;**28(1)**:5–24.

[44] Fischer D, Li Y, Ahlemeyer B, Krieglstein J, Kissel T. In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials. 2003;**24(7)**:1121–1131.

[45] Mallegni N, Phuong T, Coltelli M, Cinelli P, Lazzeri A. Poly(lactic acid) (PLA) Based Tear Resistant and Biodegradable Flexible Films by Blown Film Extrusion. Materials. 2018;**11(1)**:148.

[46] Das U, Kawase M, Sakagami H, Ideo A, Shimada J, Molnár J et al. 3–(3,4,5–Trimethoxyphenyl)–1–oxo–2–propene: A novel pharmacophore displaying potent multidrug resistance reversal and selective cytotoxicity. Bioorganic & Medicinal Chemistry. 2007;**15(10)**:3373–3380.

[47] Lyu, Schley J, Loy B, Lind D, Hobot C, Sparer R et al. Kinetics and Time–Temperature Equivalence of Polymer

Degradation. Biomacromolecules. 2007;**8(7)**:2301–2310.

[48] Lazzari S, Codari F, Storti G, Morbidelli M, Moscatelli D. Modeling the pH–dependent PLA oligomer degradation kinetics. Polymer Degradation and Stability. 2014;**110**:80–90.

[49] Zhou Q, Xanthos M. Nanoclay and crystallinity effects on the hydrolytic degradation of polylactides. Polymer Degradation and Stability. 2008;**93(8)**:1450–1459.

[50] Tsuji H, Ikada Y. Properties and morphology of poly(l –lactide) 4. Effects of structural parameters on long–term hydrolysis of poly(l –lactide) in phosphate–buffered solution. Polymer Degradation and Stability. 2000;**67(1)**:179–189.

[51] Schliecker G, Schmidt C, Fuchs S, Kissel T. Characterization of a homologous series of d , l –lactic acid oligomers; a mechanistic study on the degradation kinetics in vitro. Biomaterials. 2003;**24(21)**:3835–3844.

[52] Dias J, Ribeiro C, Sencadas V, Botelho G, Ribelles J, Lanceros–Mendez S. Influence of fiber diameter and crystallinity on the stability of electrospun poly(l–lactic acid) membranes to hydrolytic degradation. Polymer Testing. 2012;**31(6)**:770–776.

[53] Kim K, Yu M, Zong X, Chiu J, Fang D, Seo Y et al. Control of degradation rate and hydrophilicity in electrospun non–woven poly(d,l–lactide) nanofiber scaffolds for biomedical applications. Biomaterials. 2003;**24(27)**:4977–4985.

[54] Zong X, Ran S, Kim K, Fang D, Hsiao B, Chu B. Structure and Morphology Changes during in Vitro Degradation of Electrospun Poly(glycolide–co–lactide) Nanofiber Membrane. Biomacromolecules. 2003;**4(2)**:416–423.

[55] Vert M, Mauduit J, Li S. Biodegradation of PLA/GA polymers: increasing complexity. Biomaterials. 1994;**15(15)**:1209–1213.

[56] Vert M, Li S, Garreau H. More about the degradation of LA/GA–derived matrices in aqueous media. Journal Of Controlled Release. 1991;**16(1–2)**:15–26.

[57] Eyal A, Canari R. pH Dependence of Carboxylic and Mineral Acid Extraction by Amine–Based Extractants: Effects of pKa, Amine Basicity, and Diluent Properties. Industrial & Engineering Chemistry Research. 1995;**34(5)**:1789–1798.

[58] Göpferich A. Mechanisms of polymer degradation and erosion. Biomaterials. 1996;**17(2)**:103–114.

[59] Lyu S, Untereker D. Degradability of Polymers for Implantable Biomedical Devices. International Journal of Molecular Sciences. 2009;**10(9)**:4033–4065.

[60] Liu L, Li S, Garreau H, Vert M. Selective Enzymatic Degradations of Poly(l–lactide) and Poly(ε–caprolactone) Blend Films. Biomacromolecules. 2000;**1(3)**:350–359.

[61] Jarerat A, Pranamuda H, Tokiwa Y. Poly(L–lactide)–Degrading Activity in Various Actinomycetes. Macromolecular Bioscience. 2002;**2(9)**:420–428.

[62] Duek E, Zavaglia C, Belangero W. In vitro study of poly(lactic acid) pin degradation. Polymer. 1999;**40(23)**:6465–6473.

[63] Sabino M, Feijoo J, Müller A. Crystallisation and morphology of poly(pdioxanone). Macromolecular Chemistry And Physics. 2000;**201(18)**:2687–2698.

[64] Sabino M, Sabater L, Ronca G, Müller A. The effect of hydrolytic degradation on the tensile properties of neat and reinforced Poly(p–dioxanone). Polymer Bulletin. 2002;**48(3)**:291–298.

[65] Burkersroda F, Schedl L, Göpferich A. Why degradable polymers undergo surface erosion or bulk erosion. Biomaterials. 2002;**23(21)**:4221–4231.

[66] Siepmann J. Mathematical modeling of bioerodible, polymeric drug delivery systems. Advanced Drug Delivery Reviews. 2001;**48(2–3)**:229–247.

[67] Makino K, Ohshima H, Kondo T. Mechanism of hydrolytic degradation of poly(L–lactide) microcapsules: effects of pH, ionic strength and buffer concentration. Journal of Microencapsulation. 1986;**3(3)**:203–212.

[68] Zambaux M. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. Journal of Controlled Release. 1998;**50(1–3)**:31–40.

[69] Zuleger S, Lippold B. Polymer particle erosion controlling drug release. I. Factors influencing drug release and characterization of the release mechanism. International Journal of Pharmaceutics. 2001; **217(1–2)**:139–152.

[70] Varma M, Kaushal A, Garg A, Garg S. Factors Affecting Mechanism and Kinetics of Drug Release from Matrix–Based Oral Controlled Drug Delivery Systems. American Journal of Drug Delivery. 2004; **2(1)**:43–57.

[71] Mochizuki A, Niikawa T, Omura I, Yamashita S. Controlled release of argatroban from PLA film—Effect of hydroxylesters as additives on enhancement of drug release. Journal of Applied Polymer Science. 2008;**108(5)**:3353–3360.

[72] Fu Y, Kao W. Drug release kinetics and transport mechanisms of non–degradable and degradable polymeric delivery systems. Expert Opinion on Drug Delivery. 2010;**7(4)**:429–444.

[73] Andreopoulos A. Plasticization of biodegradable polymers for use in controlled release. Clinical Materials. 1994;**15(2)**:89–92.

[74] Tarvainen T, Karjalainen T, Malin M, Pohjolainen S, Tuominen J, Seppälä J et al. Degradation of and drug release from a novel 2,2−bis(2−oxazoline) linked poly(lactic acid) polymer. Journal of Controlled Release. 2002; **81(3)**:251−261.

[75] Zarzycki R, Modrzejewska Z, Nawrotek K. (2010). Drug release from hydrogel matrices. Ecological Chemistry and Engineering. 2010;**17(2)**:117−136.