



EVALUATION OF TETRABUTYLAMMONIUM BROMIDE AS A CATALYST IN POLYANHYDRIDE SYNTHESIS FOR DRUG ENCAPSULATION

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ABSTRACT

The use of tetrabutylammonium bromide as a catalyst was evaluated in polyanhydride synthesis from tridecanedioic and succinic acids to be used as controlled delivery system of drug. Polyanhydrides were synthesized for combinations of different molar ratio of tridecanedioic and succinic acids (25:75, 50:50, 75:25) by trough microwave radiation during 3 and 5 minutes, with and without catalyst. Intrinsic viscosity determined from single point measurements was highest for that polyanhydride synthesized from 75:25 molar ratio and 5 minutes of microwave radiation. Thermogravimetric analysis and differential scanning calorimetry showed polyanhydrides have high thermal stability, with melting temperature of 45.5 °C for the polyanhydride without catalyst and 61 °C for that obtained in the presence of the catalyst. The degradation capacity of the samples was evaluated by pH and temperature sensitivity, being the degradation higher in acid media (pH 7.4) at 42°C. Synthesized polyanhydrides have therefore a high potential to be used in the encapsulation of drugs.

Keywords: polyanhydride, tridecanedioic acid, succinic acid, tetrabutylammonium bromide, microwave irradiation, catalyst.

INTRODUCCIÓN

Conventional drug delivery systems have hesitations associated with its toxicity and due to their poor selectivity they may not release the drugs in the desired area causing affections to healthy organs [1]. Contrary to these, biopolymers are not toxic or mutagenic, and they are biodegradable [2], qualities that make them suitable candidates for controlled drug release, besides its degradation occurs by surface erosion [3], which is variable and depends of the biopolymer structure, so it can take days or months [4]. The use of biopolymers in drug delivery systems pretends the dosage of a drug through the polymer matrix in fluxes within its therapeutic window [5, 6].

Several investigations have been conducted about the use of polyanhydrides as controlled drug release devices in the treatment of eye diseases, antineoplastic agents, anticoagulants, and neuroactive drugs. In these clinical tests, carmustine (chemotherapeutic agent) has been loaded in poly (1, 3-bis-(p-carboxyphenoxy propane)-co-(sebacic anhydride) in 20:80 ratio, commercially known as Glidel® and used in the treatment for brain tumors [7, 8]. Moreover, polyanhydride nanoparticles were synthesized by using melt polymerization mechanism from 1, 6-bis(p-carboxyphenoxy) hexane, sebacic acid (SA), and 1,8-bis-(p-carboxyphenoxy)-3,6-dioxaoctane (CPTEG). Nanoparticles were elaborated to release therapeutic antibody [9].

The aim of this investigation is the synthesis of polyanhydrides based on tridecanedioic and succinic acid with and without tetrabutylammonium bromide as a catalyst for controlled drug release. Succinic acid has been widely used in food and pharmaceutical industry and furthermore it can be used as a monomer for biodegradable polymer synthesis [10, 11], additionally, tridecanedioic acid has been found in milk and in a variety

of biofluids including urine and saliva [12]. Tetrabutylammonium bromide is a non-toxic phase transfer catalyst widely used in diastereoselective alkylation systems at room temperature. In order to develop new biodegradable polymers, polyanhydrides from dibasic acids have been synthesized by polycondensation with microwave irradiation technology.

MATERIALS AND METHODS

Materials

Tridecanedioic acid (CAS No 505-52-2) and succinic acid (CAS No 110-20-6) obtained from Merck were used to synthesize the polyanhydride. As crosslinking agent and solvent was employed acetic anhydride (CAS No. 108-24-7) manufactured by Sigma - Aldrich. Tetrabutylammonium bromide obtained from Sigma - Aldrich (CAS 1643-19-2) was the catalyst of the reaction. Reaction was carried out in a microwave. A LAUDA Alpha thermostat together with a vacuum pump (Sportsnet TW-1) were used to remove excess of acetic anhydride and the unwanted derivate products.

Polyanhydride Synthesis from Tridecanedioic Acid and Succinic Acid through Microwave Radiation

Synthesis of polyanhydride from tridecanedioic and succinic acids was worked out using a microwave and acetic anhydride as crosslinking agent. Before the prepolymerization process, the microwave was calibrated by hot spot test. A moistening filter paper was putted into a microwave oven for 15 min resulting in the identification of some hot spots in the microwave, which were avoided for polymerization reaction. Prepolymerization process of tridecanedioic acid was carried out in a molar ratio 1:3 of each dicarboxylic acid solid and acetic anhydride. The mixture was placed in a vial and situated under microwave radiation with a power of 1100 watts (which was adjusted



to 80% of its power) for 5 min [13]. Next, the unreacted acetic anhydride was removed by using a vacuum pump system with the vial immersed in a water bath at 40°C. The same procedure was employed for prepolymerization process of succinic acid. For the copolymerization, the previously obtained prepolymers were mixed and it was added tetrabutylammonium bromide as a catalyst. Finally, the mixture in the vial was placed in the microwave during the stipulated time. In this work the effect of factors such as the ratio of prepolymers in the mixture, the presence of catalyst and the polymerization reaction time was evaluated.

Intrinsic Viscosity

Through Mark-Houwink equation, $[\eta] = kM^a$, the intrinsic viscosity (η) is related to the molecular weight of the polymer (M) [14]. Viscosity measurements have been used to determine indirectly the molecular weight of polyanhydrides, for that, at a first stage, in a 10 ml chloroform/ethanol solution (50/50) was added 1 g of each polyanhydride combination; after that, a series of new dilutions were prepared from this solution with concentrations of 0.0025, 0.005, 0.0075 and 0.01 g/ml. Measurements were performed by using a capillary viscometer submerged in a thermal bath at 25°C, each one of the samples was passed through the capillary until its upper mark and it was registered the time that the solution take to reach the bottom mark of the capillary of this. By using a pycnometer of 10 ml was determine the polyanhydrides density [15].

Intrinsic viscosity of all combinations of polyanhydrides synthesized was determined from single point measurements using Solomon-Ciuta and Kuwahara relations:

$$\text{Solomon - Ciuta (SC): } [\eta] = \frac{[2(\eta_{sp} - \ln \eta_r)]^{\frac{1}{2}}}{c} \quad (1)$$

$$\text{Kuwahara (K): } [\eta] = \frac{(\eta_{sp} + 3 \ln \eta_r)}{4c} \quad (2)$$

$$\eta_r = \frac{\rho t}{\rho_0 t_0}$$

$$\eta_{sp} = \eta_r - 1$$

Where η_{sp} is specific viscosity, η_r relative viscosity and c the concentration of solution that flows through the capillary. ρ and ρ_0 are the density of solution and pure solvent, respectively. t and t_0 are times that the sample took to go through the capillary for the solution and pure solvent.

Characterization of Polyanhydrides

Samples with the largest molecular weight were characterized to determine the morphology, stability, and degradation. Thermogravimetric analysis was used to

identify thermal stability of the polymers synthesized. The analysis was performed on a SDT Q600 Thermogravimetric Analyzer (TA Instrument); samples were heated under nitrogen gas atmosphere from 10 to 595°C at a heating rate of 10°C min⁻¹.

Differential scanning calorimetry was carried out in a DSC Q200 V24.4 Build 116, using a heating rate of 10°C min⁻¹ and heating from 0 to 140°C.

An IR Affinity Shimadzu, S/N A213749 spectrometer was used to register FTIR spectra of the polyanhydrides in a frequency range of 400 to 4000 cm⁻¹. A JEOL JSM 6490 LV scanning electron microscopy in secondary electron mode using an accelerating of 20 kV was used for analysis SEM. Images were taken with 750X and 3000X of magnifications. Samples were subjected to surface metallization.

Degradation Analysis

To evaluate the response to external stimuli, similar condition to those of the human body have been simulated. This was done through buffer solutions. For degradation test, polymer disks were prepared by compressing 0.3g of each polymer sample into a disk using a hydraulic press. Buffer were prepared as a combination of 0.2M potassium chloride and 0.2M hydrochloric acid solutions for buffer at pH 1.2, and 0.1M potassium acid phosphate with 0.1M sodium hydroxide solutions for pH 7.4 buffer. Then disks were placed in a vial with 20 ml of the corresponding buffer solution. The samples were stored in a thermostatic bath and its temperature was regulated at 37°C or 42°C. Buffer solution was replaced periodically (every 4.5 h) to maintain the conditions of the environment. After 24 h, some samples were taken out of the buffer solution and washed with deionized water; subsequently they were putted in a vacuum oven at 50°C and 250 psi for 3h to remove the water. Finally, samples were weighted to determine the percentage of mass loss. The same procedure was carried out until to reach 72 h [16]. Percentage of mass loss was obtained using the following equation:

$$\% \text{ Mass loss} = \frac{m_o - m_i}{m_i} * 100 \quad (3)$$

where m_i is the pre-degraded dry weigh of the disk and m_o is the weight of the simple after the degradation test.

RESUSLTS

Intrinsic Viscosity

Table-1 shows the average intrinsic viscosity values obtained from results produced by both correlations for all combination of polyanhydrides established.

**Table-1.** Intrinsic viscosity results from single point equations.

Molar ratio	Time	Intrinsic viscosity Poly(tridecanedioic-co-succinic) with catalyst	Intrinsic viscosity Poly(tridecanedioic-co-succinic) without catalyst
25:75	3 min	25.38	32.36
	5 min	26.98	36.21
50:50	3 min	28.22	35.82
	5 min	36.36	36.75
75:25	3 min	19.62	20.54
	5 min	45.86	55.69

According to the results of the intrinsic viscosity estimated from Solomon-Ciuta and Kuwahara, the polyanhydrides subjected to microwave radiation for 5 minutes with and without the use of catalyst are the samples with the highest viscosity, therefore, have the highest molecular weight. Moreover, polyanhydrides synthesized based on the ratio 75:25, subjected to microwave radiation for 5 min with and without the use of catalyst are the samples with highest viscosity. The obtained results suggest that the catalyst TBAB do not modify the reaction mechanism, because it is a phase-transfer catalyst facilitates the molecular interaction and acts as an initiator together with the microwave irradiation, preventing the termination and facilitating propagation. The results obtained in this analysis are like those reported by Laitano and Marrugo [15] in the study of Poly (Sebacic-co-Azelaic) anhydride for 5 minutes of reaction. As seen in results reported in Table-1, the intrinsic viscosity is affected by the relation of the co-monomers, as well as the time reaction and the addition of catalyst in a minor degree. Those samples with the highest molecular weight were selected for the other characterizations.

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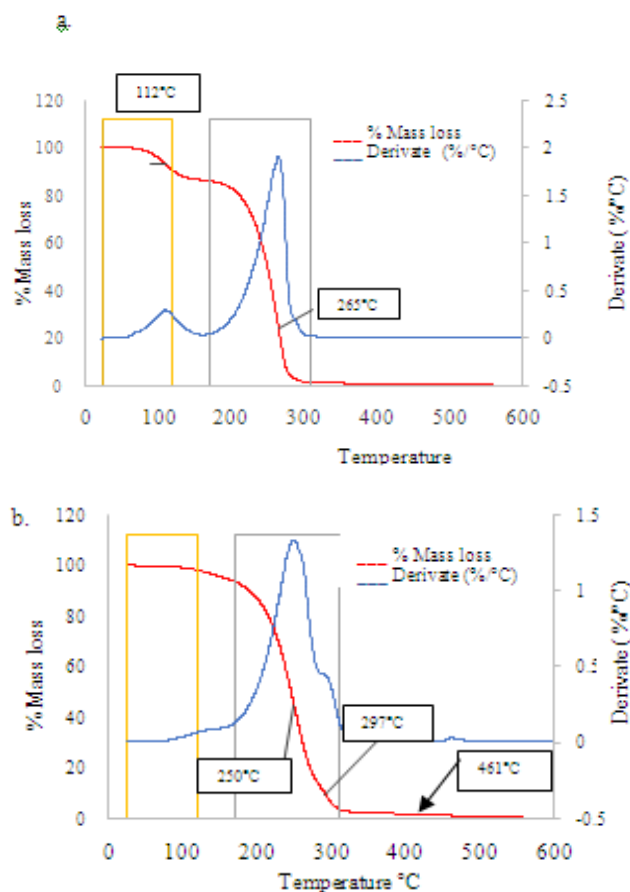


Figure-1. Thermogravimetric analysis del poly (tridecanedioic-co-succinic) anhydride. a) Without catalyst. b) With catalyst.

In the second stage from 170 until 300°C, 98% weight loss takes place. Where the thermal degradation of the polyanhydride without catalyst occurs at 265°C and that of the modified polyanhydride is 280°C. For the Poly (dodecanedioic acid-tridecanedioic acid) a thermal degradation temperature at 304 °C was obtained [18]. Last stage is between 300 and 490°C, reaching here the total weight loss. According to literature, at this temperature range can be identified cyclic and linear hydrocarbon compounds [19]. Thermal behavior of the synthesized polyanhydrides shows a gradual weight loss



with increasing the temperature, so that their thermal stability can be considered adequate. At temperatures below 100°C, weight loss is relatively low for both samples, 4% for poly (tridecanedioic-co-succinic) without catalyst and 2% for poly (tridecanedioic-co-succinic) synthesized with catalyst, the latter being the more stable throughout the analysis.

Differential Scanning Calorimetry (DSC)

DSC thermograms of 75:25 Poly(tridecanedioic-co-succinic) anhydride synthesized by microwave radiation during 5 min with and without catalyst are shown in Figure-2 A and 2. B respectively.

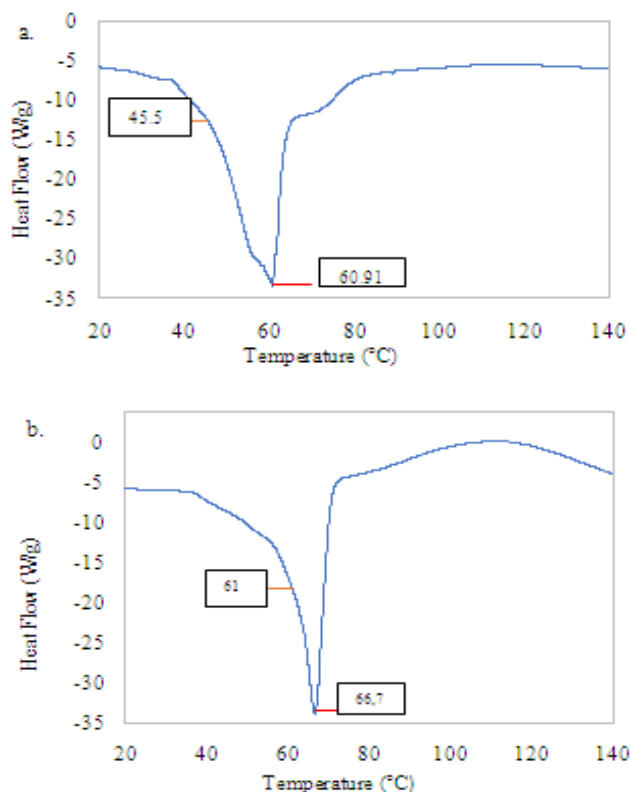


Figure-2. Thermal analysis DSC for poly (tridecanedioic-co-succinic) anhydride de 75:25 - 5 min a) without catalyst. b) With catalyst.

In both thermograms it can be identified a double peak for the melting temperature (T_m), which is due to the melting, recrystallization and subsequent melting of the polyanhydride at the time of the thermal scanning [20]. The peak at 45.5°C (Figure-2A.) and 61°C (Figure-2B.) corresponds to the melting temperature for the base polyanhydride and the catalyzed polyanhydride respectively, the second peak is attributed to the melting temperature of the crystals created during the recrystallization [21]. For a synthesized polyanhydride from sebacic acid and beaver oil (80% sebacic acid) was obtained a melting temperature of 72°C [22].

Scanning Electron Microscopy (SEM)

The energy dispersive X-ray spectroscopy (EDS) of the elemental composition of the samples are shown in

Table-2. This analysis reveals the presence of oxygen atoms, while hydrogen content was not detected, because of the device employed to carried out the analysis just detect atoms with a molecular weight greater than carbon molecular weight. Results obtained for uncatalyzed sample agree with the obtained for polyanhydrides evaluated to be used in biomedical applications as Poly(azelaic-co-decanedioic) anhydride [23]. Differences identified for catalyzed sample are attributed to a low absorption of water as was observed through TGA results and the acetic acid (reaction byproduct) removal during the washed of the sample with methanol, due to the chemical similarity between the both products.

Table-2. Quantitative elemental analysis of poly (tridecanedioic-co-succinic) anhydride 75:25 and 5 min under microwave radiation.

Spectrum	Sample	Average	Literature
O	With TBAB	28	28 ^a
	Without TBAB	23	

Figure-3 shows the representative SEM images of poly(tridecanedioic-co-succinic) anhydride with and without tetrabutylammonium bromide. It becomes evident a swelling of the sample without catalyst, which is attributed to absorption of water molecules and its amorphous characteristics. Sample with catalyst, however, shows a less swollen structure and a better ordering of the chains, which leads to a more compact appearance.

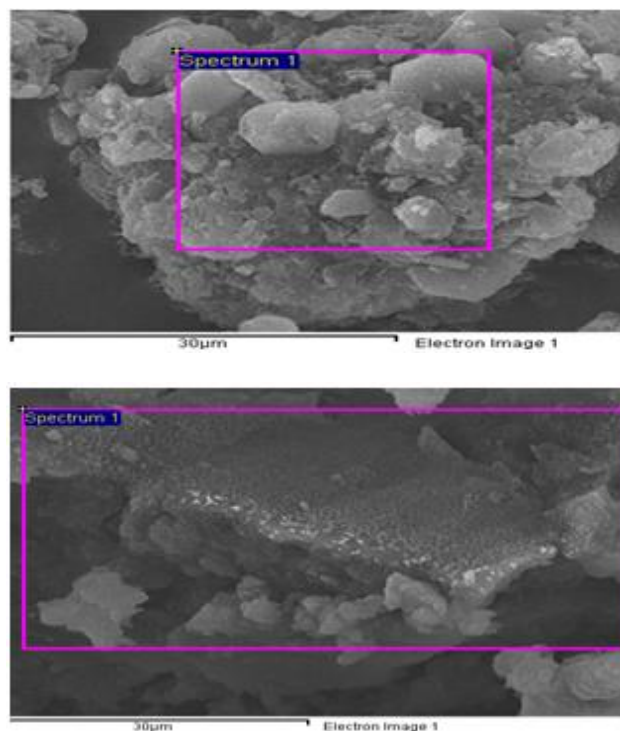


Figure-3. SEM Images a) Polyanhydride synthesized without TBAB. b) Polyanhydride synthesized with TBAB.



Fourier Transform Infrared (FTIR)

FT-IR spectrum for poly (tridecanedioic-co-succinic) anhydride is shown in Figure-4. Monomers used for the copolymer synthesis were obtained from organic acids, whose structures have two carboxylic groups and a hydrocarbon chain. Peaks corresponding to carboxylic acids are easily detected in the spectra, which suggest the presence of monomers that did not react.

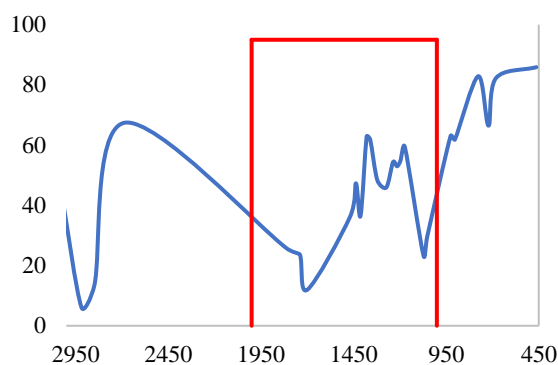
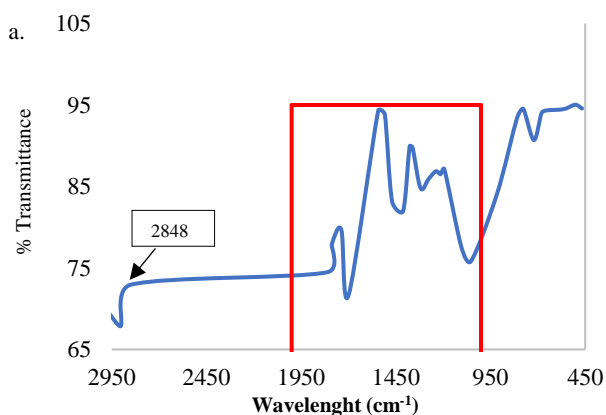


Figure-4. FTIR spectrum for a) Polyanhydride without TBAB as catalyst. b) Polyanhydride with TBAB as catalyst.

In Figure-4a, 4b for sample with and without catalyst, respectively the band around $3300 - 2500 \text{ cm}^{-1}$ is assigned to O-H bond stretching vibration, also carbonyl stretching $\text{C}=\text{O}$, C-O stretching vibration and the bending vibration of O-H group were identified in the spectra [24]. For polyanhydrides were identified three characteristics peaks attributed to vibrations of $(-\text{CO}-\text{O}-\text{CO}-)$. Table-3 presents the vibrations identified for synthesized polyanhydrides. For samples of polyanhydride synthesized from sebacic acid, 1, 3-bis- (carboxyphenoxy) propane, and methoxy polyethylene glycols, Wang, *et al.* [25] reported the presence of peaks corresponding to anhydrous carbonyl vibrations around 1810 cm^{-1} and 1739 cm^{-1} .

Table-3. Functional groups identified in FTIR analysis.

Vibration	Polyanhydride without TBAB	Polyanhydride with TBAB
Stretching of O-H	2894	2849
Stretching of $\text{C}=\text{O}$	1738	1740
Stretching of C-O	1238	1236
Bending of O-H	1412	1412
$(-\text{CO}-\text{O}-\text{CO}-)$	1738	1700
	1788	1740
	1813	1811

Degradation Analysis

Polyanhydride degradation was studied by through the mass loss test of the polymers into acid and basic media during 72 hours, the degradation media were at 37° and 42°C . Poly(tridecanedioic-co-succinic) anhydride with TBAB in basic media at 42°C had the highest degradation percentage, 97.38%. Similar results have been reported for polyanhydrides based on sebacic and azelaic acid [15] whose degradation percentage reached was 96.25% under the same conditions of degradation. For the same conditions of pH (7.4) at 37°C , poly(tridecanedioic-co-succinic) anhydride with TBAB reached a slightly lower degradation percentage, 88.95%. According the mass loss results reported in Figures 5 and

6, into alkaline media (pH 7.4) degradation is greater than in acid media, as it happens for polyanhydrides, according to the reported by [26]. At low pH, the degradation products of the polymer are in the ionized form, which does not allow its complete solubility into the medium [27].

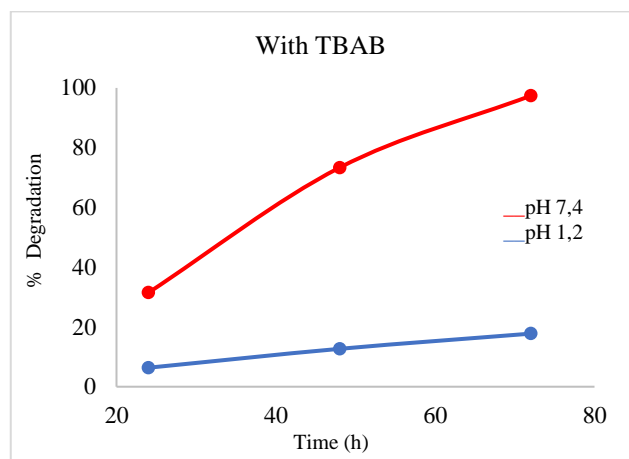
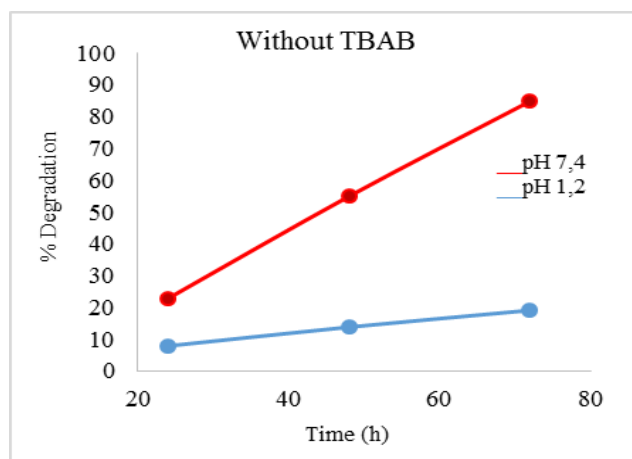


Figure-6. Degradation test by mass loss of poly (tridecanedioic-co-succinic) anhydride at pH 1.2 and 7.4 and 42°C.

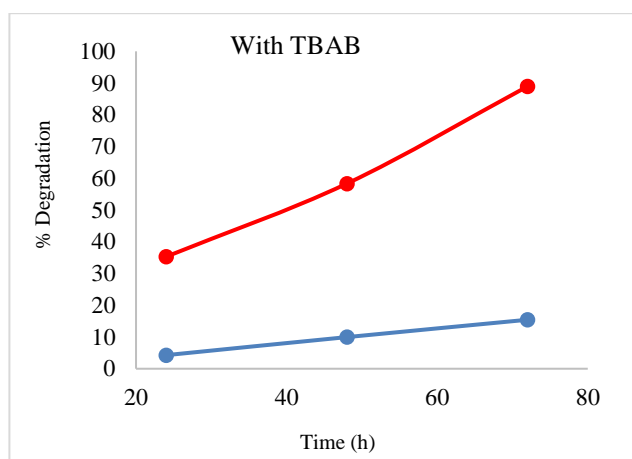
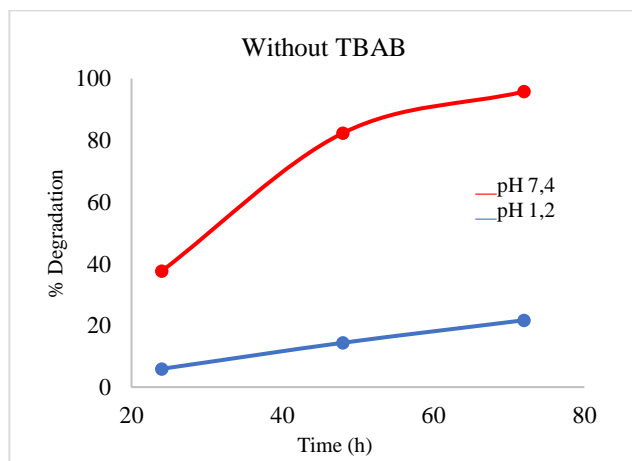


Figure-5. Degradation test by mass loss of poly (tridecanedioic-co-succinic) anhydride at pH 1.2 and 7.4 and 37°C.



Mass loss percentage increases with increasing the temperature in the degradation test, because of the temperature increase lead to an increase in the solubility of the sample in the solution, a similar behavior was reported by [15], [22] for polyanhydrides based in sebacic with azelaic acids, and azelaic with dodecanedioic acid, respectively.

CONCLUSIONS

The microwave-assisted polymerization process demonstrated to be a simple, inexpensive, fast, and effective technology for the synthesis of polyanhydride, considering that the synthesis time was reduced compared to conventional methods such as melt polymerization. Characteristics peaks of anhydride bond ($-\text{CO}-\text{O}-\text{CO}-$) were identified by FTIR analysis, evidencing the effectivity of the method of synthesis. Poly(tridecanedioic-co-succinic) with and without catalyst have wide range of melting temperatures, keeping stables at average corporal temperatures, in addition, they gradually lose mass with increasing temperature. The synthesized polyanhydride is pH sensitive, being highly degradable at pH 7.4, which is the pH condition of the human bloodstream.

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