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In Vitro Degradation of an Aromatic Polyanhydride with Enhanced Thermal Properties

Sabrina S. Snyder[a], **Theodore J. Anastasiou**[b], and **Kathryn E. Uhrich**[a,b,*]

[a]Department of Biomedical Engineering, Rutgers University, 610 Taylor Road, Piscataway, NJ 08854

[b]Department of Chemistry & Chemical Biology, Rutgers University, 610 Taylor Road, Piscataway, NJ 08854

Abstract

Polyanhydrides have been studied as a drug delivery vehicles due to their surface-eroding behavior which results in zero-order release. However, many polyanhyrides have thermal and solubility properties that make them difficult to formulate for these applications. Poly[α,α′-bis(*ortho*-carboxyphenoxy)-*para*-xylene] (*o*CPX) is an aromatic polyanhydride that has thermal and solubility properties enabling facile processing. The polymer's *in vitro* degradation profile exhibited an induction period up to 10 days in which degradation product concentration in the media was minimal, followed by a period of stable release of the biocompatible degradation product. Scanning electron microscope images and molecular weight changes of the polymer matrices confirm that this polymer is primarily surface-eroding. The combination of thermal properties, solubility, polymer degradation time, and erosion mechanism indicate that poly(*o*CPX) is be a suitable matrix candidate for extended, controlled drug delivery.

Keywords

biocompatible; biodegradable; polyanhydride; aromatic polyanhydride; surface erosion

1. Introduction

Polyanhydrides have successfully been utilized as drug delivery systems due to their excellent biocompatibility, controlled erosion, and ability to encapsulate sensitive bioactives.[1-3] Polyanhydrides as matrices for delivering admixed drugs has been widely studied because polyanhydrides typically exhibit surface erosion, thereby resulting in more stable drug release than other classes of polymers, such as polyesters, which typically exhibit bulk degradation.[1, 4]

*Corresponding author: Dr. Kathryn E. Uhrich, Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey 610 Taylor Road, Piscataway, NJ 08854-8087, USA, keuhrich@rutgers.edu, Tel.: (732) 445-0361, Fax: (732) 445-7036.

The two most commonly studied polyanhydrides studied are the linear, aliphatic poly(sebacic acid) (SA) and the aromatic poly[1,3-bis-(*para*-carboxyphenoxy)propane] (*p*CPP).[1-3] Although *p*CPP fully degrades over three years, it is insoluble in common organic solvents and has high glass transition (T_g) and melting (T_m) temperatures, leading to difficulty in device fabrication.[1, 3] Although SA has greater solvent solubility and a lower T_g and T_m, it degrades completely in a matter of days.[5]

A copolymer of *p*CPP:SA (20:80) has been used in an implantable device, Gliadel®, to control the delivery of chemotherapeutic drugs to treat brain cancer.[6] The combination of *p*CPP and SA is used to adjust the solubility, thermal properties, and degradation rates of the polymers.[1] However, this technique does not provide polymers with optimal properties as *p*CPP:SA copolymers with a high SA content still degrade within a few days, and copolymers with a high *p*CPP content have T_g values below physiologic temperature.[7] These low temperatures are advantageous for processing, but can be a problem *in vivo* as the materials deform following implantation. For drug delivery devices, the inability to retain a defined shape over time does not allow for accurate calculations of drug delivery rates.[8] Hence, polymers with T_g values above physiological temperature are desired for their ability to retain their mechanical integrity *in vivo*.

An aromatic polyanhydride, poly[α,α′-bis(*ortho*-carboxyphenoxy)-*para*-xylene] (*o*CPX), was previously synthesized by melt-condensation polymerization methods.[9] This polymer is being evaluated as a biomaterial, specifically as a drug delivery matrix, because it is soluble in organic solvents and the T_g value falls within a practical range for thermal processibility (∼68°C), while still being above physiological temperatures.[9] It is these solubility and thermal characteristics that permit the facile fabrication of microspheres, fibers, or films, and useful in biomedical applications.

As *o*CPX is an aromatic polyanhydride, it is expected to have a slower degradation rate and longer degradation profile than linear aliphatic polyanhydrides.[3] This study reports the changes that occur during hydrolytic degradation of *o*CPX. The polymers and their degradation products were evaluated via several methods: mass loss of polymer matrices during *in vitro* degradation, high pressure liquid chromatography (HPLC) identification of polymer degradation products, UV analysis of polymer degradation product concentration, and scanning electron microscopy (SEM) analysis of the degraded polymer disks. The polymer was also tested for stability toward common sterilization techniques, and the polymer degradation products tested for cytotoxicity.

2. Materials and methods

2.1. Materials

Acetic anhydride used to synthesize the polymer was purchased from Fischer (Fair Lawn, NJ). All other chemicals and reagents were purchased from Sigma-Aldrich (Milwaukee, WI) and used as received.

2.2 Polymer synthesis and characterization

The polymer was synthesized by methods previously reported (summarized in Figure 1 below),[9] resulting in a polymer with a weight average molecular weight = 5600 Da, a polydispersity = 2.1, and a T_g = 68 °C. The melting temperature (T_m) previously reported[9] was found to be due to impurities, as the poly(*o*CPX) used in this study did not exhibit a T_m The originally reported T_m = 114 °C for poly(*o*CPX) is near the *o*CPX methyl ester precursor T_m = 120-122 °C.

2.3. Polymer in vitro degradation studies

Polymer disks were prepared by compressing finely ground polymer (∼150 mg) into disks (13 mm diameter × ∼1 mm thick) using a hydraulic press (Carver Model M, Garfield, NJ). A force of 10,000 psi was applied for 10 minutes at room temperature. Degradation media consisted of phosphate buffered saline (0.01 M PBS contains 0.0027 M potassium chloride and 0.137 M sodium chloride) at pH 7.4 prepared from Sigma-Aldrich PBS tablets. Hydrolytic degradation was conducted in triplicate by placing polymer disks in 10 mL PBS in glass scintillation vials. Samples were placed in an incubator shaker (37°C, 60 rpm). Every 5 days, the media was replaced with fresh PBS. Collected media was analyzed for degradation products and their concentrations. After 90 days, the remains of the polymer disks were collected, rinsed with deionized water, dried under vacuum, and analyzed for mass loss and molecular weight. The cutoff time of 90 days was chosen because by that time point the polymer had shown constant release rates of the monitored degradation product, such that further degradation could be determined from earlier trends.

2.4. Degradation product analysis via HPLC

To determine what degradation products were being released into solution and if UV spectroscopy would be a viable method to rapidly quantify degradation product concentration, analysis of polymer degradation media was performed on the spent degradation media. One sample at each time point was analyzed using a Waters system (Milford, MA) consisting of a 2695 Separations Module with a 2487 dual λ absorbance detector. Empower software was used for data collection and processing. Samples were resolved at room temperature on an Xterra reversed phase RP18 5μm column (4.6 × 150 mm). UV detection of eluted components was performed at λ = 278 nm. Mobile phase consisted of acetonitrile:water (95:5) with ∼1% phosphoric acid, adjusted to pH =2.5 with hydrochloric acid at a flow rate of 1 mL/min. Samples were filtered through 0.22 μm PVDF filters (Fisher) prior to injection.

2.5. Diacid concentration determination via UV absorbance

Concentration of *o*CPX diacid in spent media was analyzed by UV spectrophotometry using a Perkin Elmer Lambda XLS spectrophotometer (Waltham, MA) at λ = 278 nm to specifically monitor diacid release. Data were calculated against a calibration curve of absorbance values from standard solutions of known diacid concentrations in PBS. The UV absorbance values of the degradation media were then fit to the calibration curve to determine diacid concentration in the samples. A saturated diacid solution in PBS was also

made and evaluated by UV absorbance to determine if the degradation media samples had reached saturation.

2.6. Molecular weight analysis

Molecular weight analyses of the polymers samples were performed both before and after the 90 day *in vitro* degradation study. After 90 days, the remains of the polymer disks were dried in a vacuum desiccator overnight, ground with mortar and pestle, and the resulting powder was used for final molecular weight determination. Weight average molecular weights (M_w) and polydispersity indices (PDI) of the polymer disks were determined by gel permeation chromatography (GPC) on a Perkin-Elmer (Waltham, MA) LC system consisting of a Series 200 refractive index detector, a Series 200 pump, and an ISS 200 autosampler. A DEC Celebris 466 computer running PE TurboChrom 4 software was used for data collection and processing, and to automate the analysis via PE-Nelson 900 Interface and 600 Link. Samples were dissolved in dichloromethane and filtered through 0.45 μm PTFE syringe filters then resolved on a Jordi divinyl benzene mixed-bed GPC column (7.8 × 300 mm) at 1 mL/min. Molecular weights were calibrated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

2.7. Scanning electron microscopy

Polymer disks were prepared and subjected to 30 days *in vitro* degradation as described above. At day 30, the disks were rinsed with deionized water then dried under vacuum. Once dried, the disks were bisected using a steel blade. Images of the disks were obtained using an AMRAY-1830I microscope (SEMTech, North Billerica, MA) after coating the samples with Au/Pd using a sputter coater (SCD 004, Balzers Union, Koln, Germany).

2.8. Radiation exposure

BD Falcon 5 mL polystyrene round-bottom tubes (12× 75 mm; BD Bioscience Discovery Labware, Bedford, MA) were used to ship 6 samples of polymer powder (1 g each) to Sterile Process Technology (Johnson & Johnson, Raritan, NJ) for radiation exposure. At ambient temperatures, samples were exposed to either a MDS Nordion Gamma Cell 220 Research Irradiator (Cobalt 60 source) or a Mevex 5 MeV, 2 kW electron beam linear accelerator. Samples were exposed to either 25 kGy (normal sterilizing dose) or 50 kGy (maximum sterilizing dose). Additional samples were included which were not exposed to radiation to account for any changes to the polymer that were due to factors other than sterilization (such as shipping conditions), these are denoted as ‘traveller samples’.

2.9. In vitro cytotoxicity evaluation

Cell compatibility of the polymer was performed by culturing NCTC clone 929 (strain L) mouse areolar fibroblast cells (ATCC, Manassas, VA) in medium containing the dissolved diacid, as the solubility of the polymer in aqueous media is negligible. These L929 fibroblast cells are a standard cell type for cytocompatibility testing as recommended by the ASTM. [10] The cell culture medium consisted of Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, St Louis, MO), 10 vol.% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), 1% L-glutamate (Sigma) and 1% penicillin/streptomycin (Sigma).

Diacid was dissolved at 100 mg/mL in dimethyl sulfoxide (DMSO) (Sigma, St Louis, MO) as a stock solution and serially diluted with DMSO to 50, 10, 5, and 1 mg/mL. These solutions were then diluted with cell culture medium to obtain medium with 1% DMSO and 1, 0.5, 0.1, 0.05, and 0.01 mg diacid/mL. The polymer-containing medium was distributed in a 96-well plate at 200 μL/well and seeded at an initial concentration of 2000 cells/well. Concentrations were tested in triplicate. Cell viability was determined using a CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI). Following a 90 min incubation time with 20 μL of the MTS reagent in the culture medium, the absorbance was recorded with a micro-plate reader at $\lambda = 490$ nm. Cell viability was normalized to cells grown in cell medium with 1% DMSO and 0 mg diacid/mL at 24 hr. A one-way ANOVA followed by Bonferroni's all pairs comparison was used to determine significance (KaleidaGraph 4.1, Synergy Software, Reading, PA).

3. Results and discussion

3.1. Molecular weight change due to in vitro degradation

The molecular weight of the polymer was analyzed before and after 90 days of hydrolytic degradation. The M_w of the entire polymer disk decreased over 90 days from 5600 Da (PDI: 2.1) to 1900 Da (PDI: 3.1). Before degradation, the GPC chromatogram for the polymer samples contained only one peak with a maximum peak at 4200 Da (Figure 2A). After 90 days, the chromatograms for all samples were trimodal (Figure 2B). The first peak at 3800 Da corresponds to mostly undegraded polymer, the second peak at 600 Da corresponds to dimers, and the third peak at 300 Da to diacid (see Figure 1 for structure).

3.2. Degradation product analysis

The degradation behavior of poly(*o*CPX) was studied under conditions that mimic the physiological environment (pH 7.4, 37 °C) at 60 rpm. The degradation media was analyzed by HPLC. Notably, degradation products other than the diacid (e.g., dimers or trimers) were not observed in the degradation media, as determined by the single peak detected in the HPLC spectra with a retention time (1.77 min) that corresponds to solublized diacid (Figure 3). The lack of other degradation products in the media is likely due to their poor solubility in aqueous media.

The dissolution rate of the diacid from the polymer surface can affect degradation; if the degradation media were saturated with diacid, subsequent degradation products would remain on the disk surface and impede water penetration into the matrix, thus, slowing degradation.[12] Therefore, the solubility of diacid in PBS was monitored to ensure that its concentration in the degradation media was sufficiently low, so as not to affect degradation rates. The maximum solubility of diacid in the buffered media (PBS, pH 7.4) is 1.34 mg/mL. The highest diacid concentration detected in the media samples was 1.02 mg/mL. Thus, the samples experienced sink-like conditions that might be expected in a dynamic system such as the human body.

The diacid concentration in the degradation media was used to monitor polymer disk erosion. Figure 4 shows cumulative diacid release from the polymer discs (in mg) from the initial (150 mg) discs. During the first 10 days, minimal amounts of diacid were detected in

the PBS media. This profile indicates an induction period, or lag time, during which polymer degradation products are at concentrations below the detection limit. This induction period is often observed with polyanhydrides, when polymer chains on the matrix surface are partially hydrolyzed but not yet soluble in media.[13] After this induction period, diacid was released into the media at a constant rate (∼ 1.36 mg/day) as indicated by the linear release profile (R^2 = 0.99). At this rate, these polymer disks would be expected to be fully degraded at ∼6 months (calculated by extrapolating the linear trend of release to 150 mg). This six-month degradation time is longer than that typically seen with many polyanhydrides with similar T_gs.[3, 4, 12]

Due to the constant linear trend and the expected length of the total degradation time, the *in vitro* degradation study was stopped at 90 days and the remains of the disks were collected for further analysis. The average mass loss at 90 days was 72.3%. This amount is a slightly higher mass loss than expected based on the amount of diacid monitored in the degradation media, with the calculated amount of diacid being released equal to 63.3% the weight of the disk. This additional mass loss may have occurred during the rinsing procedure used to remove salts from the disks before the mass loss measurements were taken.

3.3. Morphological Changes

Polymer disks were monitored by SEM before and after 30 days incubation for morphological changes. The virgin disk has a uniformly smooth cross section (Figure 5A). The degraded disk exhibits a porous structure (area a, Figure 5B) on the exterior surface, while internally, the polymer remains uniform (area b, Figure 5B). This porous structure, known as an erosion zone, is indicative of water penetration into the disk. This feature is typical in surface-eroding polymers such as polyanhydrides and is responsible for the zero-order release profile observed in the diacid release profile (Figure 4).[12, 14]

3.4. Ionizing radiation

As poly(*o*CPX) is being investigated as a potential biomaterial, its stability to common sterilizing procedures was evaluated. Electron beam and gamma irradiation were chosen as other common sterilizing methods involve heat and/or moisture (e.g., dry heat, autoclaving, and ethylene oxide), which cause polyanhydride degradation.[15] Polymer samples were subjected to 0, 25, or 50 kGy doses (untreated, standard sterilizing dose, and maximum sterilizing dose, respectively) of either electron beam or gamma radiation. The irradiated polymers were then analyzed for changes in molecular weight or degradation rates. No significant differences were observed between irradiated and non-irradiated samples (Figure 6) at any of the dosing regimes

3.5. Cytotoxicity

L929 fibroblasts were exposed to increasing concentrations of *o*CPX diacid for 72 hr to evaluate its effect on cell viability, proliferation and morphology. No morphological changes were observed and the only significant difference in cell viability between any of the samples and the DMSO control was the 1 mg/mL sample at 72 hr (Figure 7). In the *in vitro* studies, this diacid concentration was only reached when the disks had been in the same 10 mL of media for 5 days. *In vivo*, the turnover rate for the surrounding liquid would be

expected to be greater than this value, indicating that poly(*oCPX*) would be expected to be biocompatible. This data is supported by the minimal inflammation observed when a poly(*o*CPX) blend was implanted in a rat model.[16]

4. Conclusions

Poly(*o*CPX), an aromatic polyanhydride, was investigated for its potential as a drug delivery matrix with respect to degradation, radiation stability, and biocompatibility because it has desirable thermal and solubility properties. The polymer degraded to release only its biocompatible diacid precursor into solution. Polymer discs exhibited a 10-day lag period before releasing the diacid in a linear fashion. At this steady degradation rate, the polymer discs are expected to degrade in ∼ 6 months. SEM imaging and M_W changes indicate that the polymer is primarily surface-eroding. The combination of its thermal properties, solubility, degradation time, and erosion mechanism indicate that poly(*o*CPX) would be a suitable candidate for extended, controlled drug delivery. Poly(*o*CPX) stability to ionizing radiation is advantageous if it is to be used as a biomaterial.

Acknowledgments

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References

1. Kumar N, Langer RS, Domb AJ. Polyanhydrides: an overview. Advanced drug delivery reviews. 2002; 54:889–910. [PubMed: 12384314]
2. Gopferich A, Tessmar J. Polyanhydride degradation and erosion. Advanced drug delivery reviews. 2002; 54:911–31. [PubMed: 12384315]
3. Domb, A.; Jain, JP.; Kumar, N. Polyanhydrides. In: Lendlein, A.; Sisson, A., editors. Handbook of Biodegradable Polymers: Synthesis, Characterization and Applications. Wiley-VHC Verlag GmbH & Co.; 2011.
4. von Burkersroda F, Schedl L, Gopferich A. Why degradable polymers undergo surface erosion or bulk erosion. Biomaterials. 2002; 23:4221–31. [PubMed: 12194525]
5. Ron E, Mathiowitz E, Mathiowitz G, Domb A, Langer R. NMR characterization of erodible copolymers. Macromolecules. 1991; 24:2278–82.
6. Wang PP, Frazier J, Brem H. Local drug delivery to the brain. Advanced drug delivery reviews. 2002; 54:987–1013. [PubMed: 12384318]
7. Domb, AJ.; Kost, J.; Wiseman, DM. Handbook of biodegradable polymers. Australia; United States: Harwood Academic Publishers; 1997.
8. Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric systems for controlled drug release. Chemical reviews. 1999; 99:3181–98. [PubMed: 11749514]
9. Anastasiou TJ, Uhrich KE. Novel polyanhydrides with enhanced thermal and solubility properties. Macromolecules. 2000; 33:6217–21.
10. International, A. ASTM F813. West Conshohocken, PA: 2012. Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices.
11. Akbari H, D'Emanuele A, Attwood D. Effect of geometry on the erosion characteristics of polyanhydride matrices. International Journal of Pharmaceutics. 1998; 160:83–9.
12. Gopferich A. Mechanisms of polymer degradation and erosion. Biomaterials. 1996; 17:103–14. [PubMed: 8624387]

13. D'Emanuele A, Hill J, Tamada JA, Domb AJ, Langer R. Molecular weight changes in polymer erosion. Pharmaceutical research. 1992; 9:1279–83. [PubMed: 1448426]
14. Whitaker-Brothers K, Uhrich K. Investigation into the erosion mechanism of salicylate-based poly(anhydride-esters). Journal of biomedical materials research Part A. 2006; 76:470–9. [PubMed: 16315189]
15. Rosario-Melendez R, Lavelle L, Bodnar S, Halperin F, Harper I, Griffin J, et al. Stability of a salicylate-based poly(anhydride-ester) to electron beam and gamma radiation. Polymer degradation and stability. 2011; 96:1625–30. [PubMed: 21909173]
16. Griffin J, Carbone A, Delgado-Rivera R, Meiners S, Uhrich KE. Design and evaluation of novel polyanhydride blends as nerve guidance conduits. Acta Biomaterialia. 2010; 6:1917–24. [PubMed: 19941978]

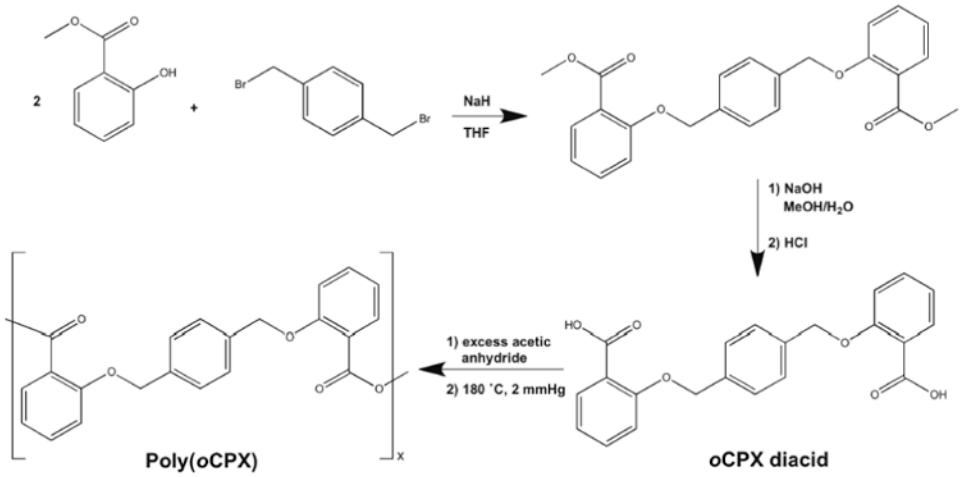

Figure 1.
Synthesis of poly(*o*CPX).[9]

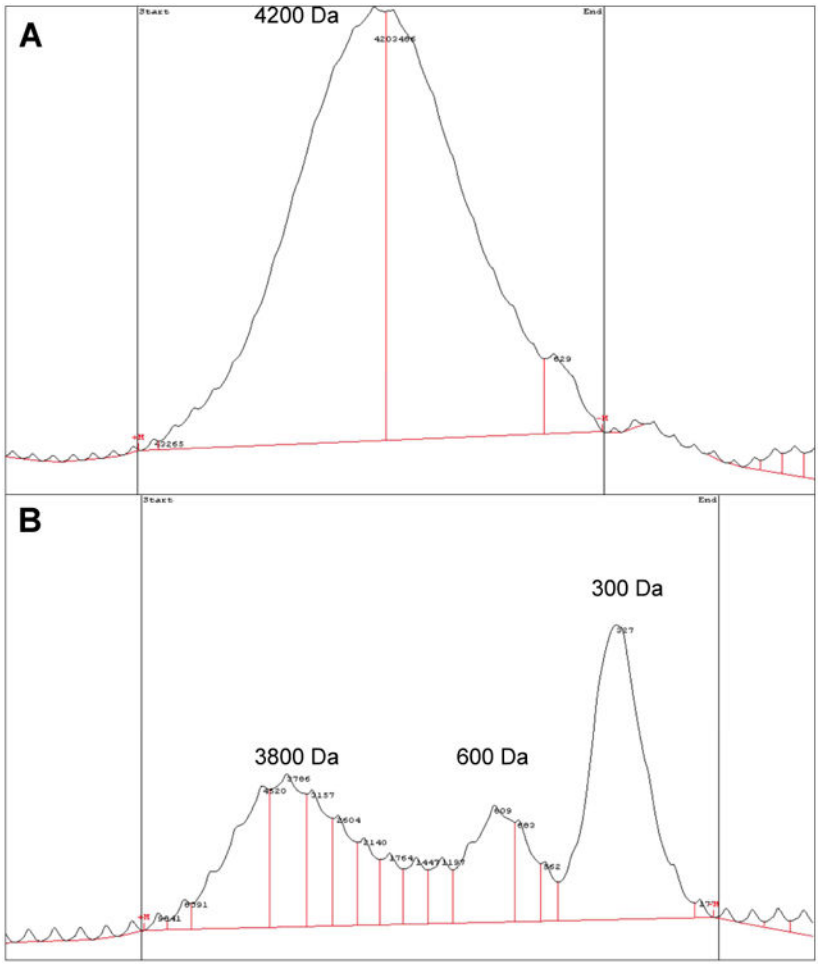

Figure 2.

Representative GPC spectra of *o*CPX (A) before and (B) after 90 day degradation (pH 7.4, 37 °C) with peak molecular weights labeled.

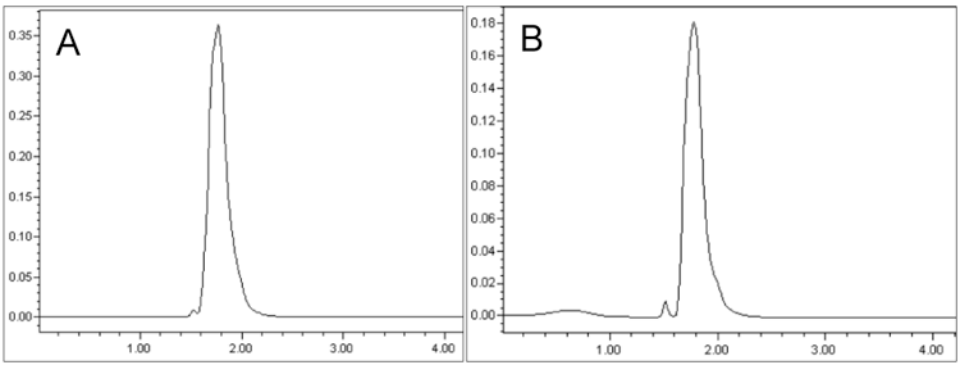

Figure 3.

HPLC spectra of a diacid standard (A) and the degradation media (B); both exhibit a single peak at 1.77 min.

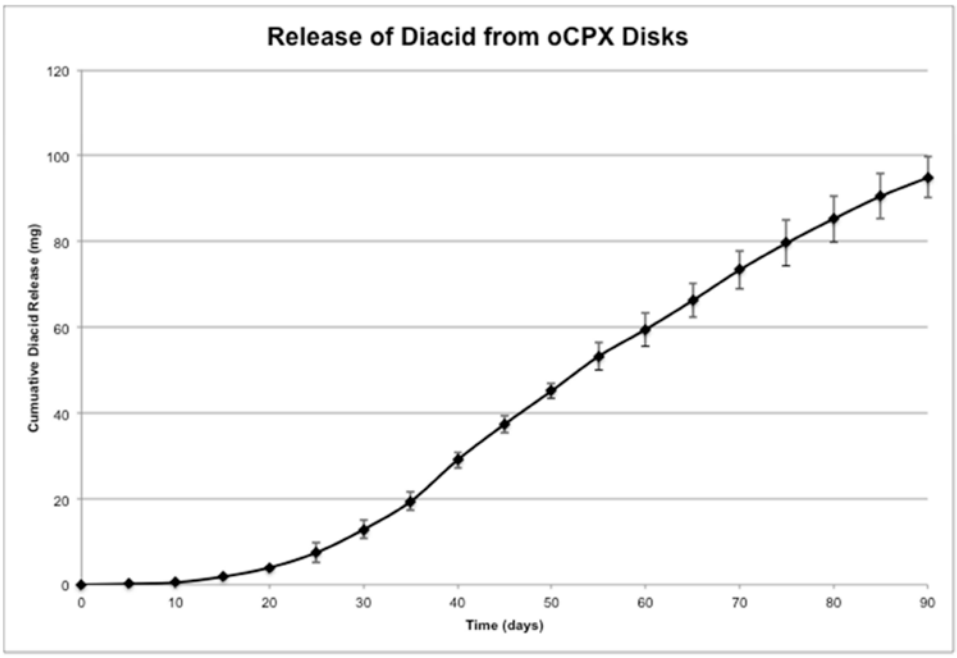

Figure 4.
Cumulative release of diacid into degradation media from poly(*o*CPX) disks in PBS (pH 7.4, 37°C)

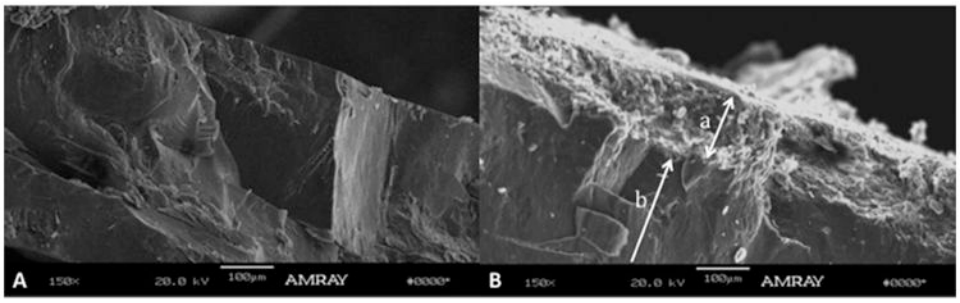

Figure 5.

SEM images of disk cross sections at day 0 (A) and day 30 (B). On Figure 5B, note the porous structure on the surface of the disk (area a), which indicative of water penetration relative to the more uniform section (area b).

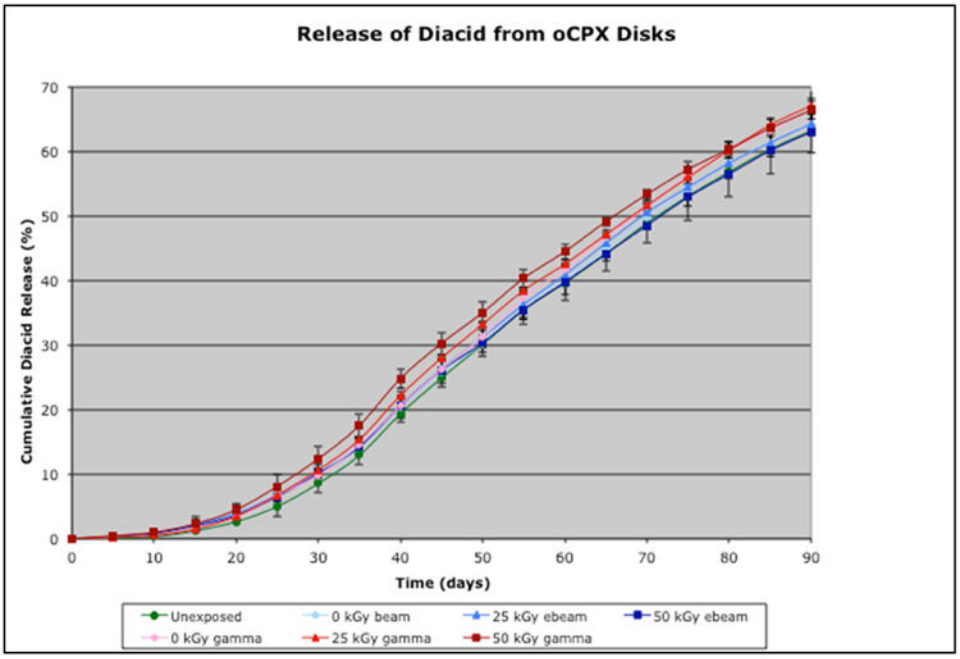

Figure 6.

Cumulative diacid release into degradation media from 150 mg irradiated poly(*o*CPX) discs in PBS following various sterilization conditions.

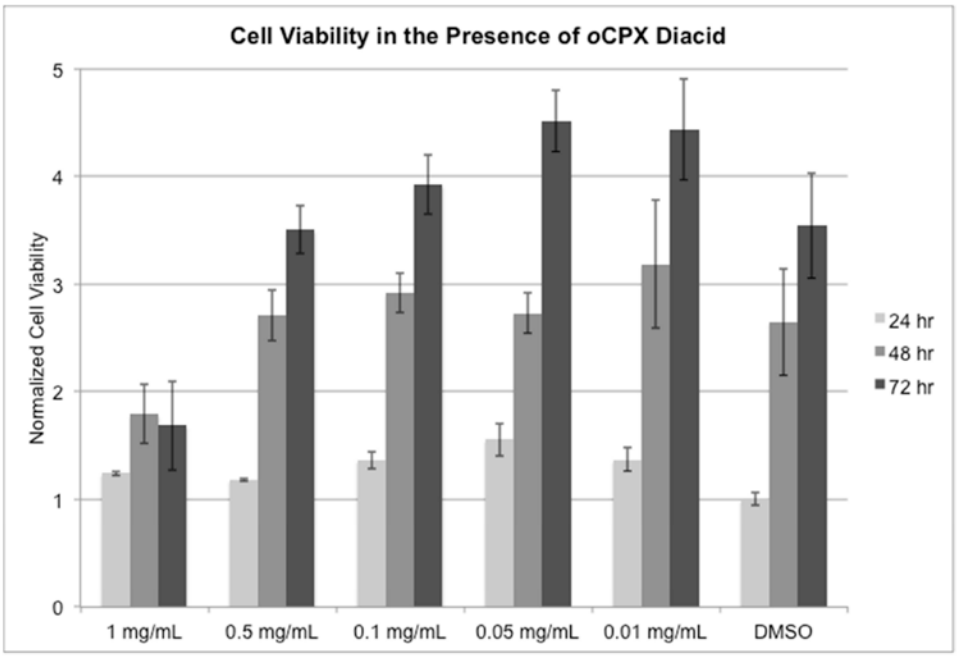

Figure 7.

*o*CPX diacid did not significantly affect cell viability at 0.5 mg/mL and lower (* indicates a significant difference ($p < 0.05$) in cell viability from the DMSO control).