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Storage Stability Study of Salicylate-based Poly(anhydride-esters)

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Abstract

Storage stability was evaluated on a biodegradable salicylate-based poly(anhydride-ester) to elucidate the effects of storage conditions over time. The hydrolytically labile polymer samples were stored in powdered form at five relevant storage temperatures (−12 °C, 4 °C, 27 °C, 37 °C, 50 °C) and monitored over four weeks for changes in color, glass transition temperature, molecular weight, and extent of hydrolysis. Samples stored at lower temperatures remained relatively constant with respect to bond hydrolysis and molecular weight. Whereas, samples stored at higher temperatures displayed significant hydrolysis. For hydrolytically degradable polymers, such as these poly(anhydride-esters), samples are best stored at low temperatures under an inert atmosphere.

Keywords

Polyanhydride; Stability; Degradation; Biodegradable; Hydrolysis

1. Introduction

Salicylate-based poly(anhydride-esters), such as poly[1,6-bis(*o*-carboxyphenoxy)hexanoate] (1, Figure 1), are polymers that have salicylic acid chemically incorporated into their backbone.[1–4] These salicylic acid-based poly(anhydride-esters) are both biodegradable and biocompatible.[1–6] They hydrolytically degrade over time to release free salicylic acid and the acid form of their linkers *via* a surface eroding, controlled release mechanism.[1–6] Previous studies determined the polymers' physicochemical properties, degradation rates, biocompatibilities, and potential biomedical applications.[1–12] To date, the ability to store the polymers as solids has not been investigated. The goal of this paper is to elucidate the influence of storage conditions on the polymer's stability.

Polymer samples were stored in scintillation vials at various storage conditions for four weeks. Storage temperatures were selected based upon relevant laboratory conditions as well as potential conditions experienced during shipping or sample preparation. An additional sample set was stored at room temperature under nitrogen and sealed with parafilm to assess polymer stability in a relatively moisture-free environment. Based upon

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previous polymer degradation and storage stability studies, Fourier transform infrared (FT-IR) spectroscopy, differential scanning calorimetry (DSC), and gel permeation chromatography (GPC) were utilized to monitor polymer degradation as a function of temperature and time.[13–27] In addition, all polymer samples were visually monitored for changes in coloration. When the labile anhydride bonds are hydrolyzed during degradation, free carboxylic acid chain ends are detected *via* FT-IR spectroscopy as a carbonyl stretch near 1700 cm⁻¹. [6,13,16] Further, anhydride bond cleavage decreases polymer chain length, leading to the formation of oligomers, which is directly monitored using GPC. The glass transition temperature may also be affected, as the polymer degrades into smaller molecules; this transition can be monitored by DSC. [24] Previously reported polyanhydride degradation studies noted decreases in molecular weights when polymers were stored in the refrigerator (4 °C) or near room temperature (21 °C). [14,17,23] The authors proposed that samples be stored at low temperatures and under nitrogen or an inert gas to preserve polymer properties upon storage. [14,17] Thus, the impact of storage under an inert atmosphere was also evaluated.

As polymer **1** is inherently biodegradable, exposure to moisture during storage will impact the polymers' physicochemical properties. [17] This study evaluates the various storage conditions to optimize storage methods and extend the polymers' shelf-lives, as needed.

2. Experimental

2.1 Sample Preparations

Poly[1,6-bis(*o*-carboxyphenoxy)hexanoate] (**1**) used for all samples was synthesized during one melt-condensation polymerization reaction and completely characterized by previously described methods.[1–4] The properties of the freshly prepared polymer are as follows: M_w : 16,600, PDI: 2.8, T_g : 44 °C, T_d : 285 °C. Prior to storage, polymer **1** was ground using a mortar and pestle to obtain particles that were 100–500 µm in diameter. Particle size was determined using U.S.A Standard Testing Sieves (ATM products). The sieves used were in compliance with ASTM E-11 specifications. [25] Powdered polymer **1** (~220 mg) was then added to 20 mL scintillation vials and sealed with caps only. Samples were stored at –12 °C, 4 °C, 27 °C, 37 °C, and 50 °C. An additional sample set was stored at room temperature (27 °C) under a nitrogen atmosphere and sealed with parafilm. All samples were prepared in triplicate.

All samples were monitored at weekly time points for changes in physicochemical properties as outlined in the Introduction. Specifically, changes in coloration, glass transition temperature, FT-IR stretch frequencies, and molecular weight were monitored over four weeks. Samples were all brought to room temperature prior to analysis.

2.2 Chemical Composition

Fourier transform infrared (FT-IR) spectra were obtained using a Thermo Nicolet/Avatar 360 FTIR spectrometer. Polymer samples (~ 15–20 mg) were solvent-cast onto NaCl plates using methylene chloride under air. Each spectrum was an average of 32 scans.

2.3 Thermal Analysis

Thermal analysis was performed using differential scanning calorimetry (DSC) to obtain glass transition temperatures (T_g). DSC was performed using a Thermal Advantage differential scanning calorimeter Q200 running on an IBM ThinkCentre computer equipped with Thermal Advantage Universal Analysis software for data collection and processing. To obtain glass transition temperatures, ~ 3–5 mg of sample was used. The sample was heated

under nitrogen from $-10\text{ }^{\circ}\text{C}$ to $200\text{ }^{\circ}\text{C}$ at a rate of $15\text{ }^{\circ}\text{C}/\text{min}$. A minimum of two heating/cooling cycles was used. The T_g was calculated as half C_p extrapolated.

2.4 Molecular Weight

Gel permeation chromatography (GPC) was used for determining molecular weights of polymers. The Perkin-Elmer LC system was equipped with a Series 200 refractive index detector, a Series 200 pump, ISS 200 autosampler, and a Jordi DVB mixed-bed GPC column ($7.8 \times 300\text{ mm}$, Alltech, Deerfield, IL). A Dell OptiPlex GX110 computer with Perkin-Elmer TurboChrom 4 software was used for collection and processing of the data and for the automation of the GPC analyses using a Perkin-Elmer Nelson 900 Series Interface and Perkin-Elmer Nelson 600 Series Link. Molecular weights (M_w) were determined with respect to polystyrene standards (Polymer Source Inc., Dorval, Canada). Samples were prepared by dissolving $\sim 15\text{--}20\text{ mg}$ of polymer in 1 mL of methylene chloride. As the solution was added to GPC vials, it was filtered using a $0.45\text{ }\mu\text{m}$ syringe filters. Samples were eluted at a rate of $1\text{ mL}/\text{min}$ and the total run time was 30 minutes.

3. Results and Discussion

3.1 Study Design and Rationale

Samples were stored at five different temperatures: freezer ($-12\text{ }^{\circ}\text{C}$), refrigerator ($4\text{ }^{\circ}\text{C}$), room temperature ($27\text{ }^{\circ}\text{C}$), physiological temperature ($37\text{ }^{\circ}\text{C}$), and an elevated temperature ($50\text{ }^{\circ}\text{C}$). An additional sample set was stored at room temperature ($27\text{ }^{\circ}\text{C}$) under a nitrogen atmosphere. The lower temperatures represent typical temperatures of laboratory refrigerators and freezers. Samples were also stored at room temperature, as it is one of the most common and least expensive storage methods. Samples stored at room temperature under nitrogen were sealed with parafilm to determine polymer stability in a relatively moisture-free environment. Samples were evaluated at elevated temperatures to mimic the range of exposure during shipping or sample preparation. Polymer **1** was synthesized from one batch of a melt-condensation polymerization reaction to ensure continuity between polymer samples.

Polymer **1** was ground using a mortar and pestle to obtain powdered samples. Polyanhydrides are known to be predominantly surface-eroding, where water penetration into the bulk of the polymer is very limited, allowing the erosion to remain mostly at the polymer-water interface.[6,26–28] As the polymer degrades, the rate of bioactive (*i.e.*, salicylic acid) released is controlled depending on the material geometry and surface area. [6,26–28] Powdered polymer samples were utilized for this study and expected to degrade relatively fast compared with other formats (e.g., disks or films) because of the higher surface area.[6,26,28] This approach enabled an accelerated storage stability study to be conducted over the course of four weeks as opposed to monitoring properties over the course of several months or years.

FT-IR spectroscopy, DSC, and GPC were utilized to monitor changes in polymeric properties. Notably, nuclear magnetic resonance (NMR) spectroscopy was not used to monitor polymer degradation. Previous studies illustrated that salicylate-based polymers synthesized by melt-condensation polymerization can undergo thermal rearrangements such as ester-ester or ester-anhydride exchanges. [17] These rearrangements lead to peak broadening in ^1H -NMR spectra of the starting polymer. [17] Considering that the hydrogen peak frequencies for the diacid and monomer are similar to the polymer, peak assignments and quantification is not viable. Thermal rearrangements also lead to an increased number of carbon peaks in ^{13}C -NMR spectra, which would make it difficult to discern with certainty

which peaks belonged to the polymer and which belonged to potential degradation products. [17]

3.2 Visual Changes: Color and Texture

Polymers were monitored for changes in color as these changes often indicate polymer degradation.[29] Specifically, color change can indicate the loss of specific functional groups or changes in glass transition.[29,30] In this study, all polymer powders were initially off-white in color. Samples stored at -12°C and 4°C , retained their original off-white coloration throughout the experiment, indicating the polymer had not significantly degraded (Figure 2A).

Samples stored at room temperature retained their original color until the third week, when the polymer samples became a light brown, tacky solid (data not shown). The light brown color and apparent texture in both cases were similar to that of the starting monomer, which may indicate that the polymer is degrading into oligomers.

By week 3 at higher storage temperatures (37°C and 50°C), the samples began to flow and coated the bottom of the storage vials. This change likely indicates extensive bond hydrolysis, leading to a decrease in glass transition temperature as shown in the next section. In addition, samples turned dark brown (Figure 2B).

3.3 Changes in Glass Transition Temperature

Samples were evaluated for changes in glass transition temperature (T_g), as a decrease in T_g is another indication of polymer degradation.[15,22,29] The average T_g changes over the four week study is illustrated in Figure 3. Polymer samples stored at lower temperatures (-12°C and 4°C) exhibited minimal change in T_g whereas samples stored at elevated temperatures displayed dramatic changes. Notably, samples stored under nitrogen displayed a relatively smaller decrease in glass transition temperature than the samples stored under air. This data supports the expectation that lowered exposure to moisture reduces polymer hydrolytic degradation. [8]

By DSC, only one distinct glass transition temperature was observed; no melting points were noted. Based upon these results, the degradation products from solid state storage are mostly oligomers. Any small molecule degradation products (e.g., salicylic acid), if present, were not in high enough concentrations to be detected.

3.4 Changes in FT-IR Spectra

Samples were also monitored at weekly time points *via* FT-IR spectroscopy. When polymers undergo hydrolytic degradation, the anhydride carbonyl stretches around 1800 cm^{-1} decrease, and the carbonyl stretches for the carboxylic acid around 1700 cm^{-1} increase. [6,13,16] For samples stored at -12°C and 4°C , the carboxylic acid carbonyl stretches were either very weak or not present. For samples stored at room temperature, the anhydride bond stretches were significantly weakened and the carboxylic acid stretch was more prominent by week 4. For the samples stored at the elevated temperatures, the carboxylic acid carbonyl stretch was strong and the anhydride carbonyl stretch was weakened.

Figure 4 displays the FT-IR spectrum of the starting polymer and representative spectra for sample sets stored at -12°C , 27°C , and 50°C after four weeks. As the storage temperature increases, the anhydride bond is less stable and hydrolyzes into the carboxylic acid. As the anhydride bonds hydrolyze, polymer chain ends (*i.e.*, carboxylic acids) are exposed as well as carboxylic acid functional groups of the degradation products.

3.5 Changes in Molecular Weight

Molecular weight data was obtained at each time point to directly monitor relative polymer size. The average molecular weight over the four week study is illustrated in Figure 5. Although polymer degradation was noted in all samples, the samples stored at elevated temperatures (37 °C and 50 °C) experienced a more dramatic decrease in molecular weight than samples stored at lower temperatures (−12 °C and 4 °C). Samples stored under nitrogen exhibited slightly less polymer degradation as noted by relatively higher molecular weight than the corresponding samples stored at room temperature not under nitrogen. Despite samples being stored under nitrogen had a lower average molecular weight at the week one time point than the samples stored just at room temperature, the overall difference in polymer degradation between weeks 1 and 4 was less for the samples stored under nitrogen. This data indicates that storing the polymer sample under an inert atmosphere stabilizes the polymers against hydrolytic degradation.

Studies previously performed by Domb and Langer corroborate this data; as the storage temperature of polyanhydrides decreased, the extent of polymer degradation decreased. [14,17] Further, the entrapment of moisture upon storage and inter- and intra-anhydride rearrangements can result in polymer degradation and a corresponding decrease in polymer molecular weight. [14,17] It is possible that the degradation observed was catalyzed by exposed carboxylate chain ends present in the polymer sample. [31] The carboxylate moieties are introduced by hydrolysis of anhydride bonds due to the entrapment of moisture upon storage or due to small molecular weight impurities (e.g., diacid or monomer) that were not removed following the re-precipitation of the polymer from dichloromethane into diethyl ether. These functional groups may accelerate the hydrolysis of the polymer. [31] In previous work, these polymers were observed to undergo more rapid degradation under basic conditions and relatively stabilize in a more acid environment. [31] Thus, our synthetic protocol requires washing the final product with acidic water; this step contrasts with protocols for polyesters, which are known to auto-catalytically hydrolyze with the carboxylate chain ends. It is likely that a combination of storage temperatures, moisture content, anhydride rearrangements, and presence of exposed carboxylate moieties may be the result of the polymer degradation observed. [14,17]

4. Conclusions

A four-week storage stability study was conducted for the biodegradable poly(anhydride-ester), **1**. Samples stored at lower temperatures retained most of their physiochemical properties over the course of the study. At elevated storage temperatures, polymer degradation increased. At room temperature, samples stored under nitrogen exhibited fewer physicochemical changes than samples stored in air.

Based on the results, polymer **1** is best stored in the laboratory under an inert atmosphere at low temperatures, either in a temperature-controlled refrigerator or freezer. However, sample storage in moisture-free packaging is likely the optimal approach to prevent polymer degradation.

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poly[1,6-bis(*o*-carboxyphenoxy)hexanoate]

Figure 1.

Chemical structure of polymer **1**, poly[1,6-bis(*o*-carboxyphenoxy)hexanoate].

Figure 2. Representative images of samples stored at temperature extremes (A) -12 °C and (B) 50 °C after 0, 1, and 4 weeks of storage. Images depict polymer samples in scintillation vials after removal from their respective storage conditions.

Figure 3. Change in sample glass transition temperature over four weeks as a function of storage condition.

Figure 4.
Comparison of IR spectrum of fresh polymer and representative stored samples after four weeks.

Figure 5.
Change in molecular weight of polymer 1 as an effect of storage temperature over four weeks.