

Preparation of biodegradable polymer nanoparticles stabilized with the poly (vinyl alcohol)-sodium dodecyl sulfate mixture: Effect of the lactide/glycolide molar ratio

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ABSTRACT

Abstract: This study examines the effects of the lactide/glycolide molar ratio on the synthesis of polymeric nanoparticles using a PVA-SDS stabilizers solution. PLA was obtained with a ratio of 100/0, while PLGA was synthesized at ratios of 88/12 and 64/36. Polymerizations employed ROP at 130°C with stannous octanoate and 1-dodecanol as catalysts. Polymerizations were conducted through ring opening at 130°C with stannous octanoate and 1-dodecanol as catalyst and co-catalyst. Characterized polymers were used to prepare emulsions stabilized with polyvinyl alcohol (15 g/L) alone or mixed with sodium dodecyl sulfate of various molecular weights (MW=13-23, 31-50 and 85-124 KDa). These emulsions were stored in phosphate-buffered saline for 28 days at 37.4°C. Colloidally stable emulsions were achieved using different poly (vinyl alcohol) concentrations, with the lactide/glycolide molar ratio influencing particle diameter. The electrostatic stabilizer formed by the poly (vinyl alcohol)-sodium dodecyl sulfate mixture demonstrated superior stabilization compared to poly (vinyl alcohol) alone, representing a novel finding. Moreover, the poly (vinyl alcohol-sodium dodecyl sulfate) mixture showed reduced water diffusion into the nanoparticles compared to poly (vinyl alcohol) alone, as evidenced by molecular weight and pH measurements. Additionally, the degradation of poly (lactic acid) and poly (lactic-co-glycolic acid) films was investigated; pH measurements in the immersed solutions showed that the degradation was increased with higher glycolide content.

Keywords: Polymer; poly; Nanoparticles; Lactide acid; Glycolide; PLGA; vinyl alcohol

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1. Introduction

Synthetic polymers have a significant advantage over natural polymers due to their high purity. Some of the synthetic polymers that have gained popularity, particularly in the medical field, and that has been approved by the Food and Drug Administration (FDA) are those of the polyester family, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and the copolymer formed between them, poly(lactic-co-glycolic acid) (PLGA). These polymers are of great interest in the biomedical field because they are biocompatible (Figure 1) and biodegradable in the human body, as well as not being toxic and being easily discarded when they are inside the body [1,16].

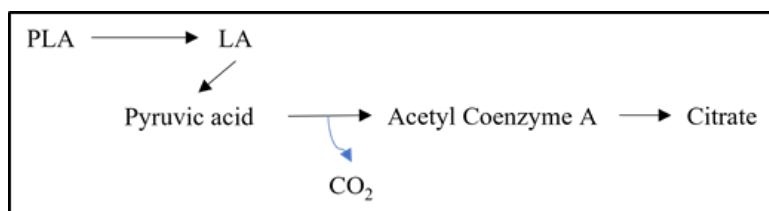


Figure 1. Degradation of PLA

Many studies have shown that the use of PLGA as nanoparticles is a promising alternative for the controlled release of certain drugs in the treatment of cancer patients [2]. One of the most common ways to make these nanoparticles is through a process called "emulsion evaporation." In this process, a polymer that has already formed is used, and high stirring speeds (>10,000 rpm) are employed. This technique involves combining an organic polymer solution with a stabilizer in an aqueous solution to produce an emulsion. The organic solvent is then taken out of the emulsion under low pressure to make the nanoparticles stabilized by surfactants and dispersed in water stiff [3]. Surfactants of the non-ionic type, especially those in the poly(vinyl alcohol) (PVA) family, are used a lot. But there are no reports in the scientific literature of combining this steric stabilizer with an electrostatic stabilizer, like sodium dodecyl sulfate (SDS), to make an electrosteric stabilizer.

Polymeric materials have important uses in medicine. Some of the most studied materials in nanoparticle form as drug delivery devices are biodegradable polymers like PLA and PLGA. There are different ways to make these nanoparticles, but the emulsion-evaporation method stands out because it starts with a polymer that is already made [17]. Additionally, Biodegradable polymer nanoparticles have gained attention in drug delivery systems, where they can be loaded with antibiotics or antimicrobial agents to enhance their targeted delivery to infection sites. These nanoparticles can provide controlled and sustained release of antimicrobial compounds, potentially improving treatment outcomes for patients with multidrug-resistant bacterial infections [24].

Stabilizers that aren't made of ions have been used, like those in the PVA family. But more research needs to be done on how changes in the PVA WAMW (Weight Average Molecular Weight) and the LA/GA (w/w) composition affect the colloidal stability of nanoparticles while they are being made and stored. In addition to what has already been said, there are no reports in the literature of PVA and SDS being mixed to cause electrosteric stabilization [4]. The disclosed method is based on emulsifying an aqueous solution of an organic polymer with a stabilizing agent to form an emulsion. The organic solvent is then removed from the emulsion under reduced pressure to provide the water-distributed surfactant-stabilized nanoparticles rigidity. The most often used non-ionic surfactants are those from the poly (vinyl alcohol) (PVA) family. No descriptions of the combination of such a steric stabilizer with an electrostatic stabilizer, notably sodium dodecyl sulphate (SDS), to create an electrosteric type stabilizer have been found in the literature.

Poly (vinyl alcohol, or PVA), which is the most frequently used to stabilize emulsions caused by the formation of relatively small particles with uniform size distribution, was selected as a stabilizing agent in the formulations. PVA has excellent stabilizing properties on the NP emulsion.

As a result, in the current study, this study intend to explore the effects of WAMW of PVA (alone or in combination with SDS) and LA/GA composition (w/w) on polymeric nanoparticles. Also, the effects of each of these effects on the degradation of these nanoparticles (via hydrolysis) and changes in the pH of the emulsions during storage, including the formation of polymeric films, will be looked at to see how similar and different the two devices are. The current study aims to investigate the colloidal stability of polymer nanoparticles by varying their LA/GA molar ratio and using the PVA-SDS mix and PVA as control colloidal stabilizers during storage. As a result, the colloidal stability of polymeric nanoparticles is reported in the current study using the LA/GA molar ratio and the PVA-SDS mix as colloidal stabilizers. The colloidal stability was studied by keeping track of how the particle size (D_p) changed in the emulsions as they were

made and stored. Also, the breakdown of PLA and PLGA nanoparticles during storage was studied by figuring out the molecular weights and changes in pH of the emulsions, as well as in the form of polymeric films [15].

This study's research spans the fields of chemical engineering and materials science, demonstrating the critical importance of chemical engineering concepts in the design and optimization of biodegradable polymer nanoparticles. The title emphasizes the "Preparation of Biodegradable Polymer Nanoparticles," a process based on chemical engineering techniques including ring-opening polymerization (ROP) at controlled temperatures and with specified catalysts. The molar ratio of lactide/glycolide is an important measure under investigation, revealing how careful control of reactant ratios can change the properties of the resulting polymers. Chemical engineers are pioneers in developing and improving such polymerization methods for a wide range of applications, ensuring reproducibility and scalability.

Furthermore, it emphasizes the function of stabilizers in preserving the stability of polymer nanoparticles, which is a topic that crosses over into both chemical engineering and materials science. The choice of stabilizer has a considerable impact on the behavior of these nanoparticles, as demonstrated by a study of polyvinyl alcohol (PVA) alone and PVA combined with sodium dodecyl sulphate (SDS). The understanding of how various stabilizers affect particle size, stability, and degradation rates provides a useful dimension to the discipline of chemical engineering, where engineers play a critical role in selecting and optimizing stabilizers for diverse industrial processes. This research emphasizes the interdisciplinary character of chemical engineering, in which material synthesis, modification, and application are critical components of advancing technology and addressing real-world difficulties.

2. Materials and methods

2.1. Reagents

3,6-dimethyl-1,4-dioxane-2,5-dione (D, L LA lactide 98%), 1,4-dioxane-2,5-dione (glycolide, GA 99%), poly(vinyl alcohol) (PVA) of different average molecular weight (WAMW= 13-23, 31-50 and 85-124 KDa, 87-89% hydrolyzed), stannous octoate (95% Sn(Oct)₂), ethyl acetate (99.5%), hexafluoroisopropanol (HFIP), anhydrous toluene (99.8%), 1-dodecanol (98%), deuterated chloroform (99.8% deuterium atoms), and phosphate-buffered saline (PBS), all provided by Sigma Aldrich, were used as received. Sodium dodecyl sulfate (SDS) was from Fluka, while chloroform and methanol, both reagent grade, were from Merck. The HPLC-grade chloroform was from the Merck brand. The water was distilled and later de-ionized by passing through ion exchange columns of the Cole-Palmer brand. Merck, India, provided ultra-high purity argon (99.999%).

2.2. Analytical Instrumentation and Miscellaneous Equipment.

2.2.1. Analytical instrumentation

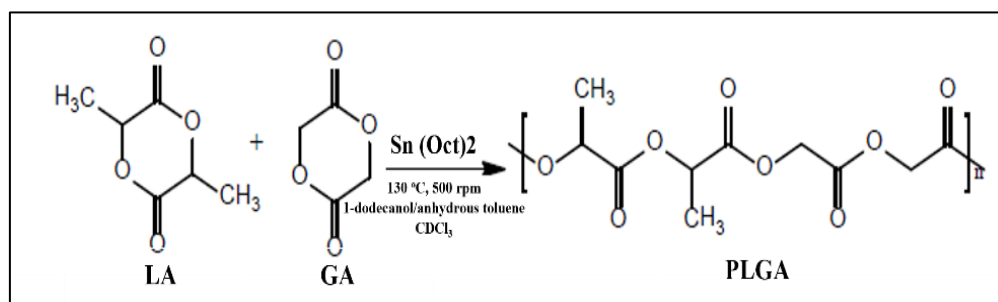
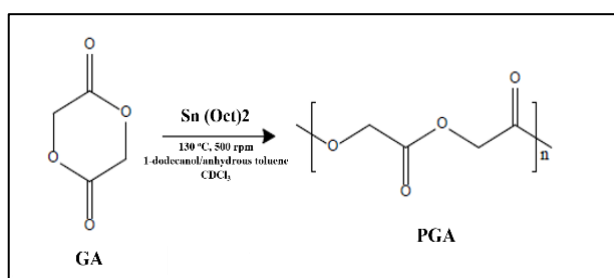
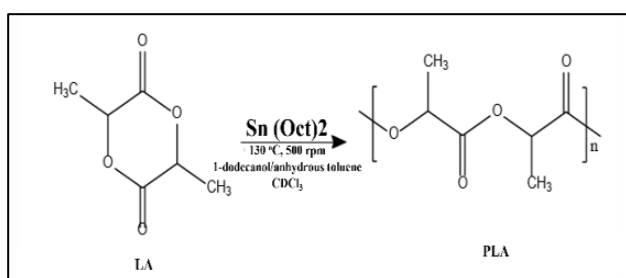
- Waters model 2695 Gel Permeation Chromatograph (GPC) with Waters model 2414 refractive index detector from Alliance. The mobile phase was HPLC grade CHCl₃ with an injection flow of 1 ml/min.
- Bruker 500MHz Advance III Proton Nuclear Magnetic Resonance (1H-NMR) using CDCl₃ or a mixture of CDCl₃/HFIP as polymer solvents.
- Differential Scanning Calorimeter (DSC) model Q200 from TA Instruments at a heating or cooling rate of 10°C/min under a nitrogen atmosphere.
- The heating or cooling rate was 10°C/min in a nitrogen atmosphere.
- Nicolet Magna 550 Infrared Spectrophotometer (FTIR), 25 scans, 4cm⁻¹ resolution, with a 4000 to 400nm wavelength.

2.3 Synthesis of polymers via ROP varying their lactide/glycolide molar ratio (La/Ga)

PLA and PLGA syntheses [varying the LA/GA ratio%w/w (100/0; 88/12; 64/36)] were carried out in groups. The total amount of dimers used was 2.6 g, which was later increased to 7 g total. In the first group shown in Table 1 an amount of total dimers (abbreviated as "M") of 2.6 g was used, while in Table 2 the amount increased to 7 g total. Polymerizations were carried out in both cases using the mass process at a stirring speed of 500 rpm and a reaction temperature of 130°C. Sn(Oct)2, abbreviated as "C", was used as a catalyst, while 1-dodecanol, abbreviated as "ROH" as initiator, both previously dissolved in anhydrous toluene (10% by weight with respect to the total amount of monomers used) in order to facilitate its addition to the reaction system due to the very small amounts used. Note how in both groups of polymerizations the molar ratios, abbreviated as "RM", of the M/C and ROH/C were kept constant at 2000 and 1, respectively. It's worth noting that the molar ratios in both groups of polymerizations were kept constant at 2000 and 1, respectively. The method used in the synthesis of polymers about formulations. After adding the respective LA and GA dimers to the reaction system, along with the catalyst and alcohol previously dissolved in 0.26g anhydrous toluene, the reactor was well sealed and kept under an inert atmosphere by passing a flow of Argon; heating the reactor to 50°C at a stirring speed of 500 rpm began at this point. After 5 minutes, the temperature was gradually increased until it reached 90°C, at which point the reaction mixture was stirred for 10 minutes to achieve better homogenization. Following this, the temperature was gradually increased until it reached 130°C and the polymerization began (Figure 2-3). After 90 minutes, the temperature was reduced to 80°C to manually add previously established volumes of CDCl₃ reagent or HFIP and begin the polymer dissolution and subsequent recovery. The temperature in the reaction system was then reduced to 50°C and maintained for the time required to recover the polymer's complete dissolution. It should be noted that reagent grade CDCl₃ was used to dissolve the polymers, with theoretical ratios of LA/GA of 100/0 and 88/12%w/w, and HFIP of 64/36%w/w.

Table 1. Formulations used in the synthesis of PLA and PLGA at 2.6 g and 7 g total.

Reactor	LA/GA ratio (% w/w)		Moles LA (MW:90.08 g/mol)		Moles GA (MW:116.07g/mol)		RM (M/C)		Moles C		RM ROH/C		Moles OH	
	2.6g	7g	2.6g	7g	2.6g	7g	2.6g	7g	2.6g	7g	2.6g	7g	2.6g	7g
1	100/0	100/0	0.018	0.0486	0	0			9.02E-06	2.43E-05			9.02E-06	9.02E-06
2	88/12	88/12	0.0159	0.0427	0.0027	0.0072	2000	2000	9.28E-06	2.50E-05	1	1	9.28E-06	9.28E-06
3	64/36	64/36	0.0115	0.0311	0.0081	0.0217			9.80E-06	2.64E-05			9.80E-06	9.80E-06



2.3.1. Polymer purification

Each polymer was cleaned by going through cycles of dissolution and precipitation, with CDCl_3 or HFIP as the solvent and reactive or industrial-grade methanol as the precipitant. At the end of the polymerization process, the polymer was already dissolved, so it was precipitated in 500 ml of methanol while stirring. Drop by drop, the polymer solution was added while watching the precipitation. This dissolution-precipitation process was repeated up to four times to get rid of any impurities in the polymer, like leftover dimer or traces of $\text{Sn}(\text{Oct})_2$ and 1-dodecanol. After the cycles were done, the wet polymer was put in a heating oven at 50°C with a vacuum to dry. This made it possible to analyze the polymer.

2.3.2. Analytical techniques used in the characterization of polymers

Different analytical techniques such as $^1\text{H-NMR}$ & FT-IR characterized the polymers of interest. The preparation of each of the samples is described below [21]:

- $^1\text{H-NMR}$: ≈ 15 mg of polymer were dissolved in 1 mL of CDCl_3 or 0.2 mL of HFIP and then made up to 1mL with CDCl_3 . The dissolution was carried out inside a glass tube for NMR which was kept in a cool place until before its analysis.
- FTIR: ≈ 15 mg of polymer and ≈ 1 g potassium bromide (KBr) was placed in a pestle for grinding. Once the mixture was achieved, it was placed in the manual press with the help of a spatula to give rise to a tablet. Said pill was carefully taken with tweezers and held in the sample holder of the equipment for study.

2.4. Preparation of polymeric nanoparticles using the emulsion-evaporation method

Using emulsion evaporation, the formulations and conditions were used to make the polymer nanoparticles [5]. Based on the theoretical ratios, three groups of emulsions with different amounts of LA/GA polymer were made: 100/0w/w, 88/12w/w, and 64/36w/w. Nine PVA formulations (Mw, KDa) were made, and three of them were 13-23, 31-50, and 85-124 in PLA 100/0 composition, 13-23, 31-50, and 85-124 in PLGA 88/12 composition, and 13-23, 31-50, and 85-124 in PLGA 64/36 composition. In the same way, 15 g/L of three different PVAs with different WAMWs were used in each group. The dispersed polymer content (CPD) was around 0.75%w in all cases. The steps for making each of the emulsions in Table 2 are explained below. PVA and PBS buffer were dissolved in 500 mL of distilled water in a beaker. On the other hand, the polymer was dissolved in ethyl acetate (alone or mixed with HFIP) in a beaker of the same volume. When the two solutions were ready, the aqueous solution was poured into the organic solution and stirred quickly for a certain amount of time to make an emulsion. Once this was done, the emulsion was split into two parts. The first part was called the control because it only had PVA as a steric stabilizer. A known amount of SDS (0.1–1.5 g/L) was added to the second part, which had been stabilized with PVA, to promote an electrostatic stabilizer as a PVA-SDS mixture [19]. After that, each emulsion was put in a separate rotary evaporator to remove the organic solvent and harden the nanoparticles, which were still spread out in the water with a surfactant to keep them stable. Lastly, the two emulsions were put into glass jars with lids so that the studies could be done on them before they were put away. So, using 9 formulations led to a total of 18 emulsions, 9 of which were stabilized with PVA (control) and the other 9 with a mixture of PVA and SDS. The difference between the two was the LA/GA (w/w) and WAMW of the PVA, as well as the type of stabilizer (steric or electrosteric), which was already mentioned.

Table 2. Formulations and conditions used in the preparation of polymer nanoparticles via emulsion-evaporation

Polymer	PLA			PLGA					
	100/0w/w			88/12w/w			64/36w/w		
Composition LA/GA, w/w	1	2	3	4	5	6	7	8	9
Formulation, no.	1	2	3	4	5	6	7	8	9
PVA Mw, KDa	13 to 23	31to50	85to124	13to23	31to50	85to124	13to23	31to50	85to124
Aqueous solution									
PVA, g	2.25								
PBS buffer, g	1.65								
water,g	148.96								
organic solution									
polymer,g	1.78								
Ethyl acetate, g	156			156			10.2		
HFIP, g	nil			nil			39.8		
Conditions									
speed stirring, rpm	24,000								
Shaking time, min	5								
Temperature, °C	27±3°C								

2.4.1. Studies carried out on PLA and PLGA emulsions

For each of the freshly made emulsions, the Dp was measured by light scattering and the dispersed polymer content (CPD) was measured by weight. To find the Dp, a drop of the emulsion was taken and put in a 1 cm plastic cell. The drop was then made to volume with a 6 g/L SDS solution in water. After 3 minutes, the cell was carefully put into the light scatterer so that the test could be done. On the other hand, the CPD was uncovered by adding a pre-weighed aluminum tray to which a known quantity of emulsion (between 2 and 5 g) had been added. The tray with the emulsion was then put on a warming rack that was set to ≈125°C. Over time, the water and ethyl acetate in the emulsion evaporated, leaving only the solid parts of the emulsion (the polymer, the surfactant(s), and the buffer). Then, using a pair of tongs, the hot tray was taken off the oven and put on the work table. Finally, the tray with the emulsion solids was weighed again. The CPD was calculated using the formula below:

$$\text{CPD (\% weight)} = \frac{[W(\text{tray+solids}) - W_{\text{tray}}] \times 0.23316843}{W_{\text{emulsion}}} \times 100$$

Where:

CPD (wt%) = content of polymer dispersed in nanoparticle form in the emulsion

W (pan + solids) = weight of aluminum pan + weight of polymer + weight of surfactant + weight of buffer, (g)

W tray = Weight of aluminium tray, g

0.23316843 = Fraction weight of the polymer in total solids

W_{emulsion} = Weight of water + weight of polymer + weight of surfactant(s) + weight of buffer, g

Once the corresponding studies (Dp and CPD) were carried out for each emulsion, the glass jars containing them were tightly covered to continue with the storage stage.

2.4.2. Studies carried out on PLA and PLGA emulsions during storage

The emulsions containing nanoparticles dispersed in water stabilized with surfactant (PVA or PVA-SDS mixture) were placed in an oven at 37.4°C for 28 days. Five samplings were carried out at different time intervals (1, 7, 14, 21 and 28 days). What was intended at this stage was to study various factors such as the colloidal stability of the nanoparticles by following the evolution of Dp by light scattering, the degradation of the nanoparticles by determining the changes in WAMW by GPC, and the pH. It should be noted that the

colloidal stability of the emulsions was also studied at room temperature. ($\approx 25^{\circ}\text{C}$) in order to compare it with the results obtained at 37.4°C .

To track how D_p changed, a sample was taken by taking the bottle of emulsion out of the heating oven and putting a drop through a Pasteur pipette. After that, the emulsion was put back in the oven at 37.4°C to keep it from going bad, while the drop was used to prepare the sample.

To find out how the WAMW and LA/GA changed in the case of PLGA, the PLA and PLGA nanoparticles had to be taken out of each of the emulsions as shown below. After taking the bottle out of the oven, 7 g of the emulsion was taken out and put into a 10 mL Teflon tube with a lid. The tube was then put in the centrifuge at 18,500 rpm for 18 minutes. After this time, the supernatant was taken out of the Teflon tube, and the white powder and film of nanoparticles that had stuck to the bottom of the tube were mixed with 7 mL of distilled water to start the washing step. This stage was done by doing three cycles of dispersion and centrifugation with the same agitation speed, but this time for 5 minutes. The goal of these cycles was to get rid of any traces of surfactant (PVA or a mixture of PVA and SDS) or PBS buffer that might be in the powder or polymeric film. This was done to get a polymer that was so pure that it wouldn't change the way it was characterized. After the treatment was done, the polymer was heated in a vacuum oven at $35\text{--}40^{\circ}\text{C}$ for 1 hour to get rid of any leftover water. Lastly, the GPC samples were made using the dried polymer.

2.5. Production of polymeric films

The technique of solvent-casting was used to make the films. In each case, the following steps were taken. 1 g of polymer was mixed with 5 ml of solvent (CDCl_3 or a mixture of CDCl_3 and HFIP in a 4/1 volume/volume ratio). It was stirred for an hour, which was long enough for the polymer to dissolve. The polymer solution was then taken up with a Pasteur pipette and put on a 35°C heating rack with a Teflon surface. The temperature of the oven was raised by 5°C every 90 minutes until it reached 60°C . During this time, a film formed as the solvent evaporated. After that, it was kept stuck for about 24 hours at 60°C to start getting rid of any leftover solvent. Last, the warming rack was turned off and carefully lifted off the Teflon surface without breaking it. Once the film was free, it was put in a vacuum oven at 50°C for 2 hours to get rid of the 0.01% of the solvent that was still on it. In the current work we employed a single injection method developed by Shah, et al (2018)[22] using a headspace gas chromatograph with flame ionization detector to determine residual solvents levels. The solvent-free film was carefully cut with scissors into a 4 x 2 cm square, and its thickness was measured with a Vernier, with an average of three readings being reported.

2.5.1. Studies carried out on films during storage

GPC characterised each of the newly developed films, after which they were placed in a phosphate buffer solution (PBS) and stored in a heating oven at 37.4°C for 28 days. Three samples were taken at different times during this treatment (1, 7, 14, 21, and 28 days). Each sample was taken by carefully extracting the film from the phosphate buffer solution (PBS) and cutting a strip with dimensions of approx. $0.3 \times 0.8 \text{ cm}$. Afterwards, the film with the largest dimensions was returned to the buffer solution to continue its storage at 37.4°C , while the strip was washed with plenty of distilled water, dried with absorbent paper and placed in a vacuum oven at 60°C for carry out sample preparation by GPC (figure 4) [20].

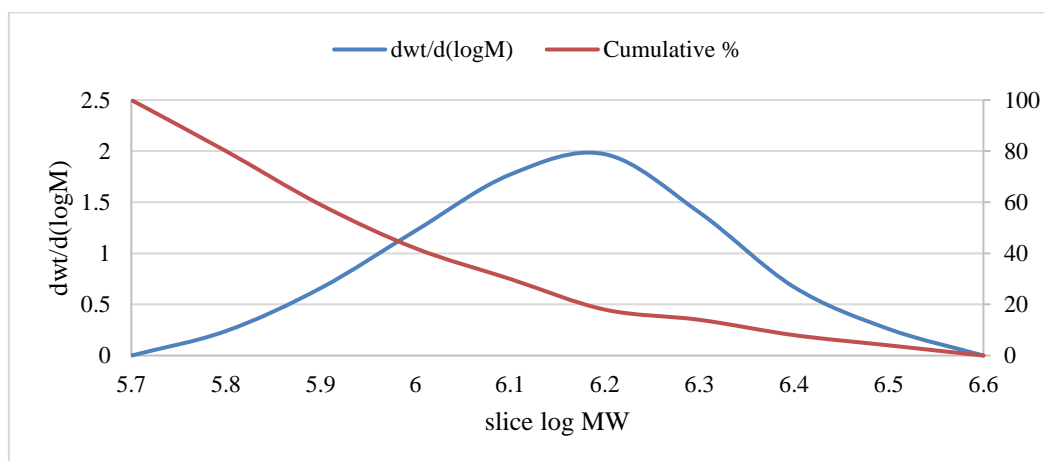


Figure 4. GPC curves showing molecular weight distribution

3. Results and discussion

3.1. Characterization of polymers with different LA/GA molar ratio obtained via ROP

For each polymer, the formulation was taken into account when it was made. The M/C (Monomer/Catalyst) and ROH/C (Alcohol/Catalyst) molar ratios were kept at 2000 and 1, respectively, while the PLA homopolymer and PLGA copolymer were polymerized. Figure 4 shows how the $^1\text{H-NMR}$ spectra change depending on the theoretical LA/GA molar ratio used (100/0, 88/12, 64/36 p/p). The lower spectrum is PLA (the control), and the higher spectra are PLGA with increasing amounts of GA in the copolymer.

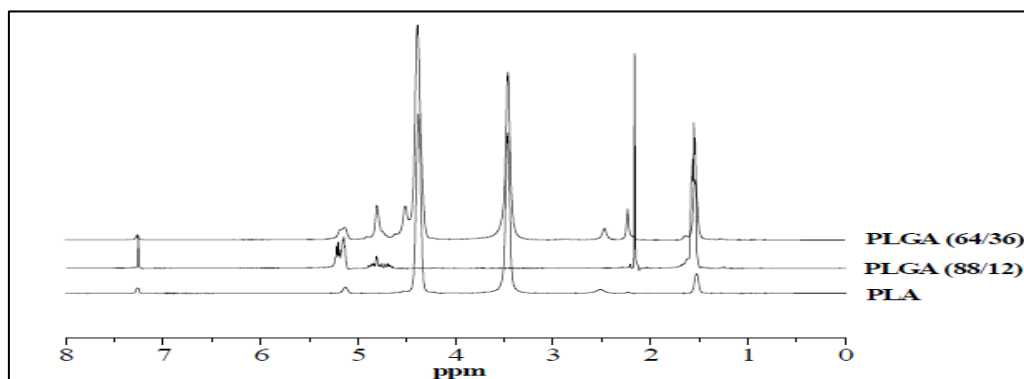


Figure 5. $^1\text{H-NMR}$ spectra of PLA and PLGA with different LA/GA molar ratios in the copolymer

It can be seen that the spectrum for PLA has two signals, one at 1.6 ppm and the other at 5.3 ppm, which are the methyl ($-\text{CH}_3$) and methine ($-\text{CH}-$) groups, respectively [6,7]. In the other spectra for PLGA, the methylene group ($-\text{CH}_2$) of the GA in the copolymer can be seen at 4.75 ppm [8], in addition to the two signals already mentioned. Based on the integration values found in $^1\text{H-NMR}$ spectra (Figure 4), the experimental relationships between LA and GA in the polymer, as well as the control (PLA), were calculated. In PLA, PLGA (88/12 & 64/36) there is a high intense common peak at 4.43 ppm representing deuterated solvent. Another common peak (singlet) at 3.5 ppm indicating the H-C-O functional group, 1.5 ppm representing H-C functional group.

Figure 6 shows the values of the molecular weights (WAMW and NAMW (Number average molecular weight)) of the polymers that were characterized by $^1\text{H-NMR}$, as a function of the experimental composition LA/GA (w/w) that was already known. The left graph shows what happened when 2.6 g of total dimers were used, while the right graph shows what happened when 7 g were used. In the figure, you can see that the WAMW and NAMW decreased when using 2.6 or 7 g of total dimers and as the amount of GA in the polymer went up [6,7]. However, keep in mind that the values for each system were not the same. When more raw material was used, the heat didn't move as well, which caused kinetic changes and, as a result, different molecular weight values.

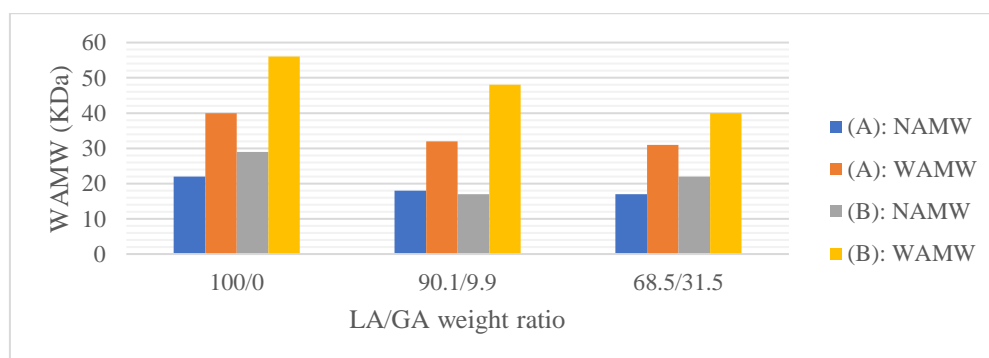


Figure 6. Evolution of WAMW and NAMW of the polymers synthesized via ROP when using different amounts of dimers to be polymerized: A) 2.6 g and B) 7 g, depending on the experimental composition LA/GA (w/w) in the polymer

On the other hand, in Figure 6, the FTIR results of the polymers characterized by $^1\text{H-NMR}$ are shown. The spectrum shown in per cent transmittance corresponds to PLA, while the signals plotted in absorbance correspond to PLA and PLGA with different experimental LA/GA ratios (p/p) as shown in Figure 7.

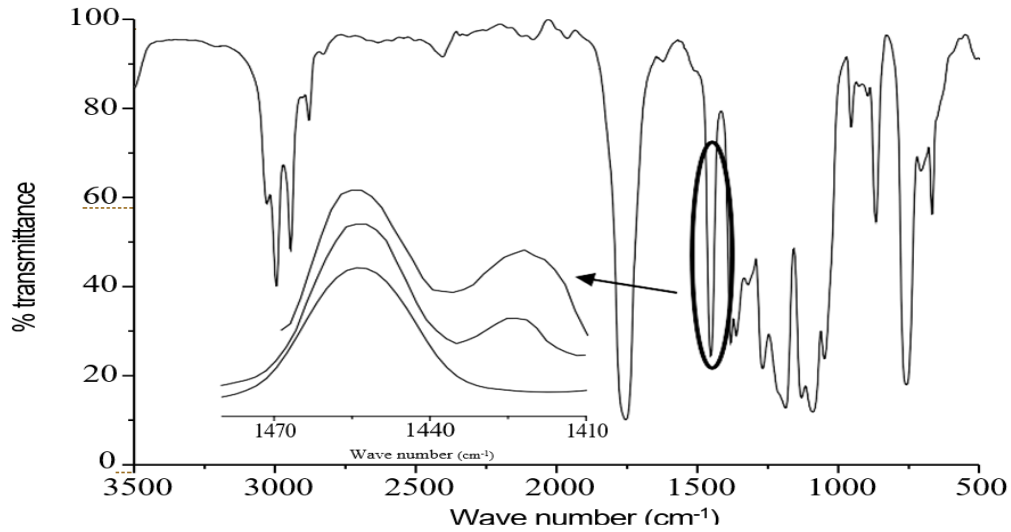


Figure 7. FTIR spectrum of the PLA (transmittance) and absorbance evolution as the GA ratio increased in the PLGA.

In the transmittance spectrum of PLA, there are two peaks near 3000 cm^{-1} (methyl-methylene region), one at 1745 cm^{-1} (carbonyl region), another at 1455 cm^{-1} ($\delta_{\text{asy}}\text{CH}_3$), and a group of peaks between 1050 and 1300 cm^{-1} (C-O bands that show the ester group is present). Also, in absorbance mode, a region of the spectrum surrounded by a circle can be seen and shown in a box for each PLGA spectrum with a different experimental LA/GA ratio. The asl CH_3 stretches are responsible for a shoulder in the lower spectrum, which is where the PLA is. This shoulder is around 1455 cm^{-1} . Also, you can see how a second shoulder starts to show up around 1425 cm^{-1} and gets stronger as the 1455 cm^{-1} signal goes down. The fact that this signal shows up means that GA ($\delta_{\text{asy}}\text{CH}_2$) is in the PLGA. What has been written about these signs is the same as what has been seen [8], as well as the bands at $2996,37\text{ cm}^{-1}$ and $2945,82\text{ cm}^{-1}$, which are attributed to $-\text{CH}_3$ C-H stretching. At $1187,45\text{ cm}^{-1}$, the absorption of ester C-O stretching is the most recognizable. The FTIR spectrum of PLA is in agreement with the described IR spectra in the literature (Singla, et al 2012) [23].

Figure 8 shows that T_g was also measured for the polymers as part of another study. All of the thermograms that were taken showed a T_g value, which suggests that a mostly random polymer was formed. None of the thermograms showed endotherms. melting, which showed that most polymers were mostly amorphous.

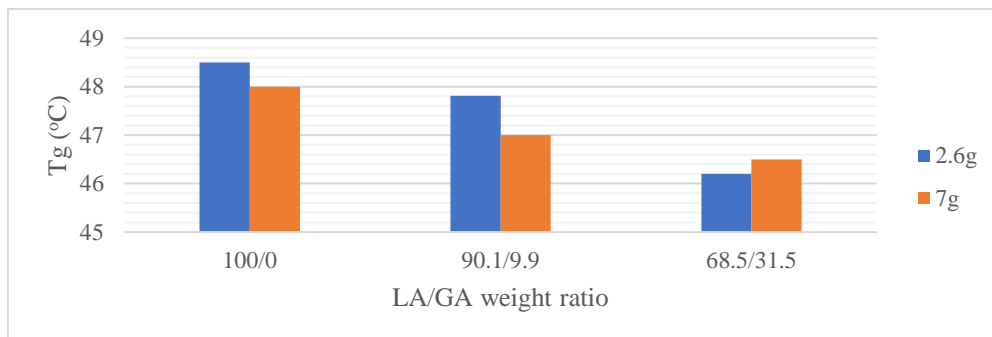


Figure 8. Evolution of the T_g as a function of the experimental LA/GA ratio in the polymer when using different amounts of dimers to be polymerized.

The T_g value goes down as the amount of GA in the polymer goes up, both when using 2.6 and 7 g of total dimers in the polymerization, which is what has been reported in the literature [9, 10]. This is a common trait

of random copolymers, where the T_g value is between the values of the pure components (T_g PLA = 50–55°C, T_g PGA = 36°C), and where the displacement of the T_g value depends a lot on the weight fractions of the monomers used. Once each polymer had been described, the emulsion-evaporation method was used to make PLA and PLGA nanoparticles with sizes of 90.1/9.9 and 68.5/31.5. As a stabilizer, 15g/L of PVA of different WAMW (alone or mixed with SDS) was used to study the effects of electrosteric (PVA-SDS mix) and steric (PVA) stabilizers both during processing and storage. It's important to note that the polymers used to make the nanoparticles were made from the 7 g of dimers (LA and GA), which were also used to make the films for each type of particle

3.2. Characterization of PLA and PLGA emulsions

The D_p values of the nanodroplets in freshly made emulsions of PLA and PLGA are 90.1/9.9 and 68.5/31.5, depending on the WAMW of the PVA and the type of stabilizer (steric or electrosteric). When the PVA-SDS mixture or PVA alone are used as colloidal stabilizers, they both help polymeric nanoparticles with a higher D_p to form. This behaviour matches what has been reported in the literature [11], which is that the viscosity of the aqueous PVA solution goes up as the value of WAMW goes up. This is because when the nanoparticles are made through emulsion-evaporation, the shear stress caused by the dispersion agent (ultraturrax) goes down, which means that the D_p values are higher. Also, show that the D_p values when the PVA-SDS mixture was used instead of PVA were lower. This is because the electrosteric stabilizer in the PVA-SDS mixture made the forces of attraction and repulsion between the particles stronger. This kept the particles from sticking together, so the D_p values were lower. When PVA was used, however, the only type of stabilization that was achieved was steric stabilization, which led to a lower force of repulsion between particles and, as a result, a higher D_p because the particles clumped together. As the amount of GA in the PLGA goes up, D_p goes up, which can be explained by the fact that the surfactants are less stable.

The CPD of PLA and PLGA emulsions (90.1/9.9 and 68.5/31.5) depends on the WAMW value of the PVA and the type of stabilization (steric or electrosteric). From the experiment results, it can be seen that the CPD value was very close for both the PVA-SDS mixture and PVA, no matter what the PVA WAMW value was or what kind of stabilization was used. In all cases, the value in the experimental CPD was around 0.69%, which is very close to the theoretical value of 0.75%. This suggests that it is possible to make colloiddally stable emulsions by changing both the WAMW value of the PVA and the type of stabilizer (steric or electrosteric). Once the characterization in D_p and CPD was done, the 18 emulsions were kept at 37.4°C for 28 days. This was done so that different things could be studied, such as the colloidal stability of the nanoparticles through changes in D_p , the degradation of the polymer through hydrolysis by measuring the molecular weights and pH in the emulsions due to the formation of acid groups in the polymer chains through hydrolysis. Here are the results and how they were analyzed and talked about. First, everything that has to do with how D_p changes when PVA with WAMW=13-23 KDa is used alone or with SDS is raised. Then, these results are compared with those from using PVA with a higher WAMW (31–50 and 85–124 KDa) to see where the colloidal stability is the same and where it is different. This same pattern of analysis and discussion is used to look at the change in molecular weight (WAMW and NAMW) and pH in the emulsions. The lowest WAMW PVA is looked at first, then those with the highest WAMW.

3.3. Characterization of PLA and PLAGA emulsions during storage

3.3.1. Evolution of the particle diameter (D_p)

Figure 9 shows how the D_p of PLA and PLGA nanoparticles (90.1/9.9 and 68.5/31.5) changes depending on the type of stabilizer (PVA-SDS or PVA mix). In all three cases, the D_p values were mostly lower when the PVA-SDS mix was used instead of PVA alone. This behavior supports the formation of the electrosteric stabilizer when a mixture of surfactants is used. This means that the nanoparticles were less likely to stick together than when PVA was used, which led to lower D_p values. On the first day of storage, the second type of stabilizer (PVA-SDS or a mixture of PVA and PVA) causes a big jump in D_p . Notice that the D_p values

were usually lower when using the PVA-SDS mixture than when using PVA alone. In all three cases, this behavior supports the formation of the electrosteric stabilizer when using a mixture of surfactants. This is because the nanoparticles repelled each other more when using the mixture of surfactants than when using PVA alone, which led to fewer clumping events and lower Dp values. Also, the Dp goes up a lot on the first day of storage (the second point in the graphs), but not as much with PLA. After that, the Dp stays pretty much the same, but the increase is more noticeable when there is more GA in the polymer. Because the number of GA groups on the surface of the nanoparticles increased, which are more water-loving than LA groups, the stabilizer (PVA) decreased in this way. its stabilizing ability. Also, it can't be ruled out that the increase in Dp is due to the nanoparticles getting bigger because there was more GA in the polymer chains, which makes them more water-loving than LA. This allowed more water to get into the nanoparticles. due to water getting into the polyester chains, which makes Dp values go up. Dp is also going down, and this is more noticeable when the amount of GA goes up in PLGA.

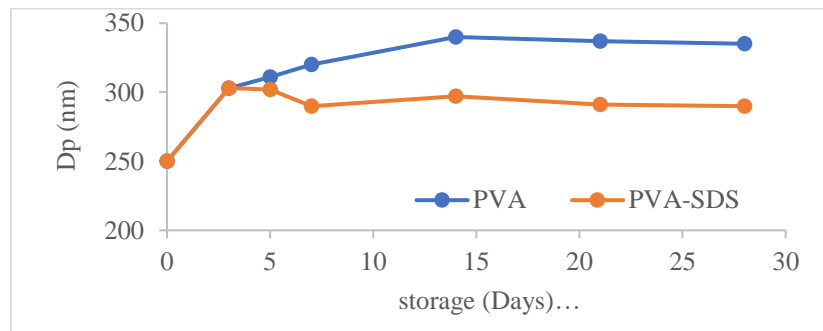


Figure 9. Dp evolution of PLA and PLGA nanoparticles (90.1/9.9 and 68.5/31.5) stored at 37.4°C using a PVA Mw= 13-23 KDa and a concentration of 15 g/L (alone or mixed with SDS)

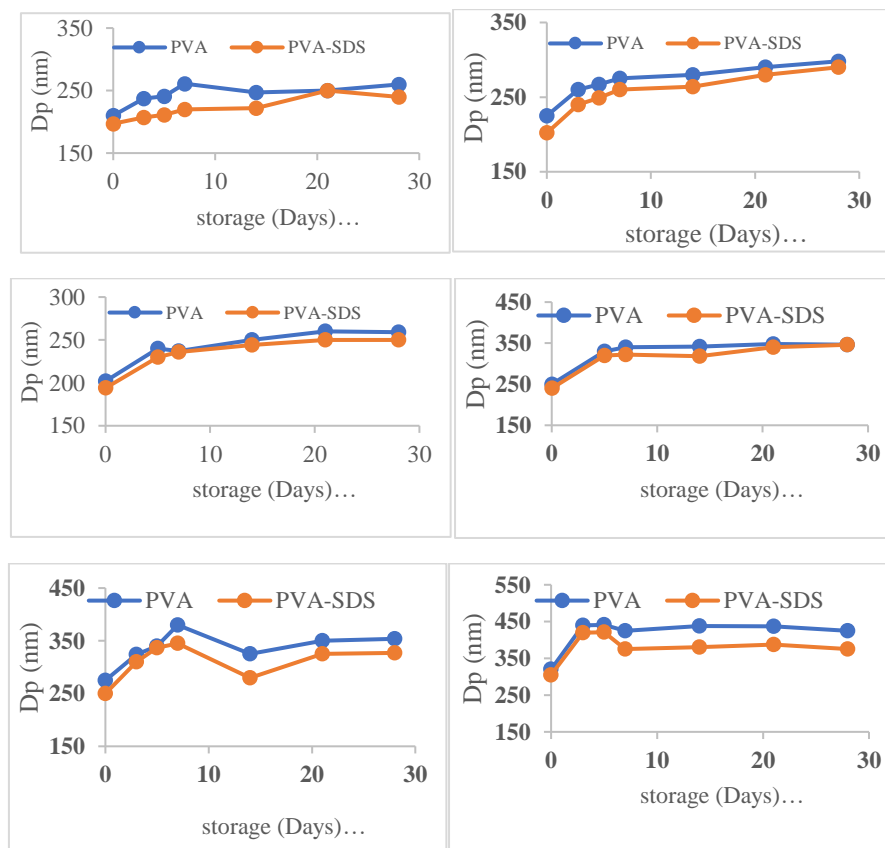


Figure 10: Dp evolution of PLA and PLGA nanoparticles (90.1/9.9 and 68.5/31.5) stored at 37.4°C using different Mw of the stabilizer: (A) PVA= 31-50 KDa and (B) PVA= 85-124 KDa. The PVA concentration is constant at 15 g/L (alone or mixed with SDS)

Also, if you look at Figure 10 and compare these results with those from using PVA with a higher Mw (alone or with SDS), you can see that the emulsions behaved very similarly in terms of colloidal stability at 37.4°C. This is because the formation of the electrosteric stabilizer in the mixture of PVA and SDS made the polymeric nanoparticles more stable than when using PVA alone. Also, Dp went up a lot on the first day of storage, which, based on what we've talked about so far, could be due to less PVA and PVA-SDS sticking to the surface and more water moving towards the surface through diffusion. Because there was more GA in the PLGA, they were more water-loving and could be found in the interior. Note that some of the emulsions shown in Figures 8 and 19 were also kept at room temperature (□25°C) to see how Dp changed over time. In this way, it was found that using a mixture of PVA and SDS stabilized the polymeric nanoparticles better than using PVA alone. Also, the increase in Dp on the first day of storage was less noticeable than when 37.4°C was used. This last behavior could be caused by several things, such as a lower temperature on the surface of the nanoparticles, which makes it easier for PVA (alone or mixed with SDS) to stick to the surface and prevent coagulation, and/or less water moving into the nanoparticles' interior because the polyester-type chains are less likely to break down, which makes them less likely to swell.

3.3.2. Evolution of molecular weight (WAMW and NAMW)

The molecular weights of PLA and PLGA nanoparticles (90.1/9.9 and 68.5/31.5) using PVA of WAMW= 13-23 KDa as a stabilizer (alone or mixed with SDS) decreased on the first day of storage, both when using the PVA-SDS mixture and PVA alone. After that, it acts differently depending on how the polymer is made. The drop in WAMW or NAMW, which was more noticeable when PLGA was used (90.1/9.9 and 68.5/31.5), can be explained by the fact that the polymeric chains on the surfaces of the nanoparticles were more attracted to water. Since they were more attracted to water than the same chains in PLA, they broke down faster, which led to more chain breaks and lower WAMW or NAMW values [14]. Also, it has been said that the molecular weight of the polymer also affects the degree of hydrolysis of the polymer chains. When the polymer chains are small, water can get to the groups that are easily broken down by water more quickly, so hydrolysis happens faster [13]. So, the fact that PLGA nanoparticles had lower WAMW or NAMW values at zero storage time than PLA nanoparticles may have had something to do with the drop in values.

On the other hand, the fact that the WAMW or NAMW of PLGA (68.5/31.5) was not lower when the PVA-SDS mixture was used as an electrosteric stabilizer instead of PVA as a steric stabilizer shows that SDS did not affect hydrolysis. The ratio of PVA to SDS has nothing to do with how many GA units are on the surface. If the PVA had been partially desorbed when it was mixed with SDS, the polymer chains that made up the nanoparticles would have been hydrolyzed more than when PVA was used alone. This is because the nanoparticles would have had more contact with the water. However, the results showed that this did not happen.

Concerning how the molecular weights changed over time and, in particular, what was seen in WAMW after the first day of storage, it can be seen that the decrease was stronger when there was more GA in the polymer. This behavior backs up what was said before, that the main causes of the decrease in WAMW due to the hydrolysis of the polymer chains were the amount of GA in the polymer (because it makes the nanoparticles more water-loving) and its value in WAMW, with the type of stabilizer (steric: PVA, or electrosteric: PVA-SDS) not making a difference because of the high concentration (15 g/L).

The development of WAMW and NAMW in PLA and PLGA nanoparticles (90.1/9.9 and 68.5/31.5) when using higher WAMW of PVA as a stabilizer (alone or mixed with SDS) during storage were compared with earlier findings to find similarities and differences, and a discussion was held based on these findings. Regarding the similarities, the following points could be made. The first one was based on the development of WAMW or NAMW, where there was a drop in values on the first day of storage (mostly higher because there was more GA in the polymer), followed by a small drop that didn't change as the storage time went on. This is what happened when WAMW= 85-124 KDa (alone or with SDS) and PVA with WAMW= 31-50 KDa were used.

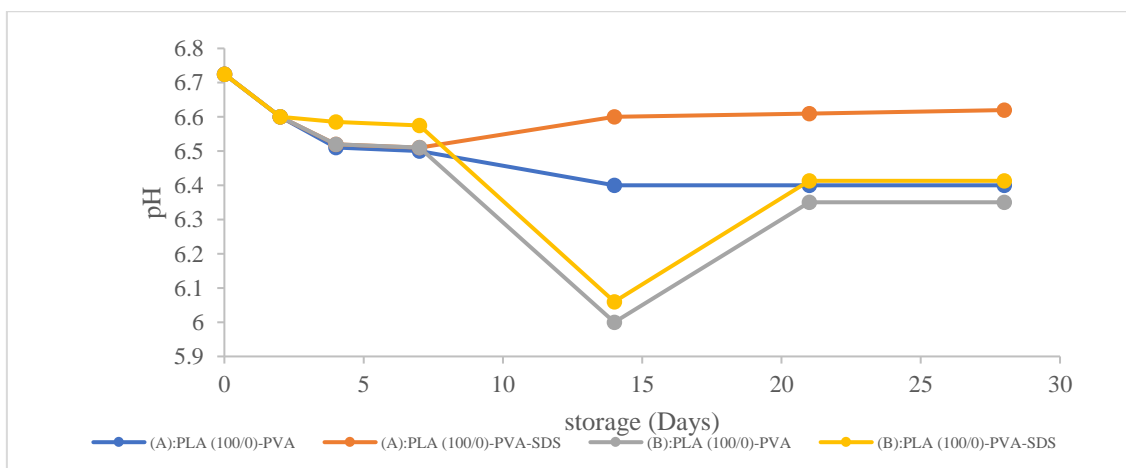
Also, it was not possible to tell how much the type of stabilization (steric or electrosteric) affected the changes in the molecular weights of the nanoparticles. This suggests that there wasn't much difference between the two stabilizers in how their molecular weight changed when using the PVA-SDS mixture. This is because neither the WAMW nor the NAMW changed clearly over time. It should be noted that very similar behavior was seen when PVA with a WAMW of 85–124 kDa was used as a stabilizer (right-hand column). When mixed with SDS, the nanoparticles had WAMW or NAMW values that were very close to those measured when only PVA was used as a stabilizer, leading to the same conclusion.

On the other hand, the next few paragraphs talk about how the results can be different. In this case, the analysis was done while keeping the same amount of GA in the polymeric nanoparticles and taking into account the difference in the WAMW values of the PVA. As the WAMW value of the PVA stabilizer grew (either on its own or when it was mixed with SDS), it was clear that the molecular weights of PLA were getting smaller. Taking into account what Olayo et al. [12] found, who used measurements of interfacial tension to find that the PVA WAMW played a big part in its adsorption at the water-organic phase interface, this behaviour makes sense. They found that PVA molecules with a higher WAMW were less effective (protected against less) than those with a lower WAMW. So, the GA groups take the chance to move to the particle surfaces, which makes the hydrolysis process go faster and decreases the WAMW. In both cases, most of them were near the ends of the chain, near the acetate groups, which are part of the chain that doesn't like water. Because of this, there was less coverage on the surface. When using PVA with a higher WAMW value (85-124 KDa), the WAMW or NAMW values of the PLA nanoparticles went down. This is because there was less covering on its surface, which made its surface area bigger. They came into more contact with water, which caused more hydrolysis and lower WAMW values.

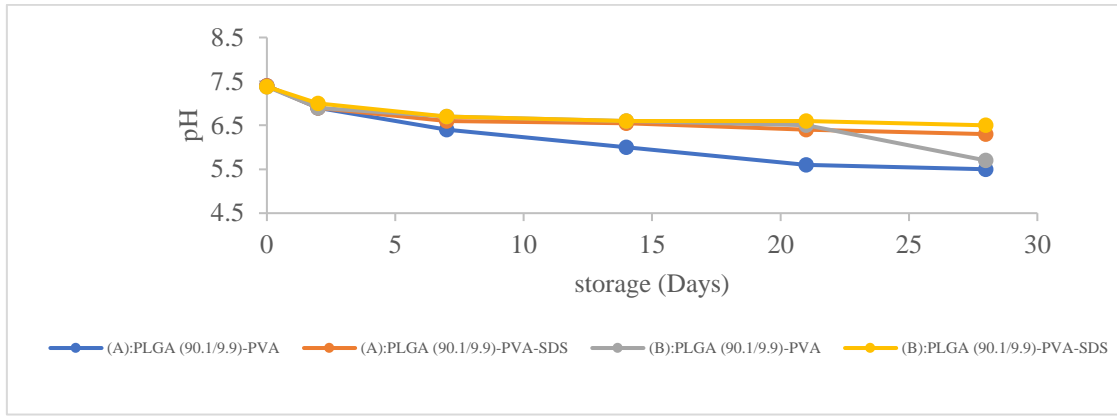
The behavior described in the last paragraph was also seen in PLGA nanoparticles, but the effect was stronger when more GA was used in the polymer, with a ratio of LA to GA of 68.5:31.5. In this case, the WAMW of the PVA (85-124 KDa) was affected by the amount of GA in the polymer, which made the polymer more hydrophilic, especially on the surface of the nanoparticles. This made the PVA less stable because its hydrophobic parts (acetate groups) didn't stick to the nanoparticles' surfaces as much [18].

3.3.3. Evolution of pH in emulsions

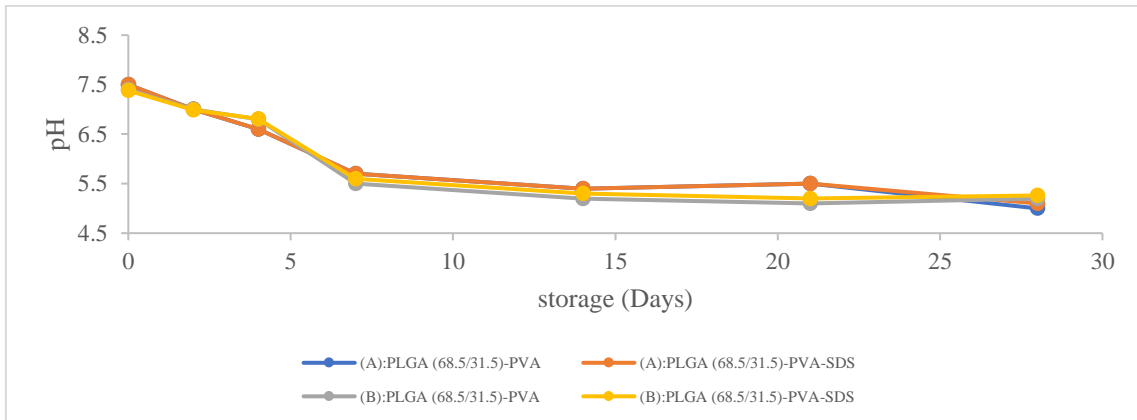
Figure 11 shows that keeping an eye on how the pH of the latexes changed indirectly confirmed what was said in the last paragraph about how water got into the nanoparticles when the PVA-SDS mixture was used. After a certain amount of time, the pH of the emulsions that had been stabilized with the PVA-SDS mixture was usually higher than those that had been stabilized with PVA. As the pH went up, the polymer chains were less likely to break down in the ester groups. This made the latexes and carboxylic acid groups less concentrated.



(a)



(b)



(d)

Figure 11: Evolution of pH in PLA and PLGA emulsions (90.1/9.9 and 68.5/31.5) stored at 37.4°C using different WAMW of the stabilizer: A) PVA= 31-50 KDa and B) PVA= 85-124 KDa. The PVA concentration was kept constant at 15 g/L (alone or mixed with SDS).

3.3.4. Characterization of PLA and PLAGA films during storage

When the films were made, they had a thickness of ≈ 0.28 mm. It can be seen that the decrease in molecular weights, especially WAMW, was more noticeable when there was more GA in the polymer. This is the same thing that was seen before with nanoparticles, which was thought to be because there was a more hydrophilic polymer. Because of this, the polymer was more attracted to water, which made it easier for water to break down the polyester groups because of their polarity.

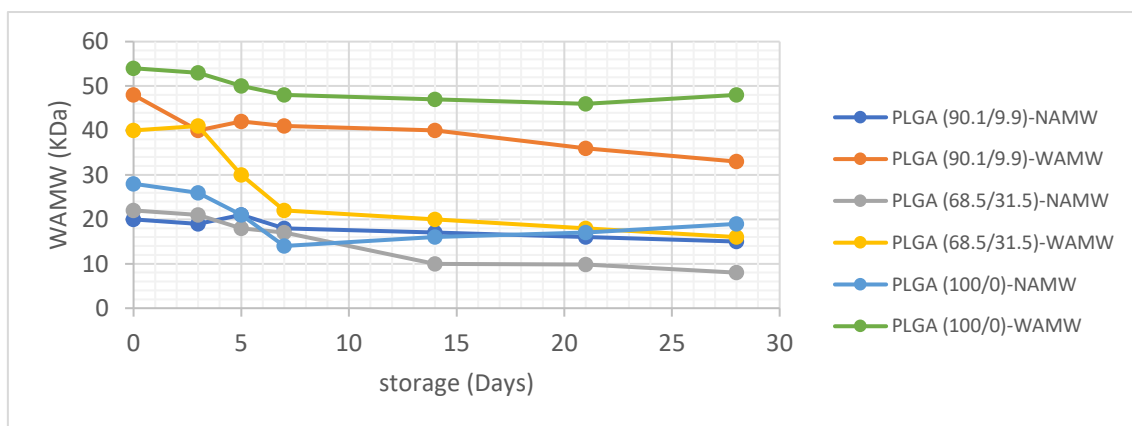


Figure 12: Evolution of WAMW and NAMW in PLA and PLGA films (90.1/9.9 and 68.5/31.5) immersed in an aqueous PBS buffer solution at 37.4°C for 28 days

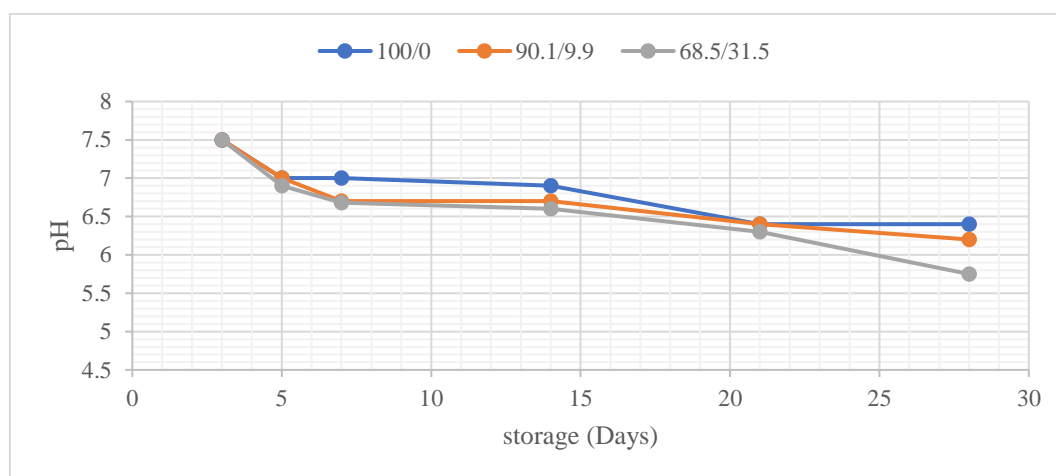


Figure 13. Evolution of the pH of the aqueous solutions containing the PLA and PLGA films (100/0; 90.1/9.9 and 68.5/31.5) during storage at 37.4°C

Finally, Figure 13 shows how the pH of the aqueous solutions with the PLA and PLGA films shown in Figure 12's right column changed over time. Notice how the pH drops quickly on the first day of storage in all three cases, no matter what kind of polymer was used, and then drops slowly but steadily for the rest of the storage. The drop in pH was the same as what was seen in the nanoparticles. It was a little stronger when there was more GA in the polymer (the arrow in the empty triangle). This behavior became more noticeable over time because there were more carboxylic acid groups and alcohols. This was shown by the decrease in WAMW or NAMW in Figure 11, which was caused by the partial hydrolysis of the ester groups.

4. Conclusions

Based on the results of this study, it was possible to make PLA and PLGA with very similar properties (LA/GA ratio, molecular weight, and T_g) by boosting the total amount of dimers from 2.6 to 7 g. Using the emulsion-evaporation method, it was possible to make colloiddally stable PLA and PLGA emulsions with a CPD of 0.75% w when using different MW of PVA at 15g/L (alone or mixed with SDS). In the same way, it was possible to confirm that the electrosteric stabilizer formed when PVA was mixed with SDS, causing D_p values to be lower than when PVA was used alone. These values depended on the molecular weight of PVA and the composition of the nanoparticles that formed. When stored, the mixture of PVA and SDS kept the particles together better than PVA alone. When mixed with SDS, these small but important differences in D_p helped us confirm that the electrosteric stabilizer was formed.

On the other hand, the type of stabilizer could be seen to affect how the molecular weights of the polymeric nanoparticles changed since the hydrolysis of the chains was mostly lower. This was shown by the fact that the pH of the emulsions was higher when PVA was used as a stabilizer. Using the solvent-casting method, PLA and PLGA films were finally made successfully. By increasing the amount of GA in the polymer, it was possible to see more hydrolysis of the polymer during storage. This was confirmed by watching how the pH changed in the aqueous solutions where the polymers were found, with the values getting lower as the amount of GA in the polymer went up.

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References

- [1] B.C. Simionescu, D. Ivanov, "Natural and synthetic polymers for designing composite materials," in Handbook of bioceramics and biocomposites, 2016, pp. 233-286.
- [2] M. Alvi, A. Yaqoob, K. Rehman, S.M. Shoaib, M.S. Akash, "PLGA-based nanoparticles for the treatment of cancer: current strategies and perspectives," AAPS Open, vol. 8, pp. 1-7, 2022.
- [3] V. Kumar, R.K. Prud'homme, "Nanoparticle stability: Processing pathways for solvent removal," Chemical Engineering Science, vol. 64, pp. 1358-1361, 2009, doi: 10.1016/j.ces.2008.11.017.
- [4] C. Vashisth, C.P. Whitby, D. Fornasiero, J. Ralston, "Interfacial displacement of nanoparticles by surfactant molecules in emulsions," Journal of Colloid and Interface Science, vol. 349, pp. 537-543, 2010, doi: 10.1016/j.jcis.2010.05.089.
- [5] Y. Wang, P. Li, T.D.T. Tran, J. Zhang, L. Kong, "Manufacturing techniques and surface engineering of polymer-based nanoparticles for targeted drug delivery to cancer," Nanomaterials, vol. 6, no. 2, p. 26, 2016, doi: 10.3390/nano6020026.
- [6] H.R. Kricheldorf, I. Kreiser-Saunders, C. Boettcher, "Polylactones: 31. Sn (II) octoate-initiated polymerization of L-lactide: a mechanistic study," Polymer, vol. 36, pp. 1253-1259, 1995, doi: 10.1016/0032-3861(95)93928-F.
- [7] B. Subramanian, C. Sanjeeviraja, M. Jayachandran, "Review on materials properties of Sn (S, Se) compound semiconductors useful for photoelectrochemical solar cells," Bulletin of Electrochemistry, vol. 18, pp. 349-366, 2002, <http://cecri.csircentral.net/id/eprint/1280>.
- [8] D. Stoilova, V. Koleva, V. Vassileva, "Infrared study of some synthetic phases of malachite (Cu₂(OH)₂CO₃)–hydrozincite (Zn₅(OH)₆(CO₃)₂) series," Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 58, pp. 2051-2059, 2002, doi: 10.1016/S1386-1425(01)00677-1.
- [9] N.R. Jadhav, V.L. Gaikwad, K.J. Nair, H.M. Kadam, "Glass transition temperature: Basics and application in pharmaceutical sector," Asian Journal of Pharmaceutics (AJP), vol. 3, 2009, doi: 10.22377/ajp.v3i2.246.
- [10] K. Nagata, H. Takase, "Effect of glass transition temperature of polymers on rheological behavior of alumina suspension and properties of alumina green sheets," Journal of the Ceramic Society of Japan, vol. 116, pp. 298-302, 2008, doi: 10.2109/jcersj2.116.298.
- [11] F. Qi, J. Wu, H. Li, G. Ma, "Recent research and development of PLGA/PLA microspheres/nanoparticles: A review in scientific and industrial aspects," Frontiers of Chemical Science and Engineering, vol. 13, pp. 14-27, 2019, <http://dx.doi.org/10.1007/s11705-018-1729-4>.
- [12] R. Olayo, E. García, B. García-Corichi, L. Sánchez-Vázquez, J. Alvarez, "Poly (vinyl alcohol) as a Stabilizer in the Suspension Polymerization of Styrene: The Effect of the Molecular Weight," Journal of Applied Polymer Science, vol. 67, pp. 71-77, 1998, doi: 10.1002/(SICI)1097-4628(19980103)67:1%3C71::AID-APP8%3E3.0.CO;2-L.
- [13] I. McCulloch and S.W. Shalaby (Eds.), "Tailored polymeric materials for controlled delivery systems," American Chemical Society, 1998, pp. 24, doi: 10.1021/bk-1998-0709.
- [14] Y. Zhang, S. Zale, L. Sawyer, H. Bernstein, "Effects of metal salts on poly (DL-lactide-co-glycolide) polymer hydrolysis," Journal of Biomedical Materials Research: An Official Journal of

- The Society for Biomaterials and The Japanese Society for Biomaterials, vol. 34, pp. 531-538, 1997, doi: 10.1002/(SICI)1097-4636(19970315)34:4%3C531::AID-JBM13%3E3.0.CO;2-F.
- [15] J.C. Ramirez, S.E. Flores-Villasenor, E. Vargas-Reyes, J. Herrera-Ordóñez, S. Torres-Rincón, R.D. Peralta-Rodríguez, "Preparation of PDLLA and PLGA nanoparticles stabilized with PVA and a PVA-SDS mixture: Studies on particle size, degradation, and drug release," *Journal of Drug Delivery Science and Technology*, vol. 60, p. 101907, 2020, doi: 10.1016/j.jddst.2020.101907.
- [16] D.T. Birnbaum, J.D. Kosmala, L. Brannon-Peppas, "Optimization of preparation techniques for poly (lactic acid-co-glycolic acid) nanoparticles," *Journal of Nanoparticle Research*, vol. 2, pp. 173-181, 2000, doi: 10.1023/A:1010038908767.
- [17] H.S. Yoo, T.G. Park, "Biodegradable polymeric micelles composed of doxorubicin conjugated PLGA-PEG block copolymer," *Journal of Controlled Release*, vol. 70, pp. 63-70, 2001, doi: 10.1016/S0168-3659(00)00339-4.
- [18] E. Sah, H. Sah, "Recent trends in preparation of poly (lactide-co-glycolide) nanoparticles by mixing polymeric organic solution with antisolvent," *Journal of Nanomaterials*, vol. 16, p. 61, 2015, doi: 10.1155/2015/794601.
- [19] J.P. Rao, K.E. Geckeler, "Polymer nanoparticles: Preparation techniques and size-control parameters," *Progress in Polymer Science*, vol. 36, pp. 887-913, 2011, doi: 10.1016/j.progpolymsci.2011.01.001.
- [20] M. G Nava-Arzaluz, E. Piñón-Segundo, A. Ganem-Rondero, D. Lechuga-Ballesteros, "Single emulsion-solvent evaporation technique and modifications for the preparation of pharmaceutical polymeric nanoparticles," *Recent Patents on Drug Delivery & Formulation*, vol. 6, pp. 209-223, 2012, doi: 10.2174/187221112802652633.
- [21] S. Felz, P. Vermeulen, M.C. van Loosdrecht, Y.M. Lin, "Chemical characterization methods for the analysis of structural extracellular polymeric substances (EPS)," *Water Research*, vol. 157, pp. 201-208, 2019, doi: 10.1016/j.watres.2019.03.068.
- [22] K. Shah, "Successful Development by Design of Experiments of a Gas Chromatography Method for Simultaneous Analysis of Residual Solvents of Classes 1 and 2," *Journal of Chromatographic Science*, 56, 2018, doi: 10.1093/chromsci/bmy026.
- [23] P. Singla, R. Mehta, D. Berek, S. Upadhyay, "Microwave Assisted Synthesis of Poly(Lactic acid) and its Characterization using Size Exclusion Chromatography," *Journal of Macromolecular Science Part A Pure and Applied Chemistry*, A49, 2012, doi: 10.1080/10601325.2012.722858.
- [24] S.S. Anaz, A.M. Tayyeh, "Characteristics of trauma patients with multidrug-resistant bacteria from an epidemiological, clinical, and microbiological perspective," *Tamjeed Journal of Healthcare Engineering and Science Technology*, vol. 1, no. 2, pp. 1-19, 2023