Enzymatic Polymerization of an Ibuprofen-Containing Monomer and Subsequent Drug Release[a]

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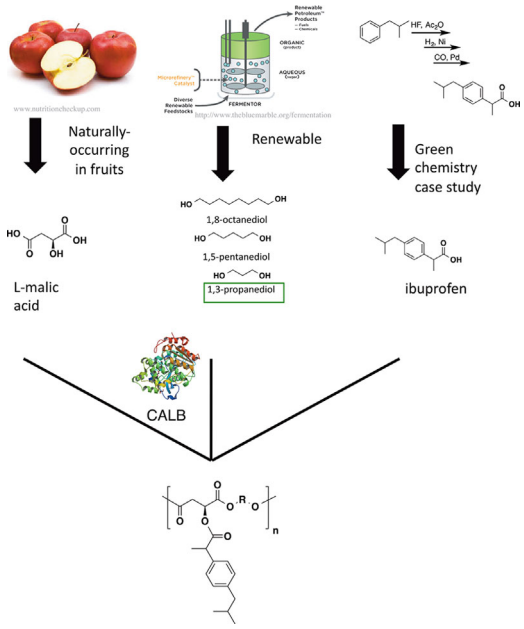
Novel ibuprofen-containing monomers comprising naturally occurring and biocompatible compounds were synthesized and subsequently polymerized via enzymatic methods. Through the use of a malic acid sugar backbone, ibuprofen was attached as a pendant group, and then subsequently polymerized with a linear aliphatic diol (1,3-propanediol, 1,5-pentanediol, or 1,8-octanediol) as comonomer using lipase B from *Candida antarctica*, a greener alternative to traditional metal catalysts. Polymer structures were elucidated by nuclear magnetic resonance and infrared spectroscopies, and thermal properties and molecular weights were determined. All polymers exhibited sustained ibuprofen release, with the longer chain, more hydrophobic diols exhibiting the slowest release over the 30 d study. Polymers were deemed cytocompatible using mouse fibroblasts, when evaluated at relevant therapeutic concentrations. Additionally, ibuprofen retained its chemical integrity throughout the polymerization and *in vitro* hydrolytic degradation processes. This methodology of enzymatic polymerization of a drug presents a more environmentally friendly synthesis and a novel approach to bioactive polymer conjugates.



1. Introduction

Lipase-catalyzed polycondensations have garnered attention due to their chemoselectivity, non-toxicity, recyclability, and derivation from renewable resources.[1] One such lipase, Novozym 435 (N435, lipase B from *Candida antarctica* immobilized on acrylic resin), has been used to synthesize a wide range of polymers with different properties. Aliphatic polyesters of diol/diacid monomers,[2,3] diol/diester monomers,[4,5] hydroxyacids,[6] and terpolymers of diacids and diols[7–9] have been synthesized, in addition to polycarbonates.[10] Silicone-containing

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polymers[11,12] and polyamides[13] have also been synthesized using N435, indicating it's broad applicability. Additionally, some enzymatically synthesized polymers contain pendant groups for future modification (i.e., coupling a bioactive or targeting moiety);[6,7,9] however, limited examples of bioactive-containing monomers polymerized via enzymatic methods are known.[14]

Using polymers for delivery of bioactives, such as drugs, has been widely studied;[15] however, the majority of research has focused on physically incorporating drugs into polymers or chemically conjugating drugs to a pre-existing polymer backbone where the polymer may – or may not – be degradable. Drug-containing monomers can allow for higher drug loading and tunability of release rates and other polymer properties not possible with using pre-existing polymers.[16] Among such researched drugs, ibuprofen is a widely used non-steroidal anti-inflammatory drug (NSAID) to treat pain, inflammation, and fever, yet it suffers from severe gastrointestinal side-effects at higher doses.[17,18] Physical incorporation of ibuprofen into a polymer has been achieved and exhibits limited drug loadings (<30%) and a burst release profile.[19–26] In systems where the drug is chemically incorporated into the polymer, non-biodegradable polymers are often used, leading to potential adverse effects when used *in vivo* as the polymer may remain.[27–30] In previous work, an ibuprofen-containing polyester was prepared using N435 but had low drug loading (3–13%) and a burst release; 35% of ibuprofen was released after 18 d, but not thereafter.[31] Polymers of sebacic acid, glycerol, poly(ethylene glycol), and ketoprofen have similarly been developed using N435; however, low drug loading (<25%) and the use of toxic, expensive vinyl moieties remain issues. Between 10 and 25% of ketoprofen was slowly released over 14 d; however, the release rate after that time was not studied.[14] Additionally, ibuprofen pro-drug micelles were developed;[32] drug loading was between 7 and 41 wt%, but toxic reagents were used for synthesis. To overcome low drug loadings, Rosario-Melendez *et al.* reported on the synthesis of ibuprofen and tartaric acid-based polymers using 1,8-octanediol as comonomer and stannous octoate as catalyst to yield polymers with 65–67 wt% ibuprofen.[33] In this work, the utility of enzyme-mediated polymer synthesis is expanded; specifically, the development of a drug-containing monomer and the utilization of enzymes (N435) to generate three different ibuprofen and malic acid-based polyesters using diols as comonomers is described.

The synthetic methodology herein utilizes "greener," more sustainable processes when compared with more traditional polycondensation methods. Of the twelve principles of green chemistry, this work focuses on (i) replacement of environmentally hazardous chemicals with safer materials, (ii) solvent elimination, (iii) product biodegradation, (iv) use of catalysts and (v) use of renewable resources[34] Thus eliminating the phrase, – the work described herein incorporates many of these principles. A high priority of this research is inclusion of renewable resources, as such, L-malic acid is a naturally occurring dicarboxylic acid found in many fruits such as apples, grapes, and berries[35,36] and serves as the polymer backbone. The diol comonomers chosen are used in cosmetic and pharmaceutical formulations.[37,38] Additionally, 1,3-propanediol is derived from biological sources.[39] In addition to the novelty of a polymer comprised of renewable monomers, the enzymatic polymerization of such a drug-containing monomer with hydrolytically degradable bioactive pendant ester groups has few literature examples.[14] In summary, this paper presents one of the few examples of synthesizing drug-containing monomers to make biocompatible polymers via green methods including enzyme-based polymerization; the only other literature examples exhibit low drug loading (<25 wt%) and utilize toxic reagents/solvents. Further, these completely biodegradable polymers are anticipated to be significant because of the environmentally sustainable synthetic methodology used to make environmentally sensitive polymers.

Polymer and precursor structures were determined by proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) and Fourier transform infrared (FT-IR) spectroscopies. Thermal properties were evaluated by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Mass spectrometry (MS) and gel permeation chromatography (GPC) were used to determine molecular weights (M_w) of precursors and polymers, respectively. To evaluate drug release from polymer, *in vitro* studies were performed under physiological conditions. Polymer cytocompatibility was determined using fibroblasts, and chemical structures of released drugs were confirmed.

2. Experimental Section

2.1. Materials

Dichloromethane (DCM) (≥99.8%), methanol (MeOH) (HPLC grade, ≥99.9%), L-malic acid (≥99%, Fluka), dimethylformamide (DMF) (anhydrous, ≥99.8%), sodium carbonate (Na_2CO_3) (≥99.5%), benzyl bromide (BnBr) (98%), magnesium sulfate ($MgSO_4$) (anhydrous, ≥99.5%), sodium bicarbonate ($NaHCO_3$) (≥99.7%), deuterated chloroform ($CDCl_3$) (99.8%, 0.03% TMS), ibuprofen (≥98%), 4-dimethylaminopyridine (DMAP) (≥99%), pivalic anhydride (99%), cyclopentylmetyl ether (CPME) (anhydrous, ≥99.9%), palladium on carbon (Pd/C) (10 wt% loading), Celite (512 medium), lipase acrylic resin from *Candida antarctica* (Novozym 435, ≥5,000 U/g), 1,3-propanediol (98%), 1,5-pentanediol (96%), 1,8-octanediol (98%), diphenyl ether (Ph_2O) (≥99%), chloroform ($CHCl_3$) (≥99.8%) phosphate buffered saline (PBS) (tablets), monobasic potassium phosphate (KH_2PO_4) (≥99%), acetonitrile (HPLC grade, ≥99.9%), concentrated hydrochloric acid (HCl) (37%), sodium hydroxide (NaOH) (≥98%), dimethyl sulfoxide (DMSO) (≥99.7%) were

obtained from Sigma–Aldrich (Milwaukee, WI) and used as received. Hydrogen (H_2) was obtained from Airgas (Piscataway, NJ). Dulbecco's Modified Eagle Medium (DMEM) was obtained from Corning (Corning, NY). (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) reagent was obtained from Promega (Madison, WI). (Poly(vinylidine fluoride) (PVDF) and polytetrafluoroethylene (PTFE) syringe filters, and Wheaton glass scintillation vials were purchased from Fisher Scientific (Fair Lawn, NJ).

2.2. Structural Characterization

NMR spectra of all compounds were recorded on a Varian 400 or 500 MHz spectrophotometer. Samples (≈5 mg $\cdot$mL^{-1} for ^{1}H; ≈40 mg $\cdot$mL^{-1} for ^{13}C) were dissolved in deuterated chloroform ($CDCl_3$), with tetramethylsilane as the internal reference. Each spectrum was an average of 16 scans for ^{1}H NMR and 256 scans for ^{13}C NMR. FT-IR absorbance spectra were measured on a Thermo Nicolet/Avatar 360 FT-IR spectrometer. Samples (1–3 wt%) were dissolved in dichloromethane (DCM) and solvent-cast onto NaCl plates. Each spectrum was an average of 32 scans.

2.3. Molecular Weight

Polymer precursors were analyzed by MS to determine molecular weights. A Finnigan LCQ-DUO running Xcalibur software and an adjustable atmospheric pressure ionization electrospray source (API-ESI Ion Source) were used. Samples were dissolved in methanol (10 μg $\cdot$mL^{-1}) and injected with a glass syringe. During the experiment, the pressure was 0.8×10^{-5} Torr and the API temperature was 150 °C. Polymer weight-averaged molecular weights (M_w) and polydispersity indices (PDI) were determined by GPC on a Waters (Watford, MA) liquid chromatography system consisting of a 515 HPLC pump and 2414 refractive index (RI) detector with Empower software. Polymer samples were prepared for auto-injection by dissolving polymer in dimethylformamide (DMF, 10 mg $\cdot$mL^{-1}) and filtering through 0.45 μm PTFE syringe filters. Samples were eluted through two PL gel columns 10^3 and 10^5 Å (Polymer Laboratories) in series at 25 °C, with DMF with 0.1% trifluoroacetic acid as eluent at a flow rate of 0.8 mL $\cdot$min^{-1}. Molecular weights were calibrated relative to narrow polystyrene standards.

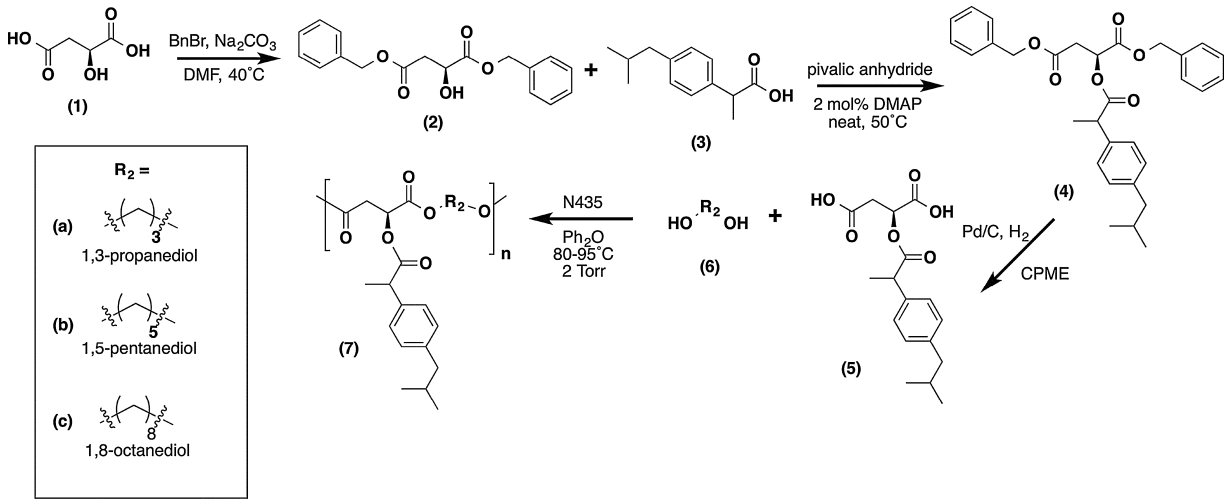
2.4. Thermal Analysis

DSC was performed using a TA DSC Q200 to evaluate melting (T_m) and glass transition (T_g) temperatures. TA Universal Analysis 2000 software was used for automation and data collection on an IBM ThinkCentre computer. Samples (5–10 mg) were heated under dry nitrogen gas to 200 °C at a heating rate of 10 °C $\cdot$min^{-1} and cooled to –50 °C at a rate of 10 °C $\cdot$min^{-1} with a two-cycle minimum. T_m was calculated at the peak of melting and T_g was defined as the midpoint of the curve.

TGA was performed on a Perkin-Elmer Pyris 1 system with TAC 7/DX instrument controller. Perkin-Elmer Pyris software running on a Dell Optiplex GX110 computer was used for automation and data collection and processing. Samples (5–10 mg) were heated under dry nitrogen gas from 25 to 600 °C at a heating rate of 10 °C $\cdot$min^{-1}. Decomposition temperatures (T_d) were measured at the onset of thermal decomposition.

2.5. Dibenzyl-l-Malate (2) Synthesis

Using the same methodology as Guo *et al.*,[40] L-malic acid (**1**, 1 eq) was dissolved in anhydrous DMF and stirred in a round-bottomed flask under nitrogen as shown in Scheme 1. Sodium carbonate (2.4 eq) was added to the reaction to form a white suspension that was stirred for 30 min. Benzyl bromide (3 eq) was then added and reaction was heated to 40 °C with stirring overnight. The reaction mixture was then concentrated in vacuo, diluted with ethyl acetate (EtOAc), and washed with saturated sodium bicarbonate (3×), deionized (DI) water, and brine. The organic layer was dried over $MgSO_4$, filtered, and the solvent removed in vacuo to yield compound **2**.



Scheme 1. Synthesis of polymer precursors and poly(ibuprofen-L-malate) polyesters using aliphatic diols of different lengths (**a**, **b**, **c**).

Yield: 95% (off-white oil). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28–7.38 (m, 10H, Ar–H); 5.10–5.20 (split, 4H, CH_2); 4.54 (s, 1H, CH); 3.23 (s, 1H, OH); 2.82–2.94 (split, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.4, 170.5, 135.4, 135.4, 128.9, 128.6, 68.0, 67.6, 67.0, 39.0. IR (NaCl, cm^{-1}): $\nu = 3\ 480$ (w; OH), 1 740 (s; C=O, ester). MS (ESI): $m/z = 315.1$ $[M+1]^+$. $T_d = 248\ ^\circ C$.

2.6. Ibuprofen Dibenzyl-l-Malate (4) Synthesis

A solvent-free esterification reported by Sakakura *et al.*[41] was used to couple the drug to compound **2**. Ibuprofen (**3**, 1.1 eq), 4-(dimethylamino) pyridine (DMAP; 2 mol%), and dibenzyl-L-malate (**2**, 1 eq) were combined in a round-bottomed flask under nitrogen. Then, pivalic anhydride (1.1 eq) was added. Reaction was heated to 55 °C and stirred overnight. Reaction was then diluted with EtOAc and washed with saturated sodium bicarbonate (3×), DI water, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to yield compound **4**.

Yield: 85% (off-white oil). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28–7.38 (m, 10H, Ar–H); 7.15 (m, 2H, Ar–H); 7.04 (m, 2H, Ar–H); 5.52 (m, 1H, CH); 4.90–5.20 (split m, 4H, CH_2); 3.72 (m, 1H, CH); 2.88 (split m, 2H, CH_2); 2.40 (dd, 2H, CH_2); 1.81 (m, 1H, CH); 1.46 (dd, 3H, CH_3); 0.87 (dd, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.9, 170.5, 169.0, 140.8, 137.2, 135.3, 129.5 (2C), 128.8 (6C), 128.6 (6C), 127.5 (2C), 68.7, 67.6, 67.0, 45.1, 38.9, 36.3, 30.4, 22.6, 18.6. IR (NaCl, cm^{-1}): $\nu = 1\ 743$ (s; C=O, ester). MS: $m/z = 502.3$ $[M+1]^+$. $T_d = 268\ ^\circ C$.

2.7. Ibuprofen L-Malic Acid (5) Synthesis

Ibuprofen dibenzyl-L-malate (**4**, 1 eq) was dissolved in anhydrous cyclopentylmethyl ether (CPME, 10 mL · g^{-1}) and 10% palladium on carbon (Pd/C, catalytic amount) was added. The reaction flask was evacuated under vacuum and purged with hydrogen gas (3×), then allowed to stir at room temperature under hydrogen overnight. The mixture was filtered through Celite to remove Pd/C. The filtrate was concentrated in vacuo to yield pure compound **5**.

Yield: 91% (light tan paste). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.19 (t, 2H, Ar–H); 7.08 (dd, 2H, Ar–H); 5.47 (split, 1H, CH); 3.78 (m, 1H, CH); 2.75 (split, 2H, CH_2); 1.83 (m, 1H, CH); 1.52 (d, 3H, CH_3); 0.88 (d, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.9, 173.9 (2C), 141.0, 136.9, 129.5, 127.5, 67.8, 45.0, 44.8, 35.9, 22.6, 18.6. IR (NaCl, cm^{-1}): $\nu = 3\ 100-3\ 300$ (w; OH, acid), 1 743 (s; C=O, ester), 1 719 (s; C=O, acid). MS: $m/z = 321.1$ $[M-1]^-$. $T_d = 211\ ^\circ C$.

2.8. Ibuprofen L-Malic Acid Polymer (7) Synthesis

Ibuprofen-L-malic acid (**5**, 1 eq) and diol (**6**, 1 eq) were added to a round-bottomed flask with N435 (10 wt% of total monomers, dried at room temperature and 2 Torr for 24 h). Diphenyl ether (200 wt% of total monomers) was added. Stirring (200 rpm) was initiated and reaction was performed in three sequential steps: (i) reaction stirred 80 °C for 4 h at atmospheric pressure under nitrogen, (ii) reaction stirred at 80 °C under vacuum (2 Torr) for 24 h, and (iii) reaction was stirred at 95 °C under vacuum (2 Torr) for an additional 24 h. Upon completion, the reaction was brought to room temperature, dissolved in chloroform ($CHCl_3$), and gravity-filtered to remove lipase. Filtrate was concentrated in vacuo to yield compound **7**.

Poly(octylene ibuprofen-L-malate) (**7a**) Yield: 82% (tan paste). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.21 (b, 2H, Ar–H); 7.09 (b, 2H, Ar–H); 5.47 (b, 1H, CH); 3.62–4.22 (b, 5H, CH, CH_2); 2.84 (b, 2H, CH_2); 2.44 (b, 2H, CH_2); 1.84 (b, 1H, CH); 1.17–1.71 (b, 15H, CH_3, CH_2, CH_2, CH_2) 0.89 (b, 6H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.2, 174.5 (2C), 139.9, 137.1, 129.9, 128.1, 68.9, 45.3, 44.7, 35.5, 31.1, 29.6 (2C), 22.3, 18.9, 17.2 (2C). IR (NaCl, cm^{-1}): $\nu = 1\ 741$ (s; C=O, ester). $M_w = 8.1$ kDa, PDI = 1.4. $T_g = -35\ ^\circ C$, $T_d = 221\ ^\circ C$.

Poly(pentylene ibuprofen-L-malate) (**7b**) Yield: 78% (brown paste). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.19 (b, 2H, Ar–H); 7.08 (b, 2H, Ar–H); 5.47 (b, 1H, CH); 3.78 (b, 1H, CH); 2.75 (b, 2H, CH_2); 1.83 (b, 1H, CH); 1.52 (b, 3H, CH_3); 0.88 (b, 6H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.1, 172.7 (2C), 139.7, 136.0, 128.4, 126.3, 67.2, 44.0, 43.8, 35.1, 29.1 (2C), 28.7, 27.0, 21.3 (2C), 17.5, 17.1. IR (NaCl, cm^{-1}): $\nu = 1\ 743$ (s; C=O, ester). $M_w = 6.4$ kDa, PDI = 1.4. $T_g = -8\ ^\circ C$, $T_d = 215\ ^\circ C$.

Poly(propylene ibuprofen-L-malate) (**7c**) Yield: 81% (brown paste). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20 (b, 2H, Ar–H); 7.08 (b, 2H, Ar–H); 5.47 (b, 5H, CH_2, CH); 3.70–4.20 (b, 1H, CH); 2.91 (b, 2H, CH_2); 2.44 (b, 2H, CH_2); 1.79–2.12 (b, 3H, CH_2, CH); 1.51 (b, 3H, CH_3); 0.89 (b, 6H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.9, 172.6 (2C), 139.7, 136.0, 128.4, 126.3, 68.2, 45.3, 44.8, 35.8, 30.4 (2C), 22.6 (2C), 18.6, 18.4. IR (NaCl, cm^{-1}): $\nu = 1\ 743$ (s; C=O, ester). $M_w = 4.8$ kDa, PDI = 1.3. $T_g = -5\ ^\circ C$, $T_d = 212\ ^\circ C$.

2.9. *In Vitro* Drug Release from Polymer

Drug release from polymers (**7a–c**) was evaluated by *in vitro* degradation in phosphate buffered saline (PBS) under physiological conditions. Polymer samples (30 mgs each, $n = 3$) were incubated in 10 mL PBS (pH 7.4) in 20 mL Wheaton glass scintillation vials (Fisher, Fair Lawn, NJ) using a controlled environment incubator-shaker (New Brunswick Scientific Co., Edison, NJ) at 60 rpm at 37 °C. At predetermined time intervals throughout the 30 d of the study, media (5 mL) was collected and replaced with fresh PBS (5 mL) and the spent media was analyzed by high-performance liquid chromatography (HPLC). Analysis was performed using an XTerra RP18 3.5 μm 4.6 × 150 mm^2 column (Waters, Milford, MA) on a Waters 2695 Separations Module equipped with a Waters 2487 Dual Absorbance Detector. All samples were filtered using 0.22 μm PVDF syringe filters and subsequently injected (20 μL) using an autosampler. The mobile phase, which was developed as a modification of a published procedure,[42] was comprised of acetonitrile (70%) and 10 mM KH_2PO_4 in DI water at pH 2.5 (30%) run at 0.5 mL · min^{-1} flow rate and ambient temperature. Absorbance was monitored at $\lambda = 223$, one of the absorption wavelengths for ibuprofen. Amounts were calculated from a calibration curve of known standard solutions.

2.10. Structural Characterization of Released Ibuprofen

Polymers (40 mgs) were placed in 20 mL Wheaton scintillation vials, then PBS (10 mL) and 1 N NaOH (2 mL) added to the vials, which were incubated in a controlled environment incubator-shaker at 37 °C with 60 rpm agitation. After complete polymer

degradation, solutions were acidified to pH 2 using concentrated HCl and ibuprofen was extracted from the samples with DCM (10 mL, 3×). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo.

2.11. Cytocompatibility Studies

In vitro cytocompatibility studies were performed by culturing 3T3 mouse fibroblasts in cell media (DMEM supplemented with 10% FBS, 1% pen/strep) containing the three different polymers. Polymers were first sterilized under UV at $\lambda = 254$ nm for 900 s (Spectronics Corporation, Westbury, NY), dissolved in DMSO to yield 10 mg $\cdot$ mL^{-1} solutions, and then diluted with cell media to reach concentrations of 0.01 and 0.001 mg $\cdot$ mL^{-1}. Cell media containing polymers were then added to allocated wells in a 96-well plate with 2 000 cells/well and incubated at 37 °C. DMSO (1%) in cell media was used as control. Cell viability was determined using CellTiter 96 Aqueous One Solution Cell Proliferation Assay. After 24, 48, and 72 h incubation with polymers, 20 μL of (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) reagent was added to each well and further incubated for 4 h at 37 °C. The absorbance was then recorded with a microplate reader (Coulter, Boulevard Brea, CA) at 492 nm.

3. Results and Discussion

3.1. Synthesis and Characterization

To prevent unwanted side reactions from occurring, the carboxylic acid groups of L-malic acid were protected before ibuprofen was coupled to the malic acid alcohol group. Thus, the selective benzyl protection of malic acid carboxylic acid groups was adapted from a previously published procedure using benzyl bromide and sodium carbonate.[40] The appearance of benzylic and aromatic signals in the ^{1}H NMR spectrum (Figure 1) and the

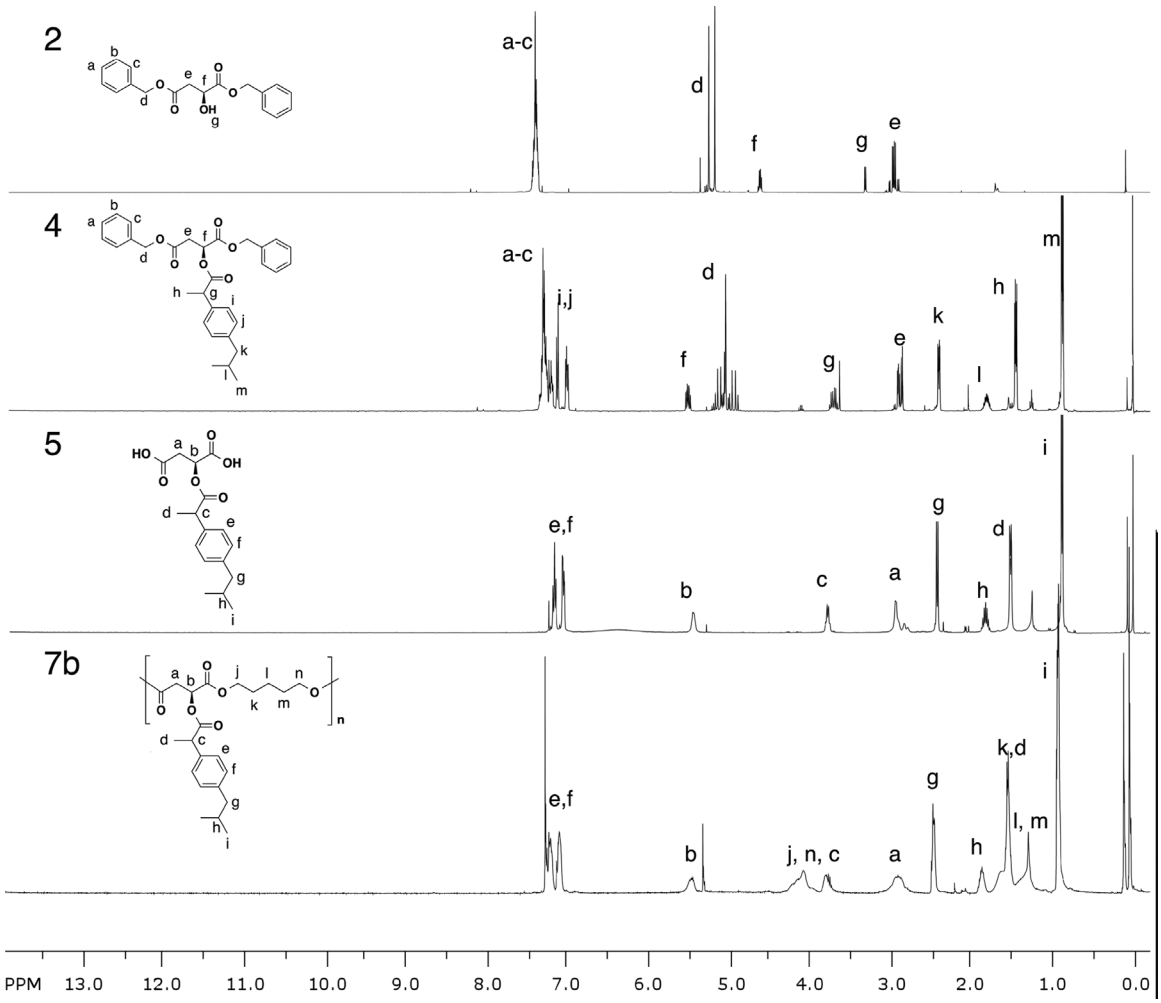


Figure 1. ^{1}H NMR spectra of compounds **2**, **4**, **5**, and **7b** showing benzyl protection, drug coupling, deprotection, and subsequent polymerization.

preservation of the FT-IR band (Figure 2) at 3 480 cm^{-1} (O-H) of **2** confirmed that successful reaction occurred at the acid groups without affecting the secondary alcohol. Thereafter, ibuprofen was coupled to the alcohol via a solvent-free esterification using catalytic DMAP and pivalic anhydride to afford **4** in high yield. The significant chemical shift of malic acid backbone protons in the NMR spectra (Figure 1) of **4** along with the formation of an IR band (Figure 2) at 1 743 cm^{-1} (ester, C=O) and disappearance of the alcohol band at 3 480 cm^{-1} indicated successful coupling of drug to **2**. Subsequent deprotection via palladium-mediated hydrogenolysis selectively cleaved the benzyl esters while leaving the pendant ester group intact. During this step, CPME was used as a greener alternative to traditional solvents such as tetrahydrofuran, dioxane, DCM, and MeOH, as it is less toxic, less volatile, and less likely to form peroxides.[43] Low volatility is preferable when choosing solvents to minimize exposure to air and laboratory workers, especially in plant-scale labs.[34,43] Diacid **5** was obtained in high yield after filtration through Celite and removal of solvent in vacuo. NMR spectrum (Figure 1) indicated the disappearance of benzylic protons while IR spectrum (Figure 2) displayed the preserved pendant ester linkage at 1 743 cm^{-1} and appearance of an additional carbonyl band at 1 719 cm^{-1} (–COOH). At each step, mass spectrometry was used to confirm product molecular weight, and ^{13}C NMR spectroscopy was used to confirm chemical structure. All polymer precursors were viscous oils or foams that did not display melting temperatures.

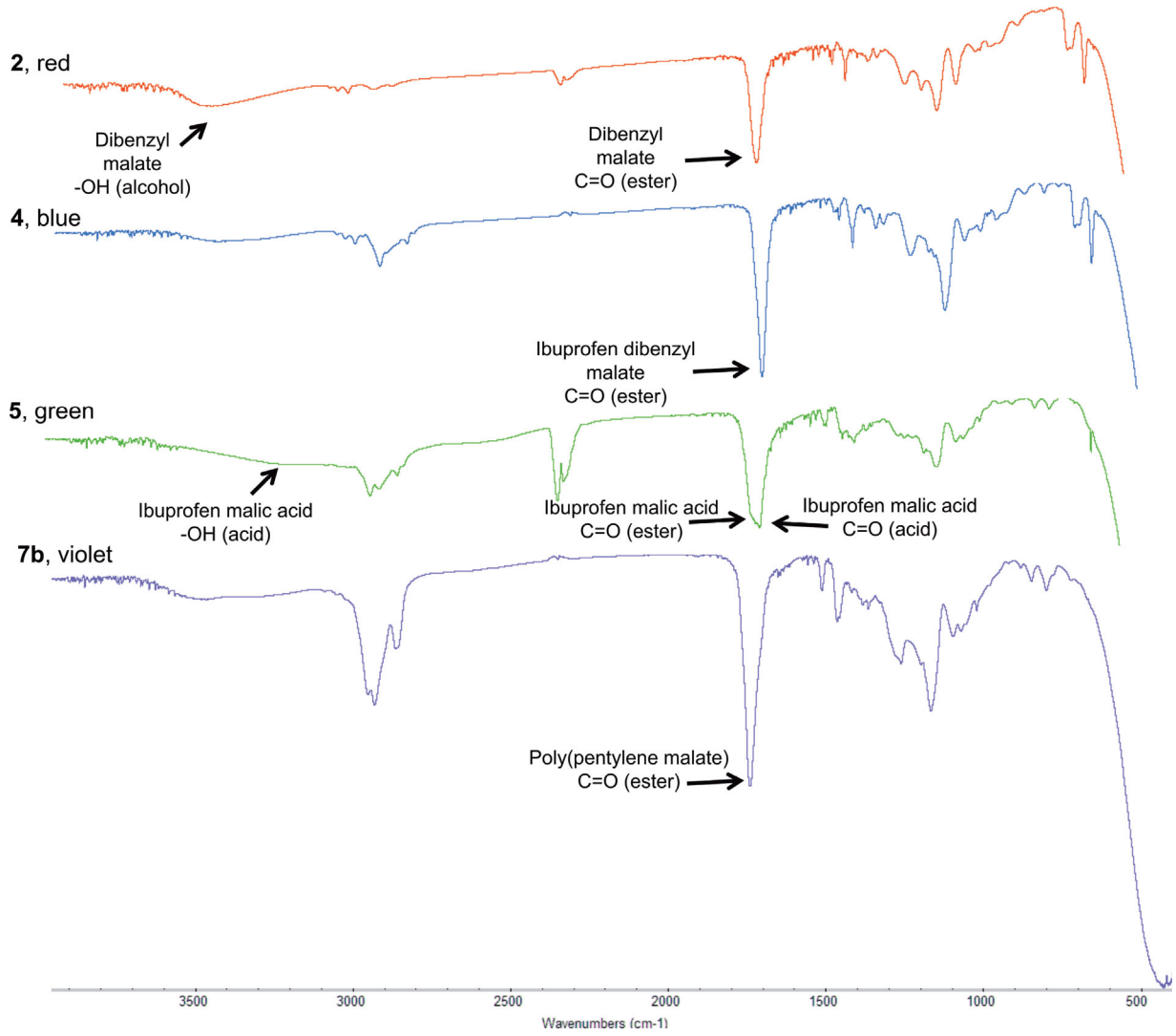


Figure 2. IR spectra of precursors **2** (red, upper), **4** (blue, mid-upper), and **5** (green, mid-lower), and polymer **7b** (violet, lower) presented as examples; key IR bands are labeled on each spectrum.

Polyester synthesis was performed using a modified polymerization procedure of aliphatic diacids and diols.[2] The polyesterification of equimolar amounts of ibuprofen-L-malic acid and diol was catalyzed by N435. This particular enzyme is readily dispersed in the reaction mixture and known to be activated by heat. In a prior study using a different dicarboxylic acid and diol, the N435 enzyme produced polymers of the same molecular weight and was stable at 70–90 °C.[44] Diphenyl ether, a hydrophobic, high-boiling solvent that has been shown to be effective for such reactions, was chosen as solvent. Upon exposure to the initial temperature of 80 °C, the reaction formed a monophasic mixture that remained for the reaction duration. After 48 h of polymerization time, the reaction mixture became solid and product was isolated after dissolution in chloroform, filtration of lipase beads, and solvent removal *in vacuo*. No further purification or precipitation steps was used; however, during enzyme removal via filtration, not all product was dissolved after 10 min, thus 100% conversion was not obtained. The lack of monomer present in the reaction mixture was confirmed by NMR and IR spectroscopies. Chloroform was chosen because it is the preferred solvent to terminate the lipase reactions;[45] despite the use of this solvent in this one step, our overall synthetic methods are greener than traditional methods; other methods to achieve similar reactions utilize excess carbodiimides,[46] chlorinated solvents,[31] and metal catalysts often used[47–49] in contrast to solvent-free, catalytic reactions, safer solvents, and enzyme catalyst. GPC analysis indicated that M_w values were moderate and PDI values narrow (Table 1) substantiating that an additional purification step was unnecessary. The longer chain length diol, 1,8-octanediol, resulted in polymers with slightly higher M_w, as expected based on previously published results.[2,3] Notably, the M_w of these enzymatically synthesized polyesters are similar to other malic acid-containing polymers;[9] due to malic acid's high acidity,[50] polymers rarely reach higher than 10 kDa. ^{1}H NMR spectroscopy of polymers **7a–c** displayed all expected peaks; **7b** is provided as an example in Figure 1. IR spectra display the only carbonyl peak at 1 743 cm^{-1}, indicative of an ester in Figure 2. ^{1}H NMR spectra of polymers **7a** and **7c** can be found in Supporting Information (Figure S1). Polymer T_g values were low (<0 °C), and decreased with increasing aliphatic diol chain length; no T_m transitions were exhibited (Table 1). T_d values were >200 °C, confirming that the polymer should be relatively stable to temperatures at which these polymers would be reacted or processed.

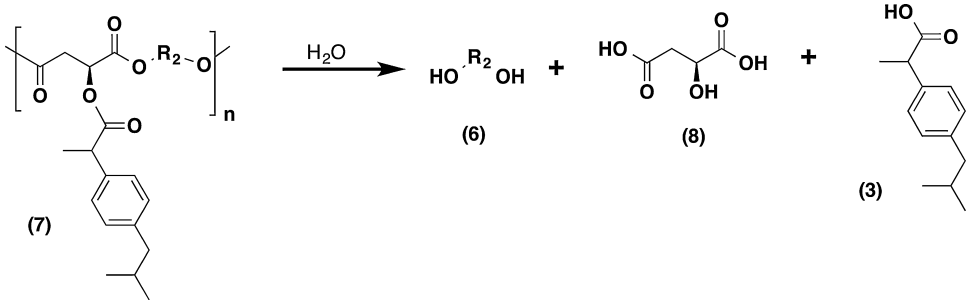
Table 1. Summary of thermal properties, molecular weights, and polydispersity indices of polymers **7a–c**.

Polymer	T_g [°C][a)]	T_d [°C][b)]	M_w [kDa][c)]	PDI[c)]
7a	−5	212	4.8	1.4
7b	−9	215	6.4	1.4
7c	−35	221	8.1	1.3

[a)]Determined on second heating cycle of DSC; [b)]determined by TGA as the onset of thermal decomposition; [c)]determined by GPC.

3.2. *In Vitro* Bioactive Release

In vitro degradation of the polymers was determined by appearance of ibuprofen in degradation media samples in PBS at physiological conditions (37 °C, pH 7.4). At predetermined times, an aliquot of release media was collected and analyzed via HPLC. The degradation rate of polymer into bioactive via ester bond hydrolysis (Scheme 2) is an important factor in obtaining controlled ibuprofen release; during degradation, the ultimate hydrolytic degradation products were the diol (**6**), L-malic acid (**8**), and ibuprofen (**3**). As an example, ibuprofen **(3),** ibuprofen-malic diacid (**5**), and malic acid (**8**) all exhibited unique retention times at 5.70, 4.29, and 2.79 min, respectively (Figure 3B). Polymers **7a–c** exhibited controlled, sustained ibuprofen release throughout the 30 d of the study. Increased aliphatic chain of the diol used decreased the drug release rate; after 30 d, 42, 58, and 82% of total ibuprofen was released from the



Scheme 2. Complete hydrolysis of polymer **7** into diol (**6**), L-malic acid (**8**), and ibuprofen (**3**).

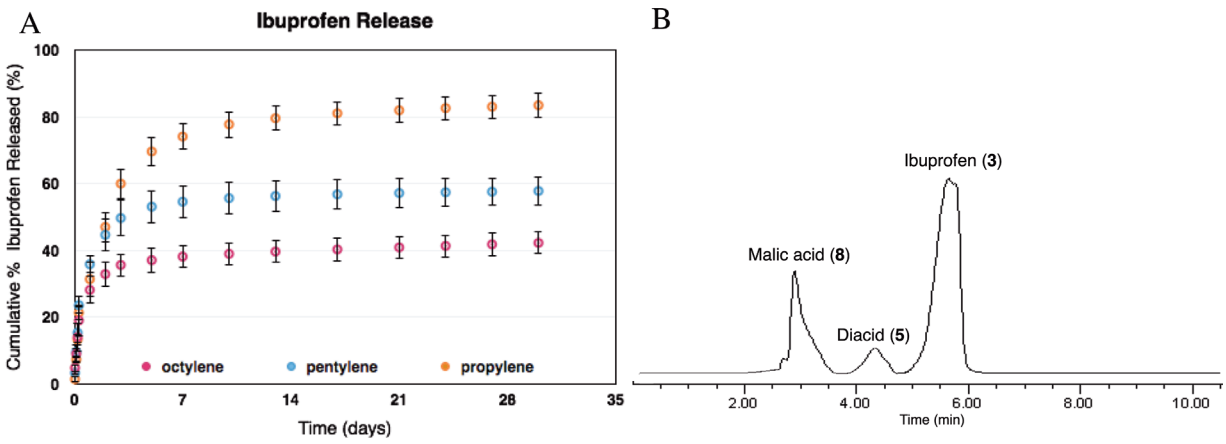


Figure 3. A. Cumulative release of ibuprofen as determined by HPLC data; B. HPLC chromatogram depicting the unique retention times of L-malic acid, **8** (R_t 2.79 min), diacid, **5** (R_t 4.29 min), and ibuprofen, **3** (R_t 5.70 min).

octylene, pentylene, and propylene polymers, respectively (Figure 3A). The release rate correlates to the water solubility of the diols; the less water-soluble, more hydrophobic 1,8-octanediol (log P = 1.44) displays the slowest release followed by intermediate 1,5-pentanediol (log P = 0.19), and 1,3-propanediol (log P = –0.68). After 30 d, the polymers were completely hydrolyzed according to the methods described above. Extracted ibuprofen was quantified in all samples to ensure a mass balance of the drug in the remaining polymer residue. Complete polymer degradation and 100% ibuprofen release is expected in 8–15 months for all three polymers. Additionally, to ensure that polymer processing did not affect the drug molecule structure, the ibuprofen extracted from media was dried under vacuum and analyzed. The ^{1}H NMR spectrum (Figure S2) of extracted drug showed all peaks at the same chemical shifts as free ibuprofen.

3.3. Cytocompatibility Studies

Based on the therapeutic plasma concentration of ibuprofen, two different concentrations of polymer were tested.[51,52] All polymers were cytocompatible at 0.01 and 0.001 mg · mL^{-1} over 72 h, i.e., no significant difference in cell viability was found between the polymer groups and the media alone control. The 0.01 mg · mL^{-1} concentration is comparable to the commonly used ibuprofen dosing.[51] This data demonstrated that these ibuprofen-based

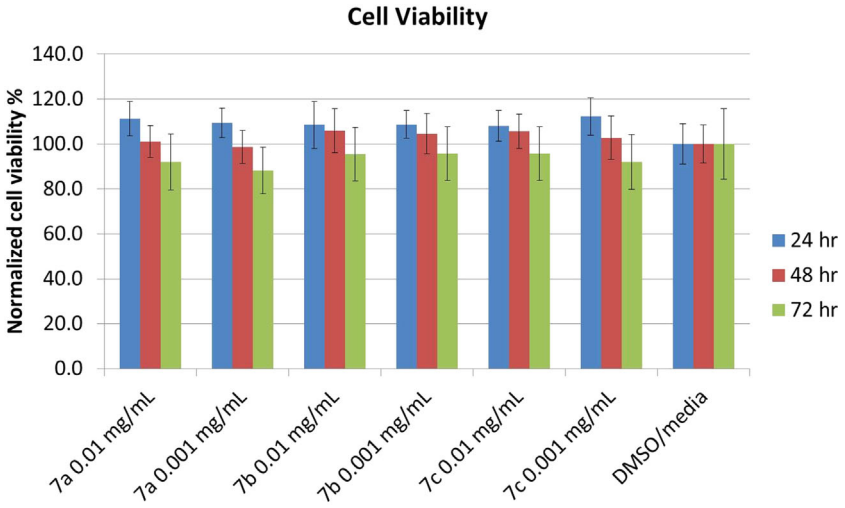


Figure 4. Cytocompatibility of polymers after 24, 48, and 72 h incubation. All groups contained 1% DMSO in cell media and control group has no polymer. Absorbance at 490 nm after MTS treatment is proportional to cell viability. Data presented as mean ± standard deviation. $n = 6$ in each group.

polymers are cytocompatible at clinically relevant concentrations and, thus, promising candidates for biomedical applications (Figure 4).

4. Conclusion

This polyester synthetic methodology moves toward a more environmentally friendly approach in developing biomaterials for drug delivery. Through the use of naturally occurring resources, safer solvents and/or eliminating solvent altogether, polymer precursors were synthesized in high yield (85–95%). A lipase-mediated polymerization, as opposed to a metal-mediated polymerization, produced three polyesters with varying aliphatic chain length with varying release characteristics. All polymers released ibuprofen over 30 d under physiological conditions; the release rate can be tuned by varying the diol used. Utilization of a shorter chain, more water-soluble diol (i.e., 1,3-propanediol) results in faster release. Significantly, the polymers are designed for hydrolytic degradation and the ultimate polymer degradation products are deemed "safe" as they are either bioactive, natural, or non-toxic. Cytocompatibility studies using fibroblasts assured the biocompatible nature of the polymers at relevant therapeutic concentrations, and NMR analysis determined that the ibuprofen structure remained unaffected throughout the synthesis procedure and polymer degradation process. The greener methodology presented here for precursor synthesis can be widely applicable to other hydroxyacids and bioactives with carboxylic acid functionalities, opening the door to other sustainable, enzymatic polymerizations of drug-containing monomers. Future works include investigating other bio-based diols to tune thermal properties and performing *in vitro* anti-inflammatory activity assays and investigating additional formulations (e.g., coatings, films).

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