POLYMERIC DRUGS FOR CONTROLLED RELEASE OF ANALGESICS

by

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ABSTRACT OF THE DISSERTATION

Polymeric Drugs for Controlled Release of Analgesics

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Pain is a physiological protective system that helps prevent damage from a harmful stimulus or detect the presence of a disease or injury. When a patient cannot tolerate the pain experienced, analgesic drugs are used to achieve pain relief. These drugs are needed for long-term, however, their analgesic effects typically have short duration and serious side effects. To address these issues, polymers that contain analgesic drugs chemically incorporated within the polymer backbone or as pendant groups were designed, synthesized, characterized, and formulated. Non-steroidal anti-inflammatory drugs (NSAIDs) [i.e., salicylic acid (SA), ibuprofen, and naproxen] and opioids (i.e., morphine and nalbuphine) were polymerized to develop drug delivery systems with potential to extend analgesic effect, reduce side effects, and prevent accidental drug withdrawal or overdose. Extended (i.e., weeks and months) NSAID release could be beneficial for treatment of chronic inflammatory diseases that cause pain and controlled release of opioids could improve chronic pain treatment.

Salicylate-based poly(anhydride-ester) (PAE) microspheres were designed as injectable delivery systems for long-term SA release. Three polymers with chemical

compositions comprised of either linear or branched aliphatic linkers were used. In addition, the formulation was optimized to improve the overall microsphere morphology. The presence of a lag time characterizes the slow-degrading salicylate-based PAEs and is unfavorable for constant long-term drug release applications. The use of copolymers and polymer blends was explored to modify the physicochemical properties and drug release profiles and thus achieve long-term SA release. The salicylate-based PAEs have great potential in various biomedical applications. However, the polymer should meet the pharmacopeial and commercial requirement of sterility. Therefore, the effect of electron beam and gamma radiation on the physicochemical properties of the salicylate-based PAEs was studied.

Morphine was chemically incorporated into a PAE backbone. The polymer termed "PolyMorphine" was designed, synthesized, and fully characterized. The hydrolytic degradation pathway of the polymer was determined by *in vitro* studies. *In vitro* studies demonstrated that PolyMorphine is non-cytotoxic towards fibroblasts. *In vivo* studies using mice showed that PolyMorphine provides analgesia for 3 days, 20 times the analgesic window of free morphine.

Novel biodegradable polyester comprised of all biocompatible elements: tartaric acid, 1,8-octanediol, and ibuprofen or naproxen as pendant groups, were synthesized and characterized. The polymers release the free drug (ibuprofen or naproxen) *in vitro* in a controlled manner without burst release. These new biomaterials are not cytotoxic towards mouse fibroblasts and human blood-derived macrophages, and the drugs retain their bioactivity after being released from the polymer.

PREFACE

"Todos podemos alcanzar metas tan altas como las estrellas, si nos proponemos y dejamos los miedos atrás" – Sor Isolina Ferré

DEDICATION

I dedicate this work to God and my family (my father Víctor M. Rosario, my sisters Aurelin and Marielin, and my brother Víctor), but especially to my mother **Ana E. Meléndez**, without you, your love, and unconditional support I would not have been able to achieve my goals.

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ABBREVIATIONS, SYMBOLS, AND UNITS

[M + 1]	Mass plus one
[M + Na]	Mass plus sodium
%	Percent
°	Degrees
°C	Degrees Celsius
°C/min	Degrees Celsius per minute
±	Plus or minus
~	Approximately
^{13}C-NMR	Carbon nuclear magnetic resonance spectroscopy
^{1}H-NMR	Proton nuclear magnetic resonance spectroscopy
ACN	Acetonitrile
API-ESI	Atmospheric Pressure ionization Electrospray Ion Source
ArH	Aromatic proton
Au	Gold
br	Broad
C	Carbon
C=O	Carbonyl
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
cm^{-1}	Wavenumber units
comp	Complex
d	Days
d	Doublet (when describing an NMR spectrum)
Da	Daltons
DCC	Dicylohexylcarbodiimide
DCM	Dichloromethane
DFDBA	Demineralized freeze-dried bone allograft
DMAP	4-(dimethylamino)pyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
dq	Doublet quartet
DSC	Differential scanning calorimetry
e-beam	Electron beam radiation
EDCI	1-[-3-(dimethylamino)propyl]-3-

	ethylcarbodiimide hydrochloride
EDTA	Ethylenediaminetetraacetic acid
eq	Equivalents
Et_3SiH	Triethylsilane
EtOAc	Ethyl acetate
EtOH	Ethanol
FBS	Fetal bovine serum
FDA	Food and Drug Administration
FT-IR	Fourier-transformed infrared spectroscopy
g	Grams
g/mL	Grams per milliliter
gamma	Gamma radiation
GPC	Gel permeation chromatography
h	Hours
H	Proton
H_2	Hydrogen
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
i.p.	Intraperitoneal
IR	Infrared spectroscopy
KBr	Potassium bromide
kg	Kilograms
kGy	KiloGray
KH_2PO_4	Potassium phosphate monobasic
$KHSO_4$	Potassium hydrogen sulfate
M	Molar
m	Multiplet
mBar	Millibar
MeOH	Methanol
mg	Milligrams
mg/kg	Milligrams per kilogram
mg/mL	milligrams per milliliter
$MgSO_4$	Magnesium sulfate
MHz	Megahertz
min	Minutes
mL	Milliliters
mm	Millimeters
mM	Millimolar

mmHg	Millimeters of mercury
mmol	Milimoles
MS	Mass spectrometry
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
MW	Molar mass
M_w	Weight-averaged molecular weight
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
nm	Nanometers
NSAID	Non-steroidal anti-inflammatory drug
O-H	Alcohol
PAE	Poly(anhydride-ester)
PBS	Phosphate buffered saline
Pd	Palladium
$Pd(OAc)_2$	Palladium (II) acetate
Pd/C	Palladium on carbon
PDI	Polydispersity index
PLGA	Poly(lactic-*co*-glicolic) acid
PLLA	Poly(lactic acid)
psi	Pounds per square inch
PTFE	Polytetrafluoroethylene
PVA	Poly(vinyl alcohol)
Py	Pyridine
RI	Refractive index
rpm	Revolutions per minute
s	Seconds
s	Singlet (when describing an NMR spectrum)
SA	Salicylic acid
SEM	Scanning electron microscopy
t	Triplet
T_d	Decomposition temperature
TEA	Triethylamine
TFL	Tail-flick latency
T_g	Glass transition temperature
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
T_m	Melting point
TNF-α	Tumor necrosis factor alpha

UV/vis	Ultraviolet/visible spectroscopy	δ	Chemical shift
		λ	Wavelength
vol%	Volume percent	μg	Micrograms
wt.%	Weight percent	μL	Microliters

1. INTRODUCTION

The development of biomaterials science has revolutionized many scientific fields, including medicine, tissue engineering, and drug delivery. Polymers have been used to release drugs in a controlled, sustained manner, thus improving patient comfort and compliance with medical treatments. Degradable polymeric biomaterials possess great potential for drug delivery applications. A good example is the extended release of analgesic drugs from biodegradable polymers, reducing side effects, preventing accidental drug withdrawals or overdose, and the reappearance of pain.

1.1. Pain and Analgesia

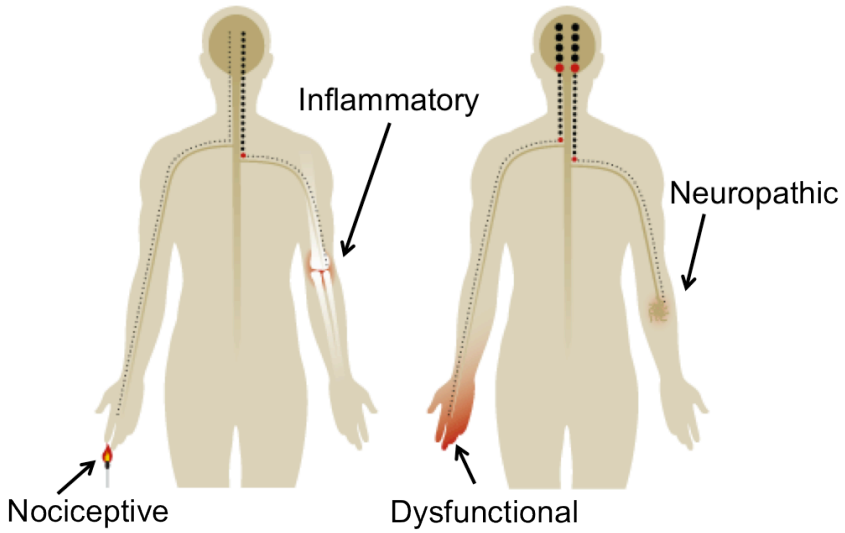


Figure 1.1. Types of pain: nociceptive, inflammatory, and pathological (neuropathic and dysfunctional). Adapted from Science News.[1]

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage.[2, 3] Pain is a physiological protective system or defense mechanism that helps prevent damage from a harmful stimulus or detect the presence of a disease or injury.[4] It is often categorized as nociceptive, inflammatory, or pathological (Figure 1.1).[5]

Nociceptive pain results from a noxious stimulus and demands immediate action.[5] The noxious stimulus activates the pain receptors, known as nociceptors, resulting in a withdrawal reflex.[6] For example, placing your hand on a hot object activates the nociceptors that send a painful message to the central nervous system; as a result you remove your hand form the object, thus minimizing tissue damage. The lack of nociceptive pain is an issue; people born with insensitivity to pain cannot feel bone fractures and pain-causing diseases and are unaware of the danger.[1, 5, 7] Not being able to feel pain may result in early death after a major injury or tissue damage (e.g., appendix rupture) not treated immediately.[7]

Inflammatory pain is also protective, however, it does not require an external stimulus.[5] This type of pain is caused by the immune system when there is an injury or infection. It helps during healing processes creating an unpleasant sensation that discourages movement or contact with the injured tissue, thus reducing the risk of further damage.[5, 8] For example, arthritis patients experience this type of pain.

The third classification, pathological pain, is the type of pain that is not a symptom of a disease or injury.[5] Pathological pain is the result of abnormal functioning of the central nervous system. It is neuropathic when it results from a lesion in the

periferal nervous system with the presence of a major injury. Pathological pain becomes dysfunctional when the nerves amplify non-painful stimuli making them extremely painful.

The absence of pain in response to a stimulus that would normally be painful is known as analgesia.[2] It should not be confused with anesthesia; analgesia relieves pain without the loss of consciousness. This painless state can be achieved with the use of analgesic drugs, also known as painkillers.

1.2. Analgesic Drugs

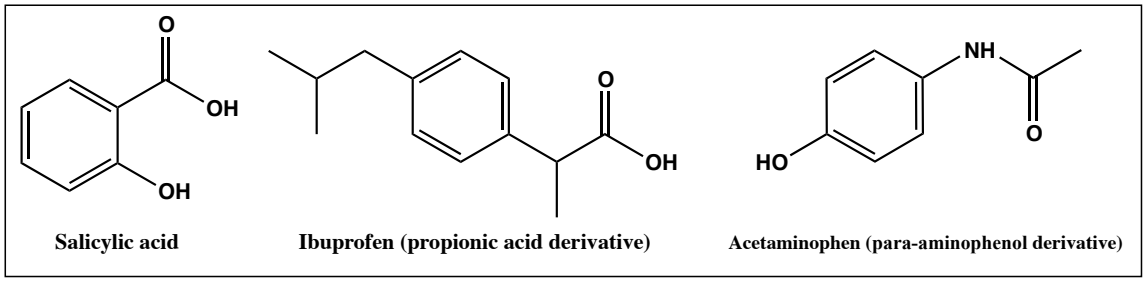


Figure 1.2. Chemical structure of NSAIDs: SA, ibuprofen, and acetaminophen.

Analgesic drugs are used to achieve analgesia when a patient cannot tolerate the pain and desires to block it. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat pain.[9-14] Salicylic acid (SA) and its derivatives, propionic acid derivatives, para-aminophenol derivatives, among others, are categorized as NSAIDs (Figure 1.2).[15] They are considered mild analgesics and are often available over-the-counter.[16, 17] They are also used to treat headaches and other non-chronic painful states.[17] NSAIDs are

effective in treating pain and swelling associated with different types of arthritis.[9, 10, 17] However, their analgesic effects have short duration.[9] Their constant use may result in serious side effects, such as stomach and intestine ulceration, bleeding, and perforation.[10]

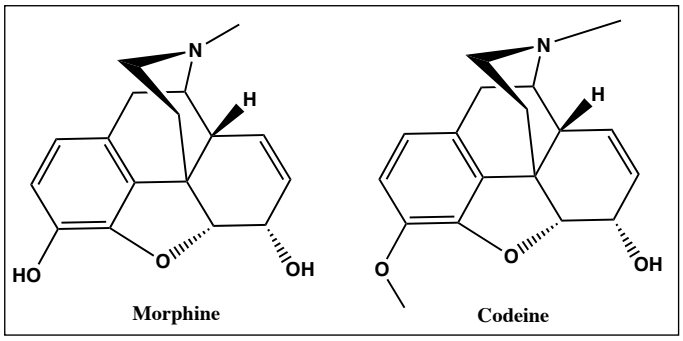


Figure 1.3. Chemical structure of opioids: morphine and codeine.

Opioids are another class of analgesics mainly used to treat severe and chronic pain.[18-21] Morphine, codeine, (Figure 1.3) and their derivatives belong to this analgesic category.[21] Opioids are potent narcotic analgesics that provide reliable analgesia.[22, 23] However, since opioids are prescription drugs, they must be used under close medical attention. Also, severe side effects are associated with their use. Addiction, tolerance, respiratory depression, somnolence, and gastrointestinal effects (e.g., nausea, vomiting, and constipation), are some of the side effects.[24, 25] In addition they must be repeatedly administered to achieve around-the-clock analgesia.[25-27]

1.3. Controlled and Localized Drug Delivery

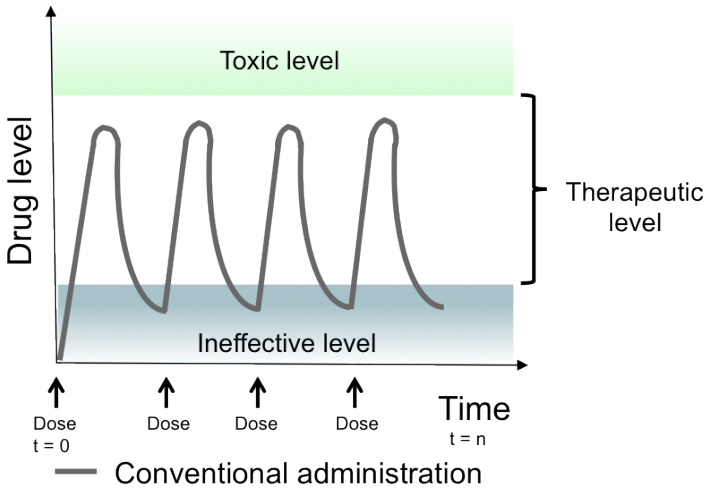


Figure 1.4. Representation of blood drug concentration after conventional drug administration (e.g., orally or intravenously).

Conventional drug administration (e.g., orally or intravenously) results in plasma drug concentrations proportional to the dose (Figure 1.4).[28, 29] Repeated administration is required to achieve long-term therapeutic effect and the drug is distributed throughout the body.[29] Both the repeated administration and the body distribution often increase the drug side effects.[29] In addition, drug administration requires following a rigorous schedule that is not convenient for all patients. Fortunately, controlled release formulations have been develop to address the aforementioned issues.[30-32]

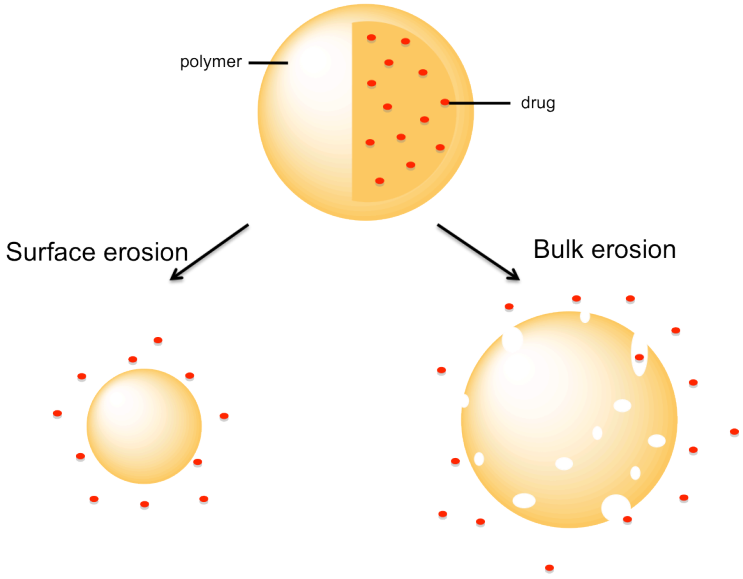


Figure 1.5. Physical encapsulation of drugs within biodegradable polymers and their degradation (surface erosion and bulk erosion) resulting in drug release.

Among the variety of materials used for controlled drug delivery, biodegradable polymers are the most advantageous.[29, 30] Biodegradable polymers have been used to physically encapsulate drugs, proteins, and other therapeutic agents and release them in a controlled manner (Figure 1.5).[30, 32] Therefore, improving medical treatments by extending the therapeutic effect (Figure 1.6), reducing side effects, preventing accidental drug withdrawals, and localizing drug release.[30, 32] Polymers such as polyanhydrides and polyesters hydrolytically degrade (by surface erosion and bulk erosion, respectively, Figure 1.5) and self-eliminate from the body without the need of surgery.[30, 33, 34] Furthermore, the polymers can be formulated into different geometries for different administration routes and localized drug release.[35] Polymer disks, for example the GLIADEL® Wafer which contains carmustine (an anti-cancer drug), are implanted into the body during surgery (e.g., craniotomy).[36] Polymer microspheres, for example

VIVITROL® a naltrexone extended release formulation, are injected into the body (e.g., intramuscularly) for the treatment of alcohol and heroin addiction.[37]

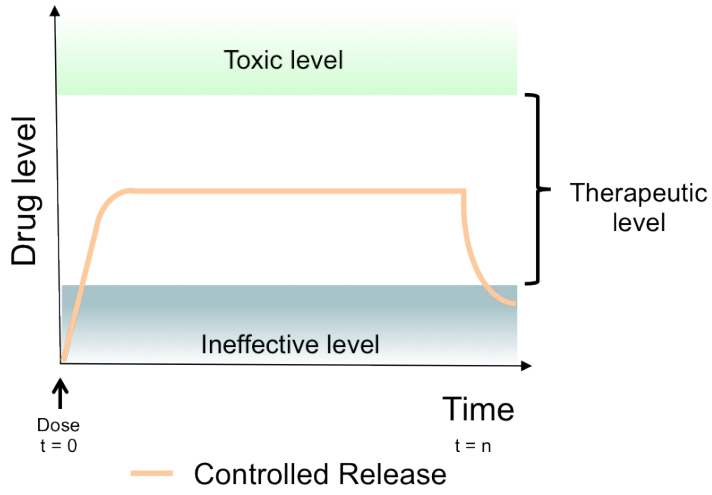


Figure 1.6. Representation of blood drug concentration in controlled release.

While these polymeric drug delivery systems are an improvement to conventional drug administration, they have drawbacks that limit their use. Most controlled release formulations are only able to encapsulate low percentages of drug.[38] Since the drug is physically associated to the polymer, the potential for their separation (accidental or intentional) is high, and therefore, the potential to disturb the controlled release mechanism increase.[39] In addition, the use of biodegradable polymers such as poly(lactic-*co*-glicolic) acid, results in side effects associated with the polymer (e.g., inflammation) and a burst drug release (i.e., rapid drug release).[40] Therefore, further improvements are needed to develop better drug delivery systems.

1.4. Polymeric Drugs

Polymers that contain drug molecules chemically incorporated within the polymer backbone or as pendant group to the backbone have been designed and synthesized by our research group for controlled drug delivery (Figure 1.7).[38, 41-46] These polymers, termed polymeric drugs, are an improvement to other controlled drug release systems. Polymeric drugs are comprised of drugs molecules bonded to one another through a "linker" molecule. The drug release rate can be manipulated by changing the structure of the polymer using different linkers.[47] Higher drug loading (i.e., more than 50 %) is achieved, compared to physical drug encapsulation.[38, 41, 43] The drug cannot be physically separated from the polymer and the drug is release in a near zero-order manner without a burst.[38, 47]

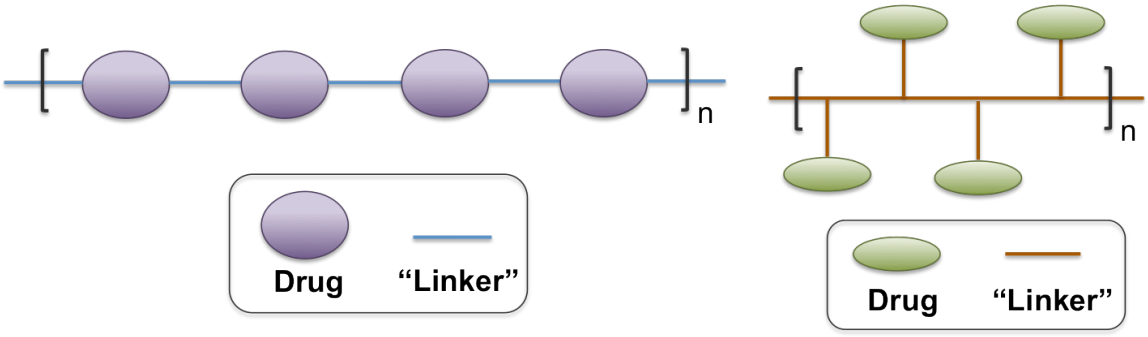


Figure 1.7. Chemical incorporation of drugs within a polymer backbone (left) or as pendant groups (right).

The first example (Figure 1.8) of this type of drug release systems are the salicylate-based poly(anhydride-esters) (PAEs).[38, 42] This PAE contains SA in the

polymer backbone and by changing the "linker" molecule, the physicochemical properties and drug release profile can be altered.[47] SA release can be manipulated to last any time from days to months.[47, 48] These salicylate-based PAEs degrade into non-toxic components and have been proven effective for controlling inflammation,[49, 50] regulating bone growth,[50, 51] and preventing biofilm formation.[49, 52] The polymers can easily be manipulated into different geometries including powders,[48] fibers,[53] microspheres,[54] disks,[47] films and cardiac stents.[55]

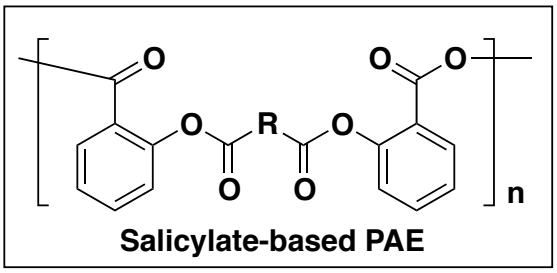


Figure 1.8. General chemical structure of the salicylate-based poly(anhydride-esters).

In addition, the salicylate-based PAEs have been used as a carrier, encapsulating other drug molecules and proteins that cannot be chemically incorporated into a polymer.[56] Furthermore, other NSAIDs (SA derivatives),[41, 43] antiseptics,[44, 46] antimicrobials,[57] and antibiotics have been chemically incorporated into polymer backbones. Polymeric drugs hold great potential for various biomedical applications, therefore, we explore the development of polymeric drugs and formulations for the treatment of pain. Polymeric drugs that can release analgesic drugs for long time periods are presented.

1.5. Research Projects

1.5.1. Formulation of Salicylate-Based Poly(anhydride-ester) Microspheres for Long-Term Salicylic Acid Release

Polymer microspheres are preferred over other formulations as they can be injected into the body rather than be surgically implanted. An injectable NSAID delivery system could be beneficial to treat pain and swelling associated with arthritis since they could be injected into the synovial cavity of a joint, localizing the drug at the target site. The formulation of salicylate-based PAEs was previously reported, however, only polymers with linear aliphatic linkers were used. For long-term drug delivery, salicylate-based PAEs with branched aliphatic linkers are more appropriate since they can achieve drug release over months. Therefore, two salicylate-based PAEs with branched aliphatic linkers and one with linear aliphatic linker were formulated into microspheres to create an injectable delivery system that could achieve long-term SA release. Furthermore, the formulation method was optimized to obtained relatively uniform microspheres.

1.5.2. Modification of the Physicochemical Properties and Drug Release Profiles of Salicylate-Based Poly(anhydride-esters) Using Copolymers and Blends

Salicylate-based PAEs used for long-term (i.e., months) SA release are characterized by the presence of a lag period. During this time, little to no drug is released from the polymer. For applications were SA release is needed at all times for extended time periods, these polymers can only achieve the later. To overcome the lag period, copolymers and polymer blends were prepared using combinations of salicylate-based PAEs (one with linear aliphatic linker and one with branched aliphatic linker). This approach maintains the high drug loading that characterizes these polymeric drugs while changing their physicochemical properties.

1.5.3. Stability of Salicylate-Based Poly(anhydride-esters) to Electron Beam and Gamma Radiation

Materials used for biomedical applications should meet the pharmacopeial and commercial requirement of sterility. Salicylate-based PAEs have great potential in various biomedical applications. Therefore, understanding the effect that common sterilization methods have on the properties of these polymers is of importance. Two salicylate-based PAEs, one with linear aliphatic linker and one with branched aliphatic linker, were exposed to electron beam and gamma radiation. After radiation exposure, the physicochemical properties and *in vitro* drug release were monitored to assess changes caused by the radiation. In addition, the polymer with linear aliphatic linker was formulated into microspheres to assess if radiation used for sterilization affects their properties.

1.5.4. Morphine-Based Poly(anhydride-ester) for Extended Pain Relief

Morphine can provide potent and reliable analgesia. However, it requires repeated administration (every 2-6 h) to maintain the drug at therapeutic levels for an extended time period. Controlled release formulations can prolong the analgesic effect of the drug and prevent accidental withdrawals due to missed doses. They could also alleviate some of the side effects associated with morphine use. Therefore, morphine was chemically incorporated into a PAE backbone. The polymer termed "PolyMorphine" was designed to degrade hydrolytically releasing morphine in a controlled manner to ultimately provide analgesia for an extended time period. The results obtained demonstrate, for the first time, a systemically administered prodrug that yields long-lasting (i.e., 3 days) analgesic effect (more than 20 times the analgesic time window of free morphine).

1.5.5. Biodegradable Ibuprofen- and Naproxen-Based Polyesters

Ibuprofen and naproxen are important propionic acid derivative NSAIDs commonly used to treat pain and swelling associated with different types of arthritis, among other uses (e.g., for minor headaches). Frequent administration of high doses is required to treat persistent symptoms (pain and swelling). Thus, severe gastrointestinal (GI) side effects such as stomach ulceration, bleeding, and perforation occur. Chemical incorporation of drug molecules into biodegradable polymer backbones has been

developed as a novel controlled delivery systems. The chemical incorporation of drugs that possess two reactive functional groups into a polymer backbone has been extensively studied in our group. Yet, minimal work has been performed on drugs containing only one reactive functional group. Here, the incorporation of ibuprofen and naproxen into biodegradable polyester backbones as pendant groups is presented. This work describes the first polymers that contain drugs with only one carboxylic acid functional group. Also, it is the first time that polyesters as polymeric drugs have been prepared.

1.6. References

1. News, S., Hurt Blocker *Science News* June 30, 2012, 2012, pp 22-25.
2. Institute of Medicine (US) Committee on Advancing Pain Research, C., and Education, *Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research*. The National Academies of Press: Washington, DC, 2011.
3. Sinatra, R. S.; Jahr, J. S.; Watkins-Pitchford, J. M., *The Essence of Analgesia and Analgesics*. First ed.; Cambridge University Press: 2011.
4. Boyce-Rustay, J. M.; Honore, P.; Jarvis, M. F., *Methods Mol Biol* **2010,** *617*, 41-55.
5. Woolf, C. J., *The Journal of Clinical Investigation* **2010,** *120* (11), 3742-3744.
6. Kidd, B. L.; Urban, L. A., *British Journal of Anaesthesia* **2001,** *87* (1), 3-11.
7. Campbell, N. A.; Reece, J. B., *Biology*. 7 th ed.; Pearson Education Inc.: San Francisco, 2005.
8. Price, D. D., *Psychological and Neural Mechanisms of Pain*. First ed.; Raven Press: New York, NY, 1988.
9. Brooks, P. M.; Day, R. O., *New England Journal of Medicine* **1991,** *324* (24), 1716-1725.
10. Laine, L.; Smith, R.; Min, K.; Chen, C.; Dubois, R. W., *Alimentary Pharmacology & Therapeutics* **2006,** *24* (5), 751-767.
11. Crielaard, B. J.; Lammers, T.; Schiffelers, R. M.; Storm, G., *Journal of Controlled Release* **2012,** *161* (2), 225-234.
12. Sieper, J.; Braun, J.; Rudwaleit, M.; Boonen, A.; Zink, A., *Annals of the Rheumatic Diseases* **2002,** *61* (suppl 3), iii8-iii18.
13. Braun, J.; Sieper, J., *The Lancet* **2007,** *369* (9570), 1379-1390.
14. Dionne, R. A.; Berthold, C. W., *Crit Rev Oral Biol Med* **2001,** *12* (4), 315-30.
15. O'Neil, M.; Heckelman, P.; Koch, C.; Roman, K., *The Merck Inex*. 14th ed.; 2006.
16. Singh, G., *American journal of therapeutics* **2000,** *7* (2), 115-121.

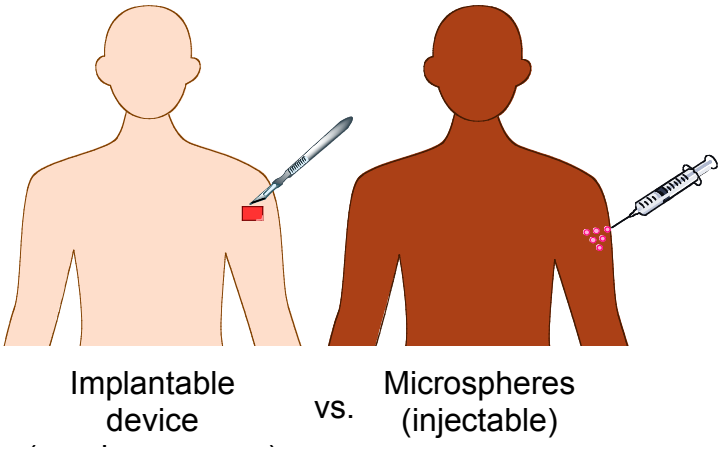
17. Hersh, E. V.; Pinto, A.; Moore, P. A., *Clinical Therapeutics* **2007,** *29* (11, Supplement 1), 2477-2497.
18. Bovill, J. G., *European Journal of Anaesthesiology (EJA)* **1997,** *14*, 9-15.
19. Akil, H.; Watson, S. J.; Young, E.; Lewis, M. E.; Khachaturian, H.; Walker, J. M., *Annu Rev Neurosci* **1984,** *7*, 223-255.
20. Pasternak, G. W., *The Opiate Receptors*. Humana Press: Totowa, New Jersey, 1988; p 1-520.
21. Davis, M. P.; Glare, P. A.; Hardy, J.; Quigley, C., *Opioids in Cancer Pain*. Oxford University Press: 2009.
22. Thompson, A. R.; Ray, J. B., *Journal of the American College of Surgeons* **2003,** *196* (2), 321-324.
23. Cox, B. M., Pharmacology of Opioid Drugs The Opiate Receptors. Pasternak, G. W., Ed. Humana Press: 2011; Vol. 23, pp 23-57.
24. Davis, M. P., *American Journal of Cancer* **2006,** *5* (3), 171-182.
25. King, C. R.; Khabazian, A., *Clinical Journal of Oncology Nursing* **2003,** *7* (4), 458.
26. Ross, E. L.; Hahn, K., *International Journal of Clinical Practice* **2008,** *62* (3), 471-479.
27. Hagen, N. A.; Thirlwell, M.; Eisenhoffer, J.; Quigley, P.; Harsanyi, Z.; Darke, A., *Journal of Pain and Symptom Management* **2005,** *29* (1), 80-90.
28. Kim, S.; Kim, J.-H.; Jeon, O.; Kwon, I. C.; Park, K., *European Journal of Pharmaceutics and Biopharmaceutics* **2009,** *71* (3), 420-430.
29. Jain, J. P.; Modi, S.; Domb, A. J.; Kumar, N., *Journal of Controlled Release* **2005,** *103* (3), 541-563.
30. Dash, A. K.; Cudworth, G. C., *Journal of Pharmacological and Toxicological Methods* **1998,** *40* (1), 1-12.
31. Langer, R.; Peppas, N. A., *AIChE Journal* **2003,** *49* (12), 2990-3006.
32. Langer, R., *Science* **1990,** *249* (4976), 1527-1533.
33. Kipper, M. J.; Shen, E.; Determan, A.; Narasimhan, B., *Biomaterials* **2002,** *23* (22), 4405-4412.
34. Kumar, N.; Langer, R. S.; Domb, A. J., *Adv Drug Deliv Rev* **2002,** *54* (7), 889-910.
35. Qiu, L. Y.; Bae, Y. H., *Pharm Res* **2006,** *23* (1), 1-30.
36. Wang, P. P.; Frazier, J.; Brem, H., *Advanced Drug Delivery Reviews* **2002,** *54* (7), 987-1013.
37. Dean, R. L., Nonclinical Pharmacology of VIVITROL®: A Monthly Injectable Naltrexone for the Treatment of Alcohol Dependence Opiate Receptors and Antagonists. Dean, R. L.; Bilsky, E. J.; Negus, S. S., Eds. Humana Press: 2009; pp 655-674.
38. Erdmann, L.; Uhrich, K., *Biomaterials* **2000,** *21* (19), 1941-1946.
39. Smith, H. S., *Pain Medicine* **2009,** *10*, S30-S38.
40. Pillai, O.; Panchagnula, R., *Current Opinion in Chemical Biology* **2001,** *5* (4), 447-451.
41. Anastasiou, T. J.; Uhrich, K. E., *Journal of Polymer Science Part A: Polymer Chemistry* **2003,** *41* (22), 3667-3679.

42. Schmeltzer, R. C.; Anastasiou, T. J.; Uhrich, K. E., *Polymer Bulletin* **2003,** *49* (6), 441-448.
43. Schmeltzer, R. C.; Schmalenberg, K. E.; Uhrich, K. E., *Biomacromolecules* **2004,** *6* (1), 359-367.
44. Schmeltzer, R. C.; Uhrich, K. E., *Polymer Bulletin* **2006,** *57* (3), 281-291.
45. Carbone, A. L.; Song, M.; Uhrich, K. E., *Biomacromolecules* **2008,** *9* (6), 1604-1612.
46. Prudencio, A.; Carbone, A. L.; Griffin, J.; Uhrich, K. E., *Macromolecular Rapid Communications* **2009,** *30* (13), 1101-1108.
47. Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E., *Macromolecules* **2005,** *38* (16), 6895-6901.
48. Carbone, A. L.; Uhrich, K. E., *Macromolecular Rapid Communications* **2009,** *30* (12), 1021-1026.
49. Bryers, J. D.; Jarvis, R. A.; Lebo, J.; Prudencio, A.; Kyriakides, T. R.; Uhrich, K., *Biomaterials* **2006,** *27* (29), 5039-5048.
50. Erdmann, L.; Macedo, B.; Uhrich, K. E., *Biomaterials* **2000,** *21*, 2507-2512.
51. Harten, R. D.; Svach, D. J.; Schmeltzer, R.; Uhrich, K. E., *Journal of Biomedical Materials Research Part A* **2005,** *72A* (4), 354-362.
52. Rosenberg, L. E.; Carbone, A. L.; Römling, U.; Uhrich, K. E.; Chikindas, M. L., *Letters in Applied Microbiology* **2008,** *46* (5), 593-599.
53. Whitaker-Brothers, K.; Uhrich, K., *Journal of Biomedical Materials Research Part A* **2004,** *70A* (2), 309-318.
54. Yeagy, B. A.; Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E.; Cook, T. J., *Journal of Microencapsulation* **2006,** *23* (6), 643-653.
55. Jabara, R.; Chronos, N.; Robinson, K., *Catheterization and Cardiovascular Interventions* **2008,** *72*, 186-194.
56. Johnson, M. L.; Uhrich, K. E., *Journal of Biomedical Materials Research Part A* **2009,** *91A* (3), 671-678.
57. Ouimet, M. A.; Uhrich, K. E., *Polymeric Materials: Science & Engineering* **2012,** *107*, 575-576.

2. FORMULATION OF SALICYLATE-BASED POLY(ANHYDRIDE-ESTER) MICROSPHERES FOR LONG-TERM SALICYLIC ACID RELEASE

2.1. Introduction

Polymer microspheres are widely used as delivery systems for encapsulated drugs,[1-4] proteins,[5] and genes/DNA[1-3, 6] for a variety of biomedical applications. Microspheres are preferred over other formulations as they can be injected into the body rather than surgically implanted (Scheme 2.1).[7] Previously, our laboratory reported the formulation of salicylate-based poly(anhydride esters) (PAEs) (Figure 2.1) into microspheres for controlled delivery of salicylic acid (SA)[8], a non-steroidal anti-inflammatory drug (NSAID).



Scheme 2.1. Representation of the implantation benefits of microspheres. Large drug delivery devices need surgical implantation whereas microspheres can be conveniently injected.

SA exhibits anti-inflammatory, anti-pyretic, and analgesic properties frequently used to treat inflammatory diseases.[9, 10] Localized delivery of an anti-inflammatory drug for an extended period of time, from microspheres could be beneficial to treat chronic inflammatory diseases that cause pain such as rheumatoid arthritis[11, 12] and osteoarthritis.[13-15] Microsphere injection can improve patient comfort and compliance, by controlling and localizing NSAID delivery, thus reducing undesired side-effects commonly observed with systemic drug administration (e.g., gastrointestinal disturbance and renal malfunction).[12, 16, 17]

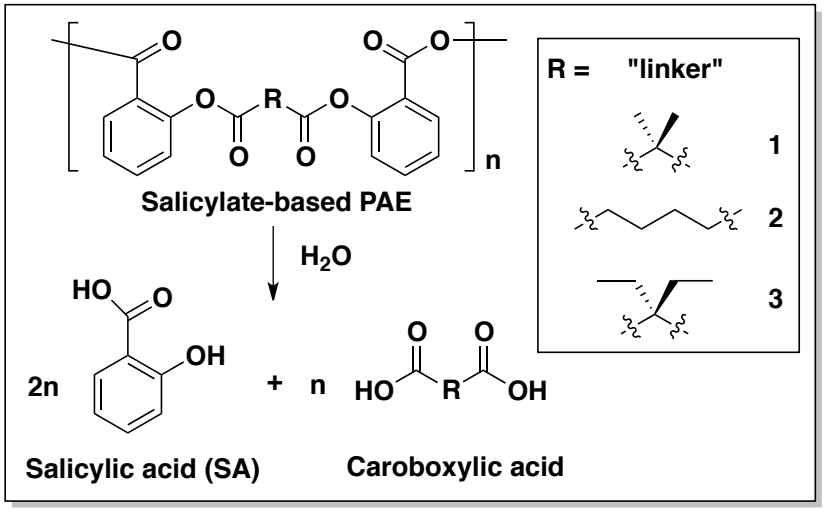


Figure 2.1. Structure of polymers **1-3** used to formulate salicylate-based PAE microspheres and their hydrolytic degradation to release SA.

The salicylate-based PAEs are unique in that SA is chemically incorporated into the polymer backbone via a "linker" molecule.[18-20] SA is released in a near zero-order fashion as the labile anhydride and ester bonds within the polymer are hydrolytically cleaved. These polymers have been extensively studied for the past decade and are biocompatible,[21-24] stable under storage conditions,[25] and can be exposed to ionizing radiation for sterilization without changing their physicochemical properties.[26] Furthermore, these PAEs allow for a higher percentage of drug loading (60-80 %) in microsphere formulations, compared to other biodegradable polymers such as poly(lactic-*co*-glycolic acid) microspheres encapsulating SA (20 %).[27]

Previous work on salicylate-based PAEs using disks has shown that SA release can be tuned to last over varying time periods by using different linkers within the polymers (Figure 2.1),[18] The use of a linear aliphatic linker (Figure 2.1, **2**) results in drug release that lasts few weeks, whereas a branched aliphatic linker (Figure 2.1, **1** and **3**) can prolong the release to months.[18] The formulation of polymer **2** into microspheres was previously reported, however, the formulation and characterization of polymer (**1** and **3**) microspheres for long-term drug release (months) are reported herein for the first time. It is important to note that as PAEs are surface-eroding polymers, the geometry of the device (disks vs. microspheres) will likely have an effect on the release profile.[28]

In this chapter, the optimization of a previously published microsphere preparation method to achieve SA release over a long time period (i.e., months), while not significantly altering physicochemical properties is presented. The initial PAE-based microsphere formulation was prepared by a well-established oil-in-water single emulsion

solvent evaporation method.[8] However, the initial microsphere formulation exhibited significant aggregation and non-spherical morphology, which are undesirable for uniform and predictable drug release. Therefore, to improve the quality of the microspheres, homogenization speed was decreased and polymer concentration was increased during formulation.

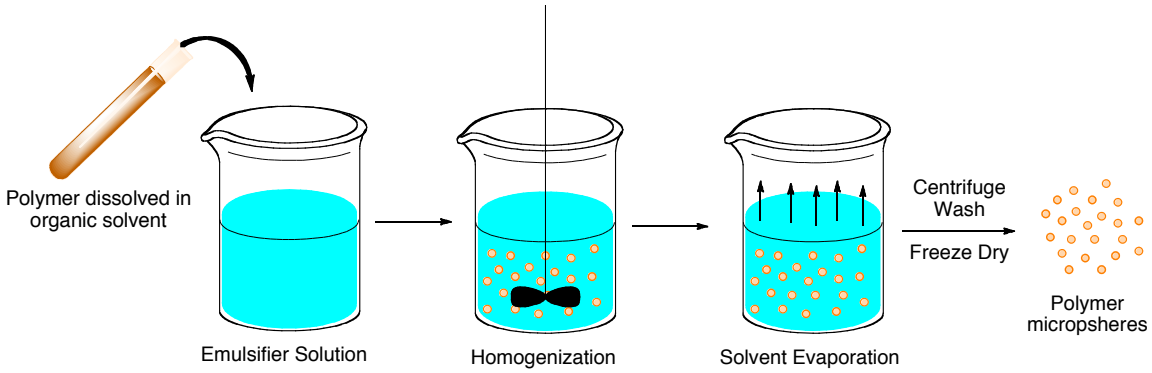
Microspheres molecular weight (M_w), polydispersity index (PDI) and glass transition temperature (T_g) were measured and compared to the pre-formulated polymer to determine the formulation effect on the polymer composition and properties. Scanning electron microscopy (SEM) was used to determine the microsphere size and morphology. *In vitro* studies were performed on the polymer microspheres to investigate SA release.

2.2. Results and Discussion

Previous work on salicylate-based PAEs using pressed disks demonstrated that the physical properties and the SA release rate can be significantly altered by changing the chemical composition of the polymer via the linker (Figure 2.1).[18] Salicylate-based PAEs with linear aliphatic linkers (including **2**) were previously formulated into microspheres, but were relatively small in size (i.e., 1-10 μm in diameter), exhibited aggregation, and achieved drug release for 12 days.[8] In this study, the formulation was improved to overcome the aforementioned issues in addition to formulating polymers **1** (linker with one degree of branching) and **3** (linker with two degrees of branching) into microspheres to achieve long- term (i.e., months) SA release.

2.2.1. Microspheres Preparation and Formulation Optimization

To optimize polymers **1-3** microsphere formulation, a modified oil-in-water single emulsion solvent evaporation technique was used (Scheme 2.2).[8] The formulation was first attempted using polymer **2** at a concentration of 0.10 g/mL and homogenization speed of ~ 25,000 rpm. To remove residual PVA, acidic water (pH 1) was used instead of neutral pH water to decrease potential polymer degradation after water exposure as the salicylate-based PAEs degrade at a slower rate under acidic conditions.[20] Two methods were used to isolate the microspheres: 1) vacuum filtration and drying *in vacuo* overnight at room temperature and 2) centrifugation and freeze-drying overnight. Microspheres of 1-10 μm in diameter with minimal aggregation were obtained (data not shown), but the yield was higher using centrifugation (80 %) compared to vacuum filtration (60 %). In an attempt to negate aggregation and increase microsphere size (surface area-to-volume ratio increases with decreasing particle size thus increasing degradation rates),[3, 29] the formulation method was further optimized by increasing polymer **2** concentration to 0.16 g/mL and decreasing homogenization speed to 10,000 rpm. With this approach, larger microspheres (4-34 μm in diameter) were obtained in 80 % yield without aggregation. This optimized method (0.16 g/mL polymer concentration, homogenization speed of 10,000 rpm, wash with acidic water, and isolation by centrifugation) was used to formulate microspheres of polymers **1** and **3**.



Scheme 2.2. Representation of the oil-in-water single emulsion solvent evaporation technique used to prepare the polymer microsphere.

2.2.2. Size and Morphology

To investigate the different time frames of complete SA release, polymers **2** and **3** were used to extend SA release. Polymer **2** was used as a reference point to compare microsphere properties against previously published data.[8] When evaluating morphology, narrow size distribution and smooth surfaces are essential properties to ensure uniform microsphere degradation and therefore, uniform drug release.[30] Also, microspheres exhibiting no aggregation are important because aggregated microspheres could be detrimental to the injection process by not passing through the needle.[30] Microspheres comprised of polymer **1-3** demonstrated size distributions of 2-46 μm, 4-34 μm, and 2-31 μm, respectively, as depicted in the representative SEM images (Figure 2.2 A-C). Overall, these microspheres are larger in size as compared to previously published data (1-10 μm in diameter).[8] No signs of aggregation were noted and all microspheres displayed smooth surfaces, and are therefore viable candidates as drug delivery systems.

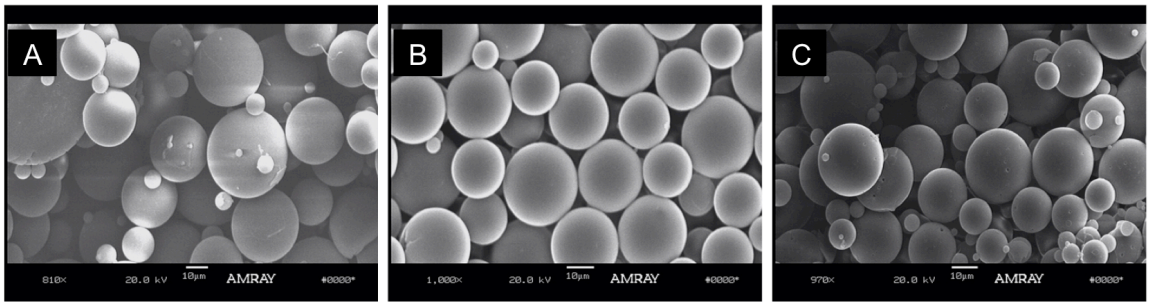


Figure 2.2. Representative scanning electron microscopy images of microspheres prepared from polymers **1** (A), **2** (B), and **3** (C).

2.2.3. Molecular Weight and Glass Transition Temperature

The M_w and T_g of the microspheres were determined and compared to the values obtained for the unprocessed polymers (Table 2.1). A large reduction in M_w may lead to lost of polymeric properties and increase PDI by the formation of oligomers. The T_g is important for the preservation of the microspheres shape after being injected in the body. A consistent decrease in M_w and increase in PDI (except for **1**) compared to the unprocessed polymer was observed for all microspheres. These changes were expected for these hydrolytically degradable polymers, as the polymers were exposed to water during formulation. However, all polymer microspheres (**1-3**) preserved a relatively high M_w (only decreasing 7 %, 7 %, and 19 %, respectively). The changes in T_g values did not follow a specific trend after formulation for all sets of microspheres. All samples had T_g values above 37 ^{o}C, which ensure their shape will remain when placed *in vivo*.

Table 2.1. Molecular weight (polymer and microspheres), polydispersity index (polymer and microspheres), glass transition temperature (polymer and microspheres), and % yield (microspheres).

	M_w (Da) polymer	M_w (Da) microspheres	PDI polymer	PDI microspheres	T_g (^oC) polymer	T_g (^oC) microspheres	% Yield microspheres
1	28,000	25,000	1.5	1.3	67	79	80
2	18,000	16,800	1.3	1.9	50	51	80
3	11,000	9,000	1.4	1.7	57	54	70

2.2.4. *In Vitro* Drug Release

In vitro drug release from the microspheres was studied to determine the SA release profiles (Figure 2.3). Microspheres prepared from polymer **2** released 100 % SA without the presence of a lag time (i.e., an initial time period where SA is not released), whereas polymer **1** and **3** microspheres released SA after a 5 and 10 day lag period, respectively. Release rates for all microspheres correlate to the linkers used: the microspheres containing the relatively more hydrophilic polymer **2** (contact angle 77°)[18] released 100 % of the drug in a shorter period of time (21 days). The microspheres containing the polymers with branched aliphatic linkers **1** and **3**, being relatively more hydrophobic (contact angle 95 and 93°, respectively)[18] and sterically hindered, released the drug at a slower rate; polymer **1** microspheres released 82 % SA in 21 days, whereas polymer **3** microspheres released 21 % SA over 21 days. Complete (100 %) SA release is projected to reach within 1 month for **1** and 3.5 months for **3** at this rate.

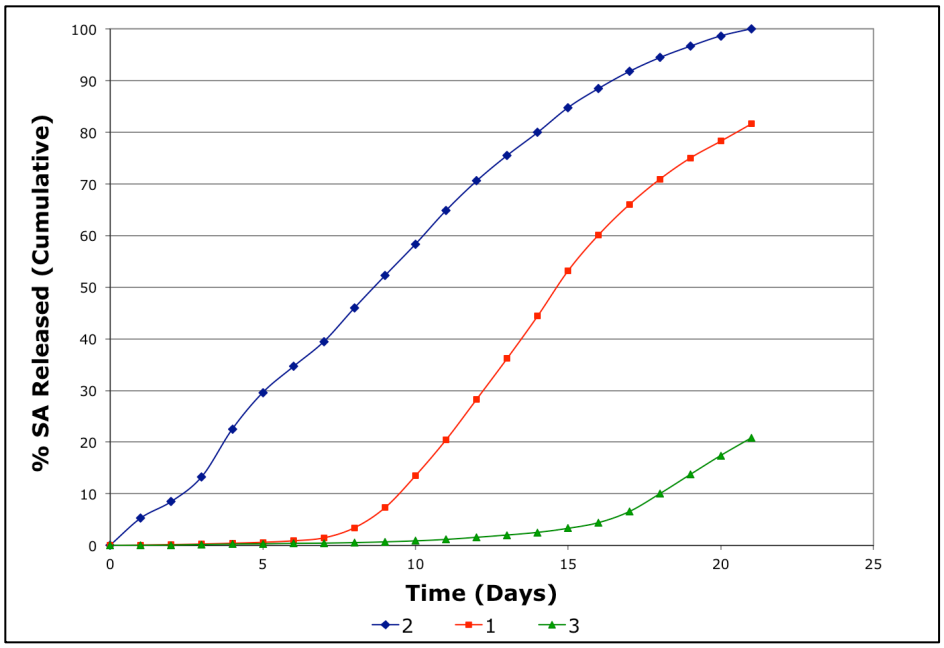


Figure 2.3. Normalized *in vitro* SA release from polymer microspheres (SA ± standard error): polymer **1** (filled squared/red), polymer **2** (filled diamonds/blue), and polymer **3** (filled triangles/green).

2.3. Conclusion

Polymer microspheres were prepared using three different salicylate-based PAEs. The modified oil-in-water single emulsion solvent evaporation method was successfully used to obtain microspheres with size distributions ranging from 2-46 μm and smooth surfaces without aggregation. The M_w, PDI, and T_g of the microspheres were studied and compared to the polymer prior to formulation where decreases in M_w, increases in PDI, and only minor changes in the T_g were observed. The SA release profile can be altered to last for months, a feature not yet attained from previous salicylate-based PAE

microspheres, by changing the chemical composition of the polymer used via the linker molecule. The ability to extend SA release over months using these microspheres provides great promise for the treatment of inflammatory diseases. Future studies will focus on the *in vivo* testing of the microspheres, overcoming the lag period observed for the slower degrading polymer microspheres, and encapsulating bioactives.

2.4. Experimental

2.4.1. Materials

All chemicals and reagents were purchased from Sigma-Aldrich (Milwaukee, WI) and used as received unless otherwise noted.

2.4.2. Polymer Synthesis

Salicylate-based PAEs were synthesized and characterized using previously reported methods.[18, 19, 22] In brief, SA (2 equivalents (eq)) was dissolved in THF and pyridine (4 eq). Diacyl chloride (R = "linkers" **1-3**, Figure 1) (1 eq) was diluted in THF and added drop-wise to the stirring reaction mixture to give a white suspension. The reaction mixture was allowed to stir overnight at room temperature and subsequently quenched by pouring over excess water and adding concentrated HCl until pH = 2. The obtained solid was filtered, washed with water, and dried *in vacuo* at room temperature.

Polymer precursors were activated in excess acetic anhydride (Fisher, Fair Lawn, NJ) at room temperature and polymerized using a previously described melt-condensation polymerization method.[18, 19, 22]

2.4.3. Microspheres Preparation

Polymers were formulated into microspheres using a modified procedure of a published oil-in-water single emulsion solvent evaporation technique (Scheme 2.2).[8] In general, salicylate-based PAEs (0.50 g) were dissolved in dichloromethane (3 mL) and added drop-wise to 1% aqueous poly(vinyl alcohol) (PVA) (80 % hydrolyzed, 30-70 kDa) solution (80 mL) at room temperature. The emulsion was homogenized for 2 min using an IKA Ultra-Turrax T8 homogenizer at approximately 10,000 rpm. The homogenized solution was left stirring for 2 h to allow microsphere formation by solvent evaporation. Microspheres were transferred to sterile 50 mL polypropylene conical tubes (30 x 115 mm style, BD Falcon, Franklin Lakes, NJ), washed with acidic water (pH 1) to remove residual PVA, and isolated by centrifugation at 3,000 rpm for 10 min. Microspheres were frozen by placing the conical tubes in a dry ice/acetone bath and lyophilized for 24 h at -40 ˚C and 133×10^{-3} mBar (LABCONO Freeze Dry System/Freezon 4.5).

2.4.4. Size and Morphology

Size and morphology of the microspheres were determined using SEM. Images

for each set of microspheres were obtained using an AMRAY-1830I microscope (AMRAY Inc.) after coating the samples with Au/Pd using a sputter coater (SCD 004, Blazers Union Limited). SEM images of each polymer microsphere sample were then analyzed using NIH ImageJ software. Distributions of particle diameter were obtained by evaluating > 50 particles per sample.

2.4.5. *In Vitro* Drug Release

SA release from polymer microspheres was studied at 37 ˚C in phosphate buffered saline (PBS) at pH 7.4 with agitation (60 rpm) to mimic physiological conditions. Triplicate samples of each set of microspheres (20.0 mg) were suspended in 20 mL of PBS. At predetermined time points, samples were centrifuged at 3,000 rpm for 5 min (Hettich Zentrifugen EBA12) to allow microspheres to settle to the bottom. Aliquots of the supernatant (15 mL) were collected and replaced with fresh PBS (15 mL). Spent media was analyzed via ultraviolet/visible spectroscopy at $\lambda = 303$ nm (wavelength at which SA is the only degradation product to absorb) using a Perkin Elmer Lambda XLS spectrophotometer to monitor SA release. Data was analyzed against known concentrations of SA and normalized to the amount of SA in the sample.

*Other methods described in Chapter 9.

2.5. References

1. Vasir, J. K.; Tambwekar, K.; Garg, S., *International Journal of Pharmaceutics* **2003,** *255* (1-2), 13-32.

2. Freitas, S.; Merkle, H. P.; Gander, B., *Journal of Controlled Release* **2005,** *102* (2), 313-332.
3. Freiberg, S.; Zhu, X. X., *International Journal of Pharmaceutics* **2004,** *282* (1-2), 1-18.
4. Edlund, U.; Albertsson, A., Degradable Polymer Microspheres for Controlled Drug Delivery Degradable Aliphatic Polyesters. Springer Berlin / Heidelberg: 2002; Vol. 157, pp 67-112.
5. Lee, K. Y.; Yuk, S. H., *Progress in Polymer Science* **2007,** *32* (7), 669-697.
6. Díez, S.; Tros de Ilarduya, C., *European Journal of Pharmaceutics and Biopharmaceutics* **2006,** *63* (2), 188-197.
7. Kipper, M. J.; Shen, E.; Determan, A.; Narasimhan, B., *Biomaterials* **2002,** *23* (22), 4405-4412.
8. Yeagy, B. A.; Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E.; Cook, T. J., *J Microencapsul* **2006,** *23* (6), 643-53.
9. Hersh, E. V.; Moore, P. A.; Ross, G. L., *Clinical Therapeutics* **2000,** *22* (5), 500-548.
10. O'Neil, M.; Heckelman, P.; Koch, C.; Roman, K., *The Merck Inex*. 14th ed.; 2006.
11. Butoescu, N.; Jordan, O.; Doelker, E., *European Journal of Pharmaceutics and Biopharmaceutics* **2009,** *73* (2), 205-218.
12. Mitragotri, S.; Yoo, J.-W., *Archives of Pharmacal Research* **2011,** *34* (11), 1887-1897.
13. Zhang, Z.; Huang, G., *Journal of Nanotechnology* **2012,** *2012*.
14. Gerwin, N.; Hops, C.; Lucke, A., *Advanced Drug Delivery Reviews* **2006,** *58* (2), 226-242.
15. Seed, S. M.; Dunican, K. C.; Lynch, A. M., *Osteoarthritis: A review of treatment options*. Advanstar Communications: Duluth, MN, ETATS-UNIS, 2009; Vol. 44, p 9.
16. Suleyman, H.; Demircan, B.; Karagoz, Y., *Pharmacol Rep* **2007,** *59* (3), 247-58.
17. Laine, L., *Journal of Pain and Symptom Management* **2003,** *25* (2), 32-40.
18. Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E., *Macromolecules* **2005,** *38* (16), 6895-6901.
19. Schmeltzer, R. C.; Anastasiou, T. J.; Uhrich, K. E., *Polymer Bulletin* **2003,** *49* (6), 441-448.
20. Erdmann, L.; Uhrich, K. E., *Biomaterials* **2000,** *21* (19), 1941-1946.
21. Erdmann, L.; Macedo, B.; Uhrich, K. E., *Biomaterials* **2000,** *21* (24), 2507-12.
22. Schmeltzer, R. C.; Johnson, M.; Griffin, J.; Uhrich, K., *J Biomater Sci Polym Ed* **2008,** *19* (10), 1295-306.
23. Reynolds, M. A.; Prudencio, A.; Aichelmann-Reidy, M. E.; Woodward, K.; Uhrich, K. E., *Curr Drug Deliv* **2007,** *4* (3), 233-9.
24. Bryers, J. D.; Jarvis, R. A.; Lebo, J.; Prudencio, A.; Kyriakides, T. R.; Uhrich, K., *Biomaterials* **2006,** *27* (29), 5039-48.
25. Deronde, B. M.; Carbone, A. L.; Uhrich, K. E., *Polym Degrad Stab* **2010,** *95* (9), 1778-1782.
26. Rosario-Meléndez, R.; Lavelle, L.; Bodnar, S.; Halperin, F.; Harper, I.; Griffin, J.; Uhrich, K. E., *Polymer Degradation and Stability* **2011,** *96* (9), 1625-1630.

27. Tang, Y.; Singh, J., *International Journal of Pharmaceutics* **2008,** *357* (1-2), 119-125.
28. Whitaker-Brothers, K.; Uhrich, K., *Journal of Biomedical Materials Research Part A* **2006,** *76A* (3), 470-479.
29. Kim, K. K.; Pack, D. W., Microspheres for Drug Delivery BioMEMS and Biomedical Nanotechnology. Ferrari, M.; Lee, A. P.; Lee, L. J., Eds. Springer US: 2006; pp 19-50.
30. Haruma, K., *Progress in Polymer Science* **2000,** *25* (8), 1171-1210.

3. MODIFICATION OF THE PHYSICOCHEMICAL PROPERTIES AND DRUG RELEASE PROFILES OF SALICYLATE-BASED POLY(ANHYDRIDE-ESTERS) USING COPOLYMERS AND BLENDS

3.1. Introduction

Salicylate-based poly(anhydride-esters) (PAEs) are innovative drug delivery systems that chemically incorporate salicylic acid (SA) into the polymer backbone (Figure 3.1).[1-4] These polymers achieve high drug loadings (60-80 %), and undergo hydrolytic degradation and releases the drug in a controlled fashion, exhibiting near zero-order release kinetics.[5]

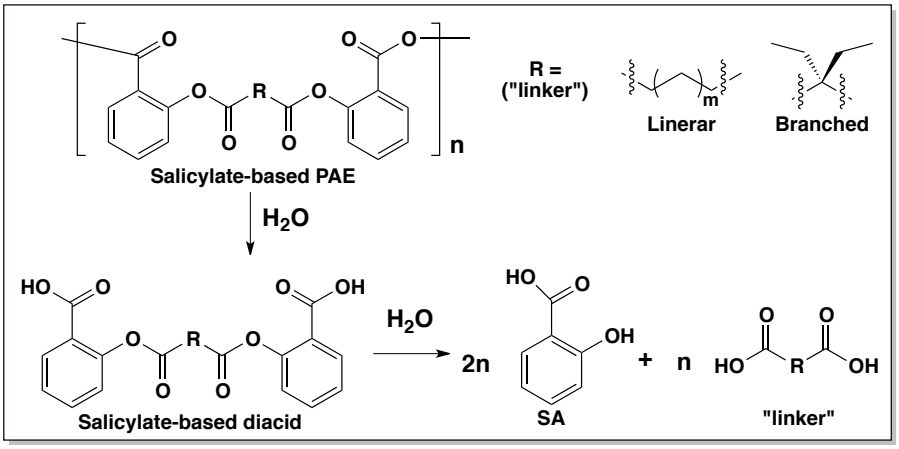


Figure 3.1. Structure and hydrolytic degradation of salicylate-based polymers.

The physicochemical properties and drug release profiles of the salicylate-based PAEs can be altered by varying the hydrophobicity of the "linker" molecule used.[3, 6, 7] Previous

work has shown that increases in the hydrophobicity of the "linker" results in slower drug release and increased lag time (time period with little to no drug release).[3] Extended release of SA could be beneficial for treatment of chronic inflammatory diseases that cause pain; for example, rheumatoid arthritis and osteoarthritis.[8-10] However, the presence of a lag time is unfavorable for constant long-term drug release. The composition of the salicylate-based PAEs must be modified to achieve this goal. To modify the physicochemical properties and drug release profiles of the salicylate-based PAEs beyond changing the "linker", we have explored the use of copolymers and polymer blends.

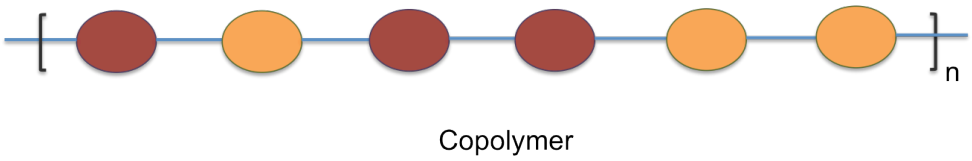


Figure 3.2. Representation of the chemical structure of a random copolymer with two different monomers (represented by brown and gold).

Copolymerization has been used to modify polymer structure and properties for various biomedical applications (Figure 3.2). Thus, it may be possible to alter the properties of the salicylate-based PAEs using copolymerization. One of the most common random copolymers used in therapeutic devices is poly(lactic-*co*-glycolic acid) (PLGA).[11, 12] Many studies have shown that the degradation profile of this copolymer can be altered by modifying the ratio of glycolic acid- and lactic acid-based monomers.[11, 13, 14] In addition, the effect of combining two or more monomers through copolymerization on the degradation kinetics has been studied with PLGA-*co*-poly(ethylene glycol),[14, 15]

poly(lactic-*co*-hydroxymethyl glycolic acid),[16] poly[(*p*-carboxyphenoxy propane)-*co*-(sebacic anhydride)],[17] poly[(*p*-carboxy phenyl)-*co*-(adipic acid)],[18] poly[(*p*-carboxy phenyl)-*co*-(succinic acid)],[18] poly[(sebacic anhydride)-*co*-caprolactone],[19] and other examples. Although copolymers of salicylate-based PAE with either *p*-carboxyphenoxyhexane or glycolic acid were previously synthesized,[6, 20] (resulting in slow- and fast-degrading copolymers, respectively) these chemical modifications compromised the high drug loading that characterize the salicylate-based PAEs.[6, 20]

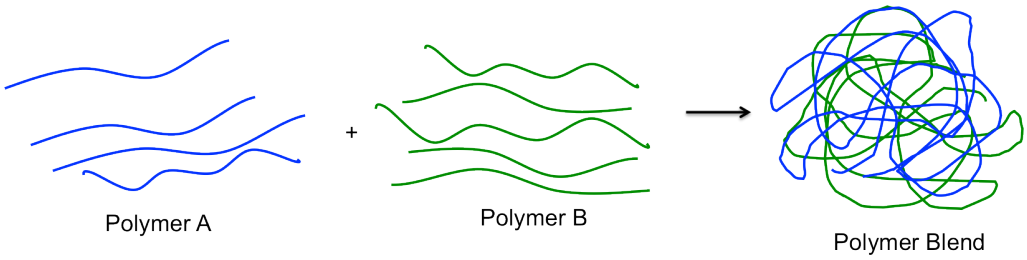


Figure 3.3. Representation of the formation of a miscible polymer blend.

Blending of two homopolymers is another method of modifying the physicochemical properties of polymers (Figure 3.3). Polymer blends do not alter the chemical structure of the polymers and have been previously examined as candidates for controlled drug delivery. Blends of polycaprolactone/polyanhydrides,[21] poly(trimethylene carbonate)/poly(adipic anhydride),[22] poly(propylene fumarate)/PLGA,[23] poly(sebacic anhydride)/poly(*p*-carboxyphenoxyhexane),[24] PLGA/poly(ethylene oxide),[25] and poly(*o*-carboxyphenoxy)-*p*-xylene)/poly(lactic acid)[26] have been prepared and has been suggested that the degradation rate of the polymer can be modified by varying the ratio of each polymer. Therefore, we explored the physical modification of the salicylate-based

PAEs using blends.

This chapter presents the modification of the physicochemical properties of salicylate-based PAEs using copolymers and blends, thus, reducing the lag time and achieving long-term drug release. In this study, two salicylate-based PAEs were used [with adipic linker (SA-adipic) and diethylmalonic linker (SA-DEM), Figure 3.4] at molar ratios of 75:25, 50:50, and 25:75. The chemical composition of the copolymers and blends was characterized by proton nuclear magnetic resonance (^{1}H-NMR). In addition, the molecular weight (M_w) (homopolymers and copolymers only), glass transition temperature [27], and contact angle were determined. The *in vitro* SA release from the copolymers and blends was studied mimicking physiological conditions.

3.2. Results and Discussion

To overcome the lag time associated with slow-degrading salicylate-based PAEs that have potential for long-term treatment of chronic inflammatory diseases, novel copolymers and blends were prepared.

3.2.1. Copolymers

To chemically modify the salicylate-based PAEs, random copolymers were synthesized (Figure 3.4). The SA-adipic and the SA-DEM diacids were combined in 75:25, 50:50, and 25:75 molar ratios, acetylated to form monomers, and polymerized *in situ* by melt-condensation polymerization.

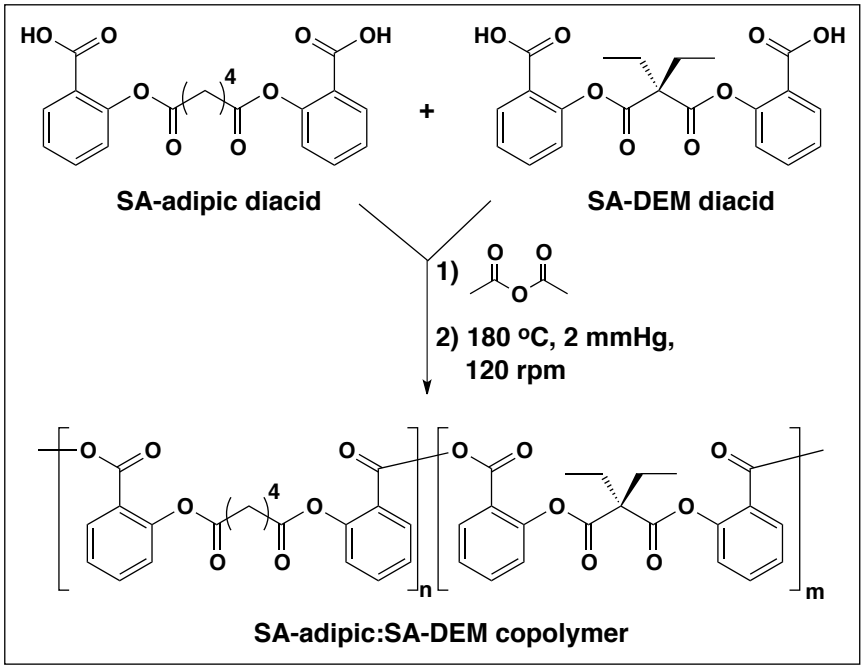


Figure 3.4. Synthesis of salicylate-based PAE copolymers by melt-condensation polymerization at 75:25, 50:50, and 25:75 molar ratios.

As summarized in Table 3.1, all polymers had M_w between 10,300 – 19,600 Da and relatively narrow PDI (1.4 – 1.6). Experimental ratios of each copolymer were determined using ^{1}H-NMR spectroscopy, calculated using the integration of the methylene protons of the SA-adipic units at δ 1.65 and the methyl protons of the SA-DEM units at δ 0.95 (Table 3.1). Consistently, a higher ratio of SA-adipic unit was obtained in each copolymer. The experimental ratios suggest that the SA-adipic monomer is more reactive (~ 1.5 times more reactive) than the SA-DEM monomer. These copolymers maintained high drug loading (69 – 71%) as compared to previously synthesized copolymers.[6, 20]

Table 3.1. Copolymers: theoretical and experimental ratios (calculated from ^{1}H-NMR spectra), M_w and PDI determined by GPC, T_g determined by DSC, and contact angle (CA) determined by goniometer.

SA-adipic:SA-DEM Theoretical	SA-adipic:SA-DEM Experimental	M_w (Da)	PDI	T_g (°C)	CA (°)
100:0	100:0	15,800	1.5	37	74
75:25	89:11	11,400	1.4	63	75
50:50	66:44	11,200	1.4	68	83
25:75	46:54	10,300	1.4	68	85
0:100	0:100	19,600	1.6	88	86

The thermal properties of the copolymers were studied finding that as the SA-DEM content increased, the T_g increased (from 37 ^{o}C for the SA-adipic to 88 ^{o}C for the SA-DEM). The thermograms displaying this trend are shown in Figure 3.5 (left). In addition to determining the T_g, contact angle measurements were used to determine the relative hydrophobicity of the polymers (using deionized water as contacting liquid). The results showed that as the SA-DEM (more hydrophobic) ratio in the copolymer, the contact angle also showed a noticeable increase. Figure 3.5 (right) shows the contact angles as a function of SA-DEM composition.

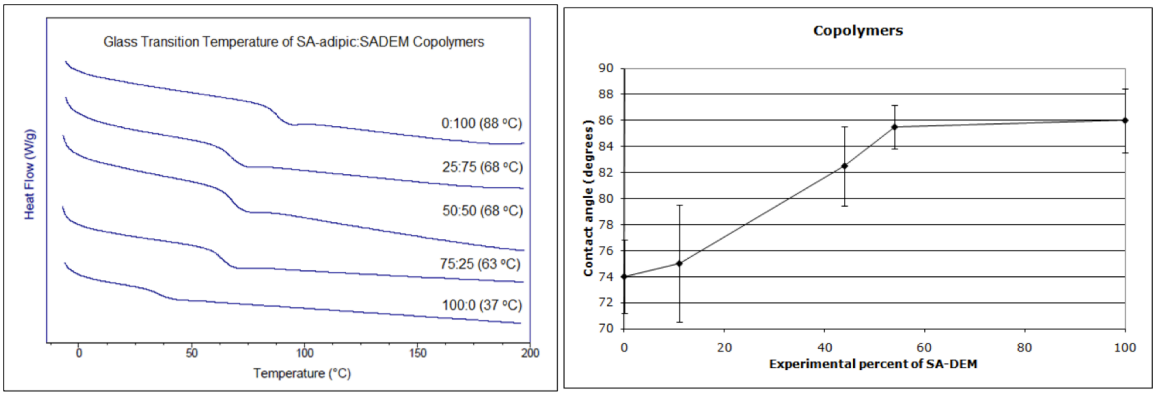


Figure 3.5. Differential scanning calorimetry thermograms of copolymers and homopolymers included for comparison (left). Water contact angle of copolymer (right) as a function of SA-DEM composition.

3.2.2. Blends

For the physical combination of salicylate-based PAEs, SA-adipic and SA-DEM homopolymers (synthesized by melt-condensation polymerization) were combined and blends prepared by solvent casting.[28] The SA-adipic polymer and the SA-DEM polymer were dissolved in DCM at 75:25, 50:50, and 25:75 weight ratios. Samples were dried and analyzed using ^{1}H-NMR spectroscopy to determine the experimental ratios of homopolymer incorporation in the blend. The same method used with the copolymers (using the integration of the methylene protons of the SA-adipic units at δ 1.65 and the methyl protons of the SA-DEM units at δ 0.95) was used to calculate blends experimental ratios (Table 3.2). The blends also preserved the high drug loading (ranging from 69 – 71 %), however, in this case the experimental ratios were closer to the theoretical values.

Table 3.2. Blends: theoretical and experimental ratios (calculated from ^{1}H-NMR spectra), T_g determined by DSC, and contact angle (CA) determined by goniometer.

SA-adipic:SA-DEM Theoretical	SA-adipic:SA-DEM Experimental	T_g (°C)	CA (°)
100:0	100:0	37	74
75:25	80:20	42	79
50:50	57:43	41	81
25:75	36:64	47	83
0:100	0:100	88	86

The T_g of the blends was studied to determine miscibility and the changes in thermal properties. When only one T_g is visible, the blend is considered miscible.[29, 30] The results (shown in Figure 3.6, left) indicate that the two polymers are miscible because a single T_g value was obtained for each blend. Comparable to the copolymers, as the content of SA-DEM component is increased, a noticeable increase in T_g is observed. The hydrophobicity of the blends was studied using contact angle measurements. Figure 3.6 (right) shows the contact angles as a function of SA-DEM composition, as the SA-DEM ratio in the copolymer and blend increased, the contact angle also increased (from 74 ^{o} to 85 ^{o}).

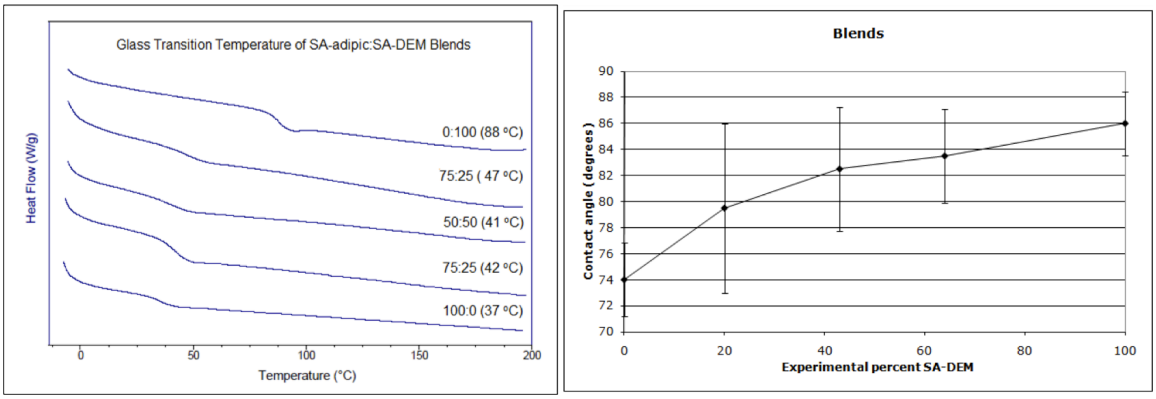


Figure 3.6. Differential scanning calorimetry thermograms of copolymers and homopolymers included for comparison (left). Water contact angle of copolymer (right) as a function of SA-DEM composition.

3.2.3. *In Vitro* Drug Release

The *in vitro* drug release profiles of the copolymers and blends under mimicking physiological conditions (i.e., pH 7.4, 37 °C, and 60 rpm) were compared to that of the homopolymers. Figure 3.7 shows the cumulative percent release of SA over 28 days from the homopolymer, copolymers, and blends. Previous work in our research group showed that the SA-adipic polymer degrades significantly faster than the SA-DEM polymer and without a lag time.[3] As shown in Figure 3.7, the lag time was decreased in all copolymers and blends. The SA-DEM ratio is directly proportional to the lag time for all samples. When the copolymers and blends of the same ratios are compared, the blends consistently displayed shorter lag times. The shorter lag time may be due to the physical interactions, rather than chemical, taking place. The copolymers containing 75:25 ratios and the 50:50 copolymer released equal or higher percentages of SA than the SA-adipic homopolymers

after 28 days, which suggests that long-term drug release may not be achieved. The blend containing 50:50 ratio of SA-Adipic:SA-DEM and the 25:75 copolymer samples are promising in terms of reducing lag time and sustaining drug release for an extended time period.

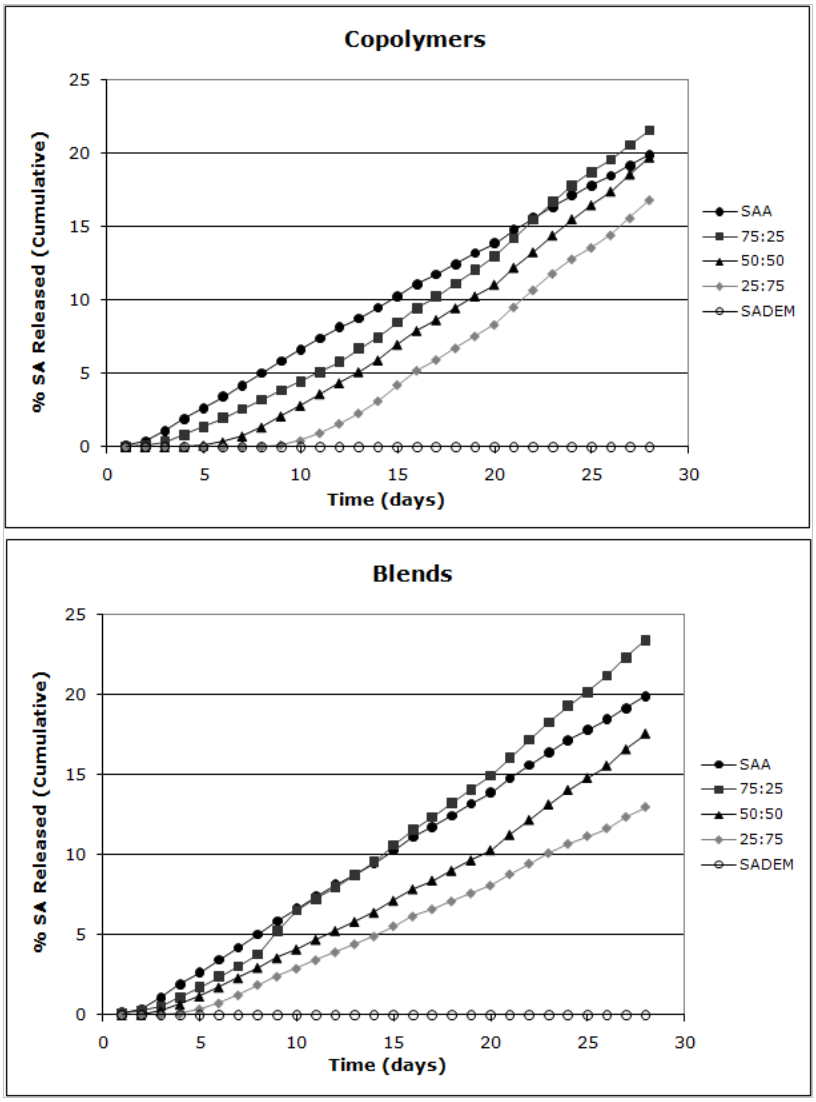


Figure 3.7. *In vitro* salicylic acid release profiles from copolymers (top) and blends (bottom). Homopolymers release profiles were included for comparison. SAA, 75:25, 50:50, 25:75, and SA-DEM.

3.3. Conclusion

The physicochemical properties of salicylate-based PAEs were modified through blending and copolymerization. By chemically and physically combining the salicylate-based PAEs, it was possible to obtain a range of physicochemical properties proportional their composition (SA-Adipic:SA-DEM ratio), without lowering the drug loading capacity. It was also possible to decrease the SA release lag time associated to the SA-DEM release profile. The chemical composition of the copolymers and blends showed a consistently higher ratio of SA-adipic component. The T_g and contact angles increased for all samples as the SA-DEM fraction was increased. Also, the SA-DEM ratio is directly proportional to lag time of SA release seen in all samples.

3.4. Experimental

3.4.1. 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.

[Leonid Garber (Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ) contributed to this work]

3.4.2. Polymers and Copolymers Synthesis

Polymers were synthesized using previously reported methods.[2, 3] In brief, to synthesize the diacids, SA (2 eq) was dissolved in tetrahydrofuran and pyridine (4 eq). Adipoyl chloride or diethylmalonyl chloride (1 eq) was diluted in THF and added drop-wise to the stirring reaction mixture to yield a white suspension. The reaction mixture was allowed to stir overnight at room temperature and subsequently quenched by pouring over excess water and adding concentrated HCl until pH ~2. The obtained solid was filtered, washed with water, and dried under vacuum at room temperature. Each diacid (for the copolymers, both diacids were combined in one flask) was activated in excess acetic anhydride at room temperature overnight. Acetic anhydride was removed under reduced pressure, and the monomers were polymerized by melt-condensation at 180 °C under vacuum and mechanical stir for 15-24 h. Homopolymers and copolymers were isolated by precipitation over excess diethyl ether, followed by decantation and vacuum drying.

3.4.3. Polymer Blends Preparation

SA-adipic and SA-DEM polymer blends were formulated with a modified procedure of solvent casting.[28] Homopolymers were combined in weight ratios of 75:25,

50:50, and 25:75 (750 mg total) and placed into polytetrafluoroethylene (PTFE) evaporating dishes, followed by addition of 2mL of anhydrous dichloromethane. The mixtures were manually stirred using a PTFE spatula for 15 min until the polymers were dissolved. The solutions were dried in a desiccator under atmospheric pressure for 6 h and then left to dry under vacuum overnight.

3.4.4. Contact Angle Measurements

Sessile-drop contact angles were measured on 13 mm diameter x 1 mm thickness disks with a model 100 Goniometer (Rame-Hart, Mountain Lakes, NJ). The angle formed at the polymer disk/water interface was measured as the contact angle. A digital camera using DROPimage Advance software on a Dell Dimension 3000 computer was used to measure the contact angles. A total of 6 measurements were performed on each disk.

3.4.5. *In Vitro* Drug Release

Disks (triplicate) were placed into scintillation vials and 10 mL phosphate buffered saline (PBS) pH 7.4 added. Samples were incubated at 37 °C under constant shaking (60 rpm) in an Excella E25 Incubator Shaker (New Brunswick Scientific, New Brunswick, NJ). PBS was removed daily and replaced with fresh PBS. Spent media was analyzed by UV/visible spectroscopy (Perkin-Elmer Lambda XLS) at $\lambda = 303$ nm.

*Other methods described in Chapter 9.

3.5. References

1. Erdmann, L.; Uhrich, K. E., *Biomaterials* **2000,** *21* (19), 1941-1946.
2. Schmeltzer, R. C.; Anastasiou, T. J.; Uhrich, K. E., *Polymer Bulletin* **2003,** *49* (6), 441-448.
3. Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E., *Macromolecules* **2005,** *38* (16), 6895-6901.
4. Schmeltzer, R. C.; Schmalenberg, K. E.; Uhrich, K. E., *Biomacromolecules* **2004,** *6* (1), 359-367.
5. Whitaker-Brothers, K.; Uhrich, K., *Journal of Biomedical Materials Research Part A* **2006,** *76A* (3), 470-479.
6. Carbone, A. L.; Uhrich, K. E., *Macromolecular Rapid Communications* **2009,** *30* (12), 1021-1026.
7. Rosario-Meléndez, R.; Ouimet, M. A.; Uhrich, K. E., *Polymer Bulletin* **2012,** *Manuscript accepted*.
8. Loren, L., *Journal of Pain and Symptom Management* **2003,** *25* (2, Supplement), 32-40.
9. Halis, L.; Berna, D.; Yalcin, K., *Anti-inflammatory and side effects of cyclooxygenase inhibitors*. Polish Academy of Sciences, Institute of Pharmacology: 2007; Vol. 59, p 12.
10. Hersh, E. V.; Moore, P. A.; Ross, G. L., *Clinical Therapeutics* **2000,** *22* (5), 500-548.
11. Makadia, H. K.; Siegel, S. J., *Polymers* **2011,** *3* (3), 1377-1397.
12. Thomas, C. M.; Lutz, J.-F., *Angewandte Chemie International Edition* **2011**, 2-5.
13. Mittal, G.; Sahana, D. K.; Bhardwaj, V.; Ravi Kumar, M. N. V., *Journal of Controlled Release* **2007,** *119* (1), 77-85.
14. Cheng, Y.; Deng, S.; Chen, P.; Ruan, R., *Frontiers of Chemistry in China* **2009,** *4* (3), 259-264.
15. Avgoustakis, K.; Beletsi, A.; Panagi, Z.; Klepetsanis, P.; Livaniou, E.; Evangelatos, G.; Ithakissios, D. S., *International Journal of Pharmaceutics* **2003,** *259* (1-2), 115-127.
16. Ghassemi, A.; van Steenbergen, M.; Talsma, H.; van Nostrum, C.; Crommelin, D.; Hennink, W., *Pharmaceutical Research* **2010,** *27* (9), 2008-2017.
17. Determan, A. S.; Trewyn, B. G.; Lin, V. S. Y.; Nilsen-Hamilton, M.; Narasimhan, B., *Journal of Controlled Release* **2004,** *100* (1), 97-109.
18. Jiang, H. L.; Zhu, K. J., *Biomaterials* **2001,** *22* (3), 211-218.
19. Miao, H.; Hao, J.; Liu, Y.; Liu, Y.; Deng, X., *Polymer International* **2008,** *57* (2), 316-323.
20. Whitaker-Brothers, K.; Uhrich, K., *Journal of Biomedical Materials Research Part A* **2004,** *70A* (2), 309-318.
21. Ben-Shabat, S.; Abuganima, E.; Raziel, A.; Domb, A. J., *Journal of Polymer Science Part A: Polymer Chemistry* **2003,** *41* (23), 3781-3787.
22. Edlund, U.; Albertsson, A.-C., *Journal of Applied Polymer Science* **1999,** *72* (2), 227-239.

23. Kempen, D. H. R.; Lu, L.; Zhu, X.; Kim, C.; Jabbari, E.; Dhert, W. J. A.; Currier, B. L.; Yaszemski, M. J., *Journal of Biomedical Materials Research Part A* **2004,** *70A* (2), 293-302.
24. Kipper, M. J.; Seifert, S.; Thiyagarajan, P.; Narasimhan, B., *Polymer* **2004,** *45* (10), 3329-3340.
25. Santander-Ortega, M.; Csaba, N.; González, L.; Bastos-González, D.; Ortega-Vinuesa, J.; Alonso, M., *Colloid & Polymer Science* **2010,** *288* (2), 141-150.
26. Griffin, J.; Carbone, A.; Delgado-Rivera, R.; Meiners, S.; Uhrich, K. E., *Acta Biomaterialia* **2010,** *6* (6), 1917-1924.
27. Mathiowitz, E.; Jacob, J.; Jong, Y.; Carino, G.; Chickering, D.; Chaturvedi, P.; Santos, C.; Vijayaraghavan, K.; Montgomery, S.; Bassett, M.; C, M., *Nature* **1997,** *386* (6623), 410-414.
28. Chan, C.-K.; Chu, I. M., *Biomaterials* **2002,** *23* (11), 2353-2358.
29. Lu, X.; Weiss, R. A., *Macromolecules* **1992,** *25* (12), 3242-3246.
30. Song, M.; Hammiche, A.; Pollock, H. M.; Hourston, D. J.; Reading, M., *Polymer* **1996,** *37* (25), 5661-5665.

4. STABILITY OF SALICYLATE-BASED POLY(ANHYDRIDE-ESTERS) TO ELECTRON BEAM AND GAMMA RADIATION

4.1 Introduction

Salicylic acid (SA) is the active metabolite of aspirin and an anti-inflammatory, anti-pyretic, keratolytic and analgesic drug widely used to treat different health conditions.[1, 2] A new mode of SA delivery is possible by the chemical incorporation of SA into a polymer backbone to yield salicylate-based poly(anhydride-esters) (PAEs).[3-5] The physicochemical properties of such polyanhydrides have been investigated over the last decade.[5-14] The ability to formulate these polymers into different geometries such as powders,[6] disks,[5, 9, 15] fibers,[7] microspheres,[10, 16, 17] etc. contributes to their wide scope of applications. In addition, these polymers allow for moderate to high SA loading, ranging from 60 - 80% active drug content, because of the direct insertion of the bioactive molecule into the polymeric backbone.[3] Upon exposure to water, the PAEs undergo hydrolytic degradation, releasing SA at different rates as a function of polymer composition.[5-7, 11] Temperature can also influence the polymer degradation rate; polymer **1** (Figure 4.1) is relatively stable in powder form when stored at low temperatures under an inert atmosphere. Yet, the PAEs degrade faster at temperatures above 25 ^{o}C[15] and in the presence of water (Figure 4.1). Further, the PAEs are shown to be non-toxic *in vitro*[14] and *in vivo*[9, 16, 18] and, therefore, have great potential in various biomedical applications.[6, 9, 13, 14, 18] However, the polymer should meet the pharmacopeial and commercial requirement of sterility.[19-23]

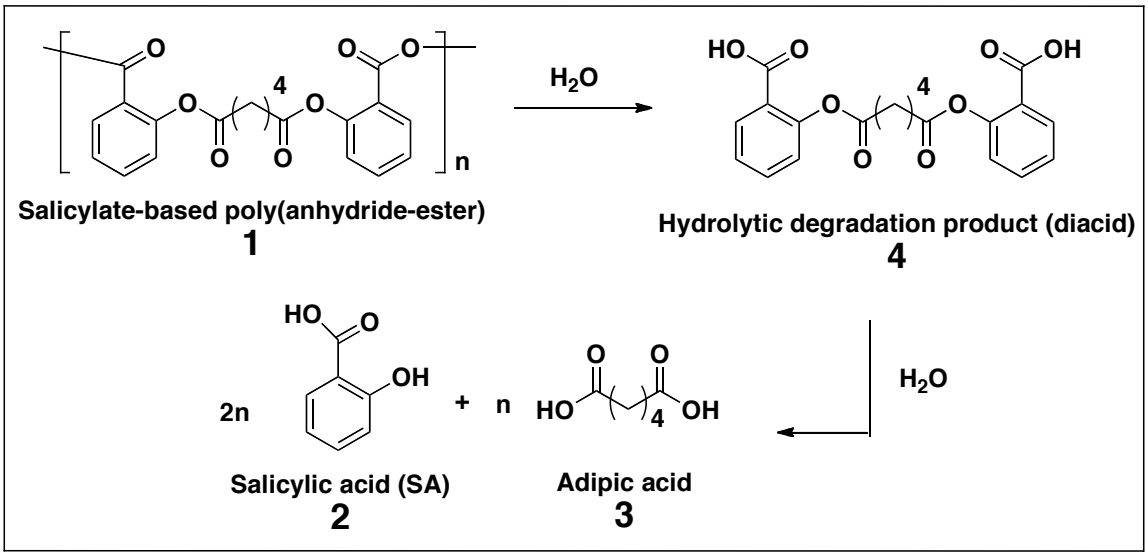


Figure 4.1. Hydrolytic degradation of salicylate-based poly(anhydride-ester) (**1**).

To manufacture these polymers for *in vivo* administration, the physicochemical properties and the SA release profile must remain relatively unchanged after sterilization and processing. Common sterilization methods for medical devices and drugs include dry heat at 150-170 °C, saturated steam at 115-132 °C and ethylene oxide exposure at 35-60 °C.[24, 25] All three options are not viable for sterilization of salicylate-based PAE, **1** because it is "designed to degrade", when placed in the body, which is an aqueous environment maintained at 37 ^{o}C.

As alternate sterilization methods, ionizing radiation such as electron-beam and gamma do not involve high temperatures or the presence of excessive moisture. Electron-beam (or e-beam) is an ionizing radiation generated using electricity and magnetism to accelerate electrons to a high energy level, whereas gamma is electromagnetic radiation emitted by man-made isotopes ^{60}Co and ^{137}Cs.[20] Both e-beam and gamma radiation are

also successfully employed for the sterilization of thermolabile medical devices and drug delivery systems.[26, 27] However, ionizing radiation can induce polymer chain scission. For example, gamma radiation reduced the molecular weight of poly($_{DL}$-lactic-*co*-glycolic acid) (PLGA),[24] poly($_{L}$-lactic acid) (PLLA),[28] and biodegradable polyurethanes.[19] Similarly, e-beam radiation increased polymer degradation rate by chain scission of PLGA and PLLA.[29] E-beam and gamma radiations can also change the polymer properties by cross-linking the chains.[20, 21] Both forms of ionizing radiation have been shown to cause polymer cross-linking of a PLLA copolymer[22] and the corresponding homopolymers, PLLA and PLGA.[28, 30]

To investigate the effect that e-beam and gamma radiation have on the physicochemical properties of salicylate-based PAEs, samples were exposed to both ionizing radiation processes (e-beam and gamma). Radiation exposure was performed at 25 (typical sterilization dose is 25 kGy)[31, 32] and 50 kGy (typical maximum processing dose) in each process by Linda Lavelle, Stanko Bodnar, Frederick Halperin, and Ike Harper, from Johnson & Johnson Sterile Process Technology (Raritan, NJ).

4.2 Results and Discussion

To determine the effect of e-beam and gamma radiation (25 and 50 kGy) on the physicochemical properties of polymer **1**, samples were analyzed for changes in color and texture. The weight-average molecular weight (M_w) and glass transition temperature (T_g), and decomposition temperature (T_d) were evaluated for polymer chain scissioning and/or crosslinking as well as changes in thermal properties. Proton nuclear magnetic

resonance (^{1}H-NMR) and infrared (IR) spectroscopies were also used to determine polymer degradation and/or chain scission. *In vitro* cell toxicity and SA release studies of polymer **1** were conducted following exposure. Furthermore, polymer **1** was formulated into microspheres to study the effect of the ionizing radiation on size and morphology of the microspheres. A second polymer (**2**), containing a branched aliphatic linker was also exposed to radiation and the changes in physicochemical properties evaluated.

4.2.1. Qualitative Assessment

Qualitatively, polymer **1** was visually analyzed for changes in color and texture (not shown). No color change was observed in the irradiated samples (25 and 50 kGy, e-beam and gamma) compared to the unexposed (samples that traveled and were treated with 0 kGy of each radiation) polymer controls. Further, all samples remained as loose powders following irradiation.

4.2.2. Physicochemical Properties

Changes in M_w and T_g values are potential indicators of polymer degradation and/or crosslinking following radiation exposure. As shown in Table 4.1, sample M_w values were slightly reduced after exposure to e-beam and gamma radiation with samples exposed to gamma having a consistently lower M_w. This difference is possibly due to the differences in dose rate, as the time needed to deliver the desired dose is longer for the

gamma process than in an e-beam process. Approximately 10% loss in the M_w was observed at the highest exposures (i.e., 50 kGy). The M_w of the sample exposed to 50 kGy gamma was the only statistically different compared to the unexposed control.

Table 4.1. Molecular weight and thermal properties of polymer **1** (mean ± standard deviation). Melting point (T_m = 174-176 ^oC) of the degradation product (diacid **4**) was not observed in any sample. † Result is statistically different compared to the unexposed sample (0 kGy).

Exposed Sample	M_w (Da)	T_g (^oC)	T_d (^oC)
0 kGy	16,500 ± 200	51 ± 0	272 ± 1
25 kGy e-beam	16,300 ± 700	50 ± 1	273 ± 0
50 kGy e-beam	15,100 ± 400	48 ± 0	272 ± 0
25 kGy gamma	15,700 ± 70	50 ± 1	†269 ± 1
50 kGy gamma	†14,700 ± 70	48 ± 1	273 ± 1

Relatedly, polymer samples exposed to a 25 kGy dose displayed no change in T_g values whereas samples exposed to 50 kGy dose displayed a 3 degree drop in T_g (Table 4.1), corresponding to a 6 % change from the unexposed polymer. Only one distinct T_g was observed and no melting points for the degradation product **4** (T_m = 174 – 176 ^oC) were noted. All T_d values remained relatively constant following radiation exposure (Table 4.1) with only one statistical significantly different sample (25 kGy gamma). The minimal decrease in M_w and T_g values as well as the absence of degradation product melting points indicate minimal polymer chain degradation occurs upon radiation exposure.

^{1}H-NMR and FT-IR spectroscopies were also used to monitor polymer degradation and crosslinking. The samples did not display the characteristic acid proton

(COOH) signal at ~13 ppm that appears upon hydrolysis of the anhydride linkages. In the IR spectra, the anhydride carbonyl bond (C=O) stretches at ~1810 and ~1790 cm^{-1} would decrease with polymer **1** hydrolysis with a corresponding increase in C=O bond stretches for the carboxylic acid at ~1700 cm^{-1}. As shown in Figure 4.2, the carboxylic acid stretches were not observed in the polymer samples (compared to the diacid, **4**). The C=O anhydride and ester stretches were clearly retained (1747 cm^{-1}) in all polymer samples.

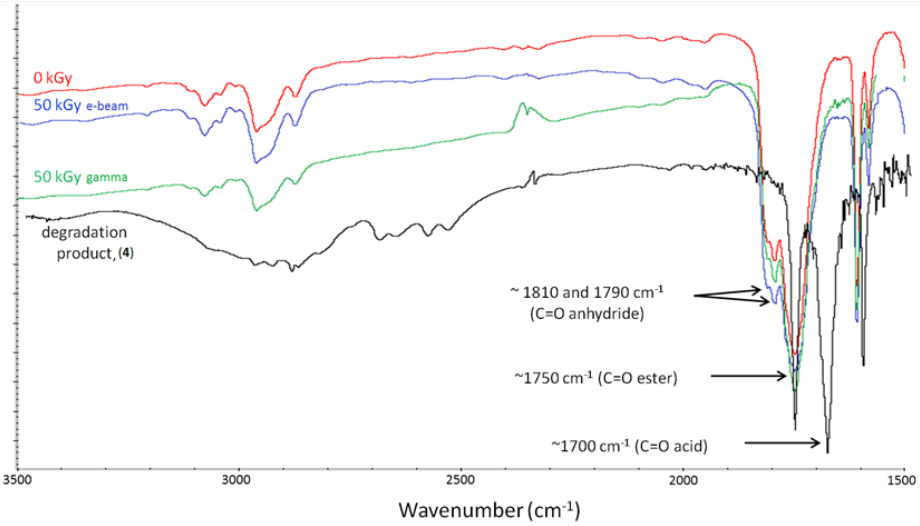


Figure 4.2. Infrared spectra of representative samples: from top to bottom: (red) unexposed sample (0 kGy); (blue) sample exposed to 50 kGy e-beam; (green) sample exposed to 50 kGy gamma; and (black) hydrolytic degradation product (diacid **4**).

4.2.3. *In Vitro* Drug Release

The most critical criteria may be the maintained ability of polymer **1** to release SA *in vitro* after radiation exposure. Retention of the drug release profile after irradiation is

important to determine what polymer property does not change upon exposure. Figure 4.3 shows the cumulative percent release of SA, where the SA was released over 20 days and all the samples (unexposed controls and exposed [25 and 50 kGy, e-beam and gamma]) displayed similar release profiles. The controlled degradation of polymer **1** to release SA remained unchanged after radiation exposure.

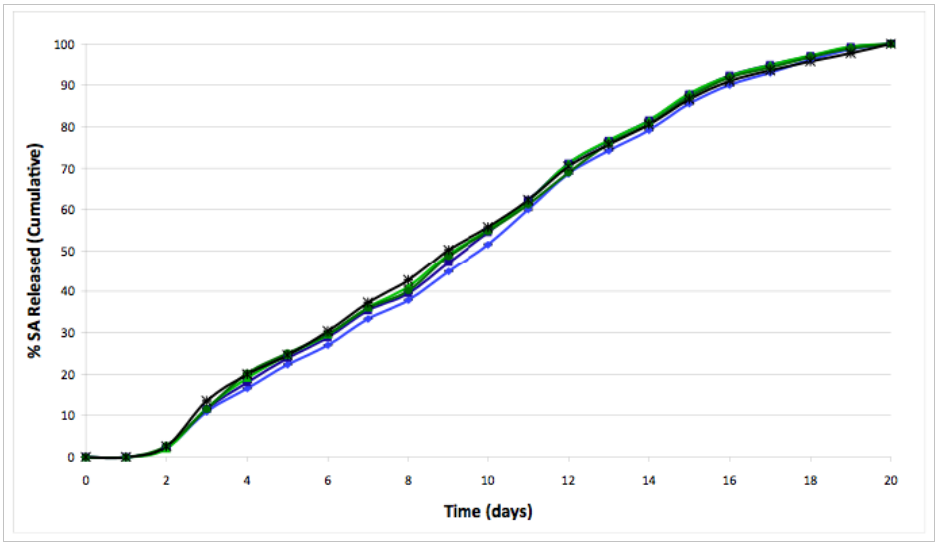


Figure 4.3. *In vitro* salicylic acid release profiles from radiation exposed and unexposed polymer. 25 kGy gamma, 50 kGy gamma, 25 kGy e-beam, 50 kGy e-beam, and unexposed (0 kGy), all displayed similar release profiles.

4.2.4. Cytocompatibility Studies

To ensure that radiation did not alter the cytocompatibility of polymer **1**, cytotoxicity experiments were performed (by Jeremy Griffin, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ). Two polymer concentrations were

chosen to mimic early-stage (0.01 mg/mL) and late-stage (0.10 mg/mL) polymer degradation. Studies were performed over a 96 h time period, during which cell viability (Figure 4.4) and morphology (Figure 4.5) were evaluated.

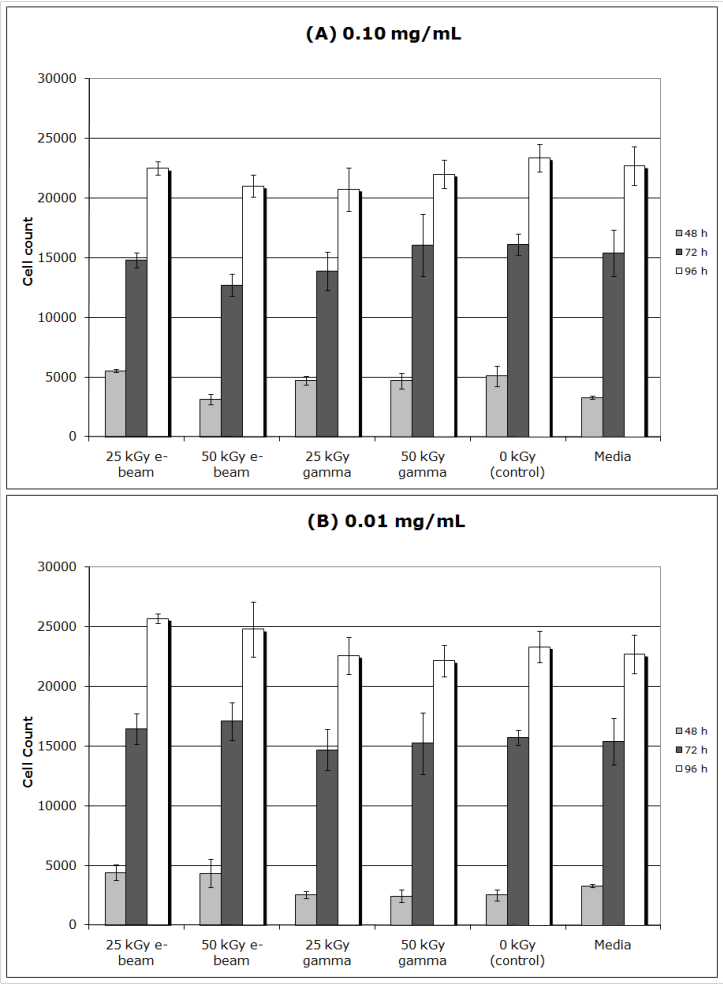


Figure 4.4. Cell viability at 48, 72 and 96 h in culture media with polymers at concentration of (A) 0.10 mg/mL media and (B) 0.01 mg/mL media. Data represent mean and standard deviation of 3 samples. No significant differences against the media control were observed.

Cell viability for the polymer-containing media and the media control at 0.10 and 0.01 mg/mL at all three time points is shown in Figure 4.4. Statistical analysis showed no significant differences at 95 % confidence level for any sample compared to the media control at both concentrations used and all time points. Comparison between the polymer-containing samples and the media control indicate normal to higher cell viability suggesting that the polymer remained non-cytotoxic after radiation exposure. Figure 4.5 shows representative light microscopy images, comparing the media control and the polymer containing samples at 0.10 mg/mL and 96 h. For all conditions, the cell images demonstrate the typical proliferation and morphology expected to a healthy fibroblast cell line. After 96 h of culture, proliferating viable cell are visible with stellate morphology and extending filopodia.

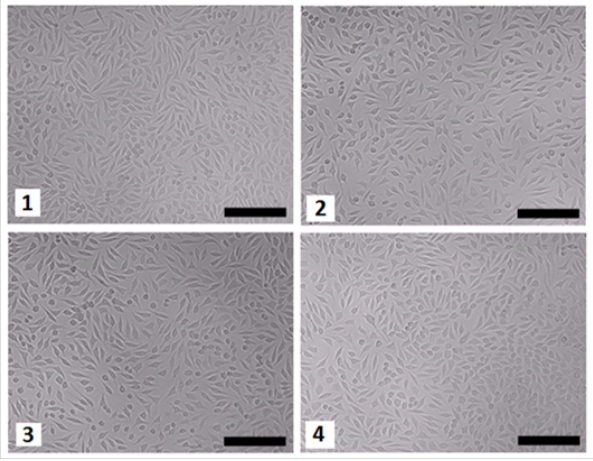


Figure 4.5. Light microscope images (10X magnification) of L929 mouse fibroblasts after 96 h of culture in polymer 1 (0.10 mg/mL) exposed under various conditions: (1) 50 kGy e-beam, (2) 50 kGy gamma, (3) unexposed polymer control, (4) media control. Scale bar is 200 μm in all images.

4.2.5. Radiation Exposure of Polymer Microspheres

Based upon our interest on an injectable drug delivery system (described on Chapter 2), the salicylate-based PAE **1** (Figure 4.1) was formulated into microspheres using a previously published oil-in-water single emulsion solvent evaporation technique.[10, 17] The microspheres obtained were exposed to ionizing radiation processes. Polymer microspheres were exposed to 25 and 50 kGy in each process (e-beam and gamma radiation). Changes in size and morphology of the microspheres and visual changes were studied to determine the effect of e-beam and gamma radiation on the formulated polymer.

No color change was observed in the irradiated samples (25 and 50 kGy, e-beam and gamma) compared to the unexposed (0 kGy) microspheres control. Further, all samples remained as loose powders following irradiation. Exposure to e-beam and gamma radiation (25 and 50 kGy) did not substantially affect size (2-34 μm) and morphology (spherical shape and smooth surface) of the microspheres. Figure 4.6, shows representative SEM images of the microspheres. Radiation doses up to 50 kGy are suitable as a sterilization dose for salicylate-based PAE microspheres.

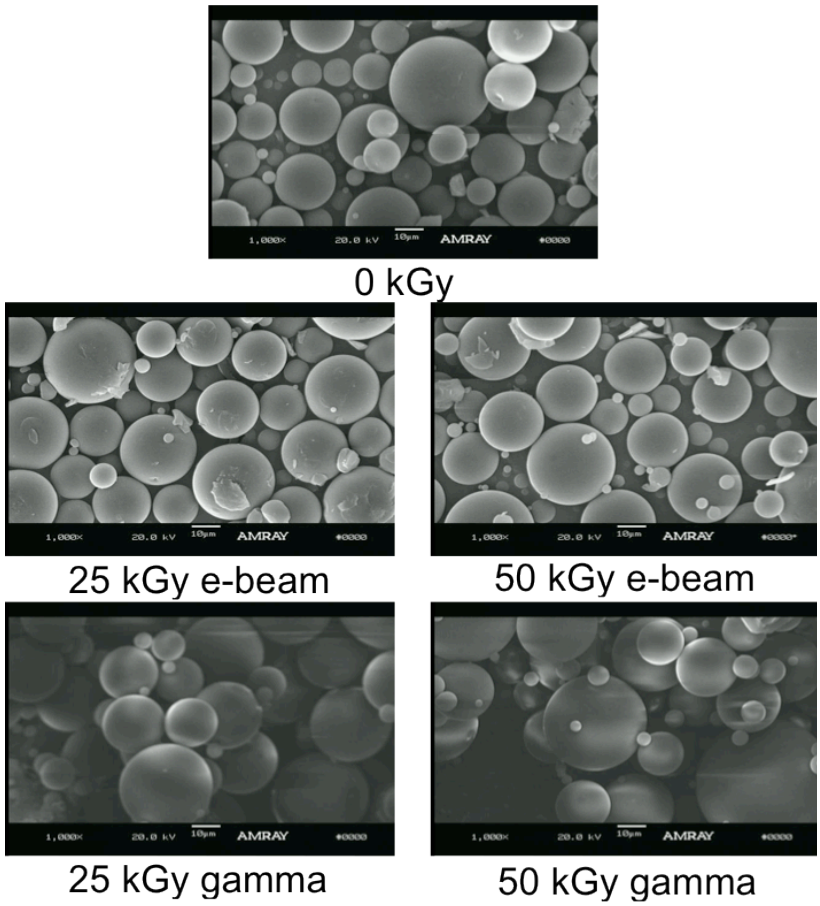


Figure 4.6. Scanning electron microscopy images of microspheres exposed to 0, 25, and 50 kGy e-beam and gamma radiation.

4.2.6. Radiation Exposure of a Slower-degrading Salicylate-based Poly(anhydride-ester)

The effect that e-beam and gamma radiation (25 and 50 kGy) have on the physicochemical properties of polymer **2** (Figure 4.7) was determined evaluating the changes in M_w, T_g and T_d after radiation exposure. ^{1}H-NMR and IR spectroscopies were used to determine polymer degradation and/or chain scission. Polymer **2** was used to test

the effect of radiation exposure on a slower-degrading salicylate-based PAE (that could provide long-term drug release).

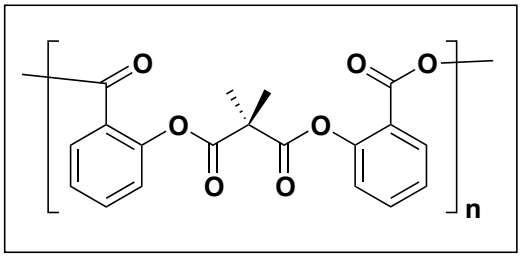


Figure 4.7. Chemical structure of polymer **2**.

The results obtained are similar to those for polymer **1**. Sample M_w values were slightly reduced after exposure to e-beam and gamma radiation with samples exposed to gamma having lower M_w. The sample exposed to gamma radiation at 50 kGy showed the greatest decrease in M_w (5 %), however, this decrease was not significantly different compared to the control. Polymer samples exposed to radiation displayed slight increase or decrease in T_g values, none of them being significantly different (Table 4.2). All T_d values remained relatively constant following radiation exposure (Table 4.2). On the ^{1}H-NMR spectra, the samples did not display the COOH signal at ~13 ppm that appears upon hydrolysis of the anhydride linkages. IR spectra showed that the anhydride C=O stretches at ~ 1790 and 1760 cm^{-1} and the ester C=O stretch at ~ 1730 cm^{-1} were preserved. No carboxylic acid C=O stretch at ~ 1700 cm^{-1} was observed.

Table 4.2. Molecular weight and thermal properties of polymer **2** (mean ± standard deviation). Melting point (T_m = 188 ^oC) of the degradation product was not observed in any sample.

Exposed Sample	M_w (Da)	T_g (^oC)	T_d (^oC)
0 kGy	28,300 ± 400	50 ± 1	265 ± 0
25 kGy e-beam	28,200 ± 1100	47 ± 0	271 ± 0
50 kGy e-beam	28,200 ± 400	51 ± 1	266 ± 1
25 kGy gamma	27,200 ± 900	51 ± 1	278 ± 0
50 kGy gamma	27,000 ± 700	50 ± 2	272 ± 1

4.3 Conclusion

PAEs such as polymers **1** and **2** are designed to hydrolytically degrade, releasing SA in a controlled fashion. For clinical use, however, the polymers must withstand sterilization and processing methods. This study demonstrated that exposure to e-beam and gamma radiation (25 and 50 kGy) did not substantially affect polymer composition, molecular weight, thermal properties, degradation characteristics, or cytocompatibility of polymer **1**. Relatedly, the size and morphology of polymer **1** microspheres were not affected. Similarly, polymer **2** did not suffer changes in its physicochemical properties after radiation exposure. Therefore, e-beam or gamma radiation doses up to 50 kGy are suitable as a sterilization dose. For potential future products consisting of salicylate-based PAE, the minimum and maximum sterilization doses will need to be determined on a per product basis.

4.4 Experimental

4.4.1. Materials

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

4.4.2. Sample Preparation

Polymer **1** and **2** were synthesized using previously reported methods.[5] Properties of the raw polymer **1** were as follows: Color = off-white, M_w = 16,800 Da, T_g = 50 ^oC, T_d = 279 ^oC. Polymer **1** was ground into a powder using a mortar and pestle and placed (1.00 g) into BD Falcon 5 mL polystyrene round-bottom tubes (12 x 75 mm style; BD Bioscience Discovery Labware, Bedford, MA) and capped. Samples were sent to Sterile Process Technology (Johnson & Johnson) for radiation exposure. Samples were then analyzed within one week after exposure. A visual assessment for changes in color and texture was immediately performed following each radiation exposure. Material characterization studies were performed in triplicate. Properties of the raw polymer **2** were as follows: Color = light yellow, M_w = 25,800 Da, T_g = 50 ^oC, T_d = 264 ^oC. Polymer **2** was treated as described above for polymer **1**.

4.4.3. Radiation Exposure

[Radiation exposure was performed by Linda Lavelle, Stanko Bodnar, Frederick Halperin, and Ike Harper, Sterile Process Technology, Johnson & Johnson, Raritan, NJ].

Samples designated for gamma irradiation were exposed under ambient conditions using a MDS Nordion Gamma Cell 220 Research Irradiator with a Cobalt 60 source. The dose rate in gamma radiation processes was approximately 0.002 kGy/sec for these studies. Temperatures during gamma exposure ranged from 30 ºC to 37 ºC, up to a maximum exposure time of 9 hrs. Samples slated for e-beam irradiation were processed under ambient conditions in the Mevex 5 MeV, 2kW electron beam linear accelerator. Samples were placed upright in an ethafoam jig and presented single-sided to the beam. The dose rate for e-beam ranged from 12.5 kGy/sec (25 kGy) to 25 kGy/sec (50 kGy). The temperature ranged from 38 ºC (25 kGy dose) to 55 ºC (50 kGy) during the e-beam exposures. Notably, this temperature increase was an instantaneous spike, not a prolonged exposure. Samples designated as controls were not exposed to ionizing radiation.

4.4.4. *In Vitro* Drug Release

After radiation exposure, polymer **1** (150 mg, powder) was compressed into a disk (10 mm diameter x 1 mm thick) using a hydraulic press (Carver model M, Garfield, NJ) applying pressure (10,000 psi) for 10 min. Disks (triplicate) were placed into scintillation

vials and 10 mL phosphate buffered saline (PBS) pH 7.4 added. Samples were incubated at 37 ^{o}C under constant shaking (60 rpm) in an Excella E25 Incubator Shaker (New Brunswick Scientific, New Brunswick, NJ). PBS was removed daily and replaced with fresh PBS. Samples were analyzed by UV/visible spectroscopy (Perkin-Elmer Lambda XLS) at λ = 303 nm.

4.4.5. Cytocompatibility Studies

[Cell cytotoxicity studies were performed by Jeremy Griffin, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ].

Cytocompatibility of polymer **1** following radiation exposure was evaluated by culturing NCTC clone 929 strain L mouse areolar fibroblast cells (L929) (ATCC, Manassas, Virginia) in media containing the dissolved polymers. These L929 cells are a standard cell type for cytocompatibility testing as recommended by the American Society for Testing and Materials (ASTM). Each polymer sample was dissolved in 10 mg/mL dimethyl sulfoxide (DMSO) as a stock solution and serially diluted with cell culture media to two concentrations (0.01 mg/mL and 0.10 mg/mL), based on standard cytotoxicity protocols.[33] Cell culture media consists of Dulbecco's Modified Eagle's Medium, 10% v/v fetal bovine serum, 1% L-glutamate and 1% penicillin/streptomycin. The polymer-containing media was distributed into a 96-well plate (Fisher, Fair Lawn, NJ) and seeded at an initial concentration of 2,000 cells per well (triplicate). The media

containing the dissolved polymer was compared to two controls: DMSO-containing media and media only.

Cellular morphology was observed and documented at 10X original magnification using a light microscope (Olympus, IX81, Center Valley, PA) at 48, 72, and 96 h post seeding. Cell viability was determined by using a CellTiter 96®AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI). The MTS tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-7 sulfophenyl)-2H-tetrazolium, inner salt; MTS(a)] is bioreduced by cells into a colored formazan product that is soluble in the tissue culture medium. Following the appropriate incubation time, 20 μL of the MTS reagent was added to 100 μL of culture medium and further incubated for four hours. The absorbance was then recorded with a microplate reader (Model 680; Bio-Rad, Hercules, CA) at 490 nm. Cell numbers were calculated based upon a standard curve created 24 hours after original cell seeding.

4.1.6. Microspheres Preparation

Polymers were formulated into microspheres using a modified procedure of a published oil-in-water single emulsion solvent evaporation technique.[10] In general, polymer **1** (0.50 g) was dissolved in dichloromethane (3 mL) and added drop-wise to 1% aqueous poly(vinyl alcohol) (PVA) (80 % hydrolyzed, 30-70 kDa) solution (80 mL) at room temperature. The emulsion was homogenized for 2 min using an IKA Ultra-Turrax T8 homogenizer at approximately 10,000 rpm. The homogenized solution was left stirring for 2 h to allow microsphere formation by solvent evaporation. Microspheres

were transferred to sterile 50 mL polypropylene conical tubes (30 x 115 mm style, BD Falcon, Franklin Lakes, NJ), washed with acidic water (pH 1) to remove residual PVA, and isolated by centrifugation at 3,000 rpm for 10 min. Microspheres were frozen by placing the conical tubes in a dry ice/acetone bath and lyophilized for 24 h at -40 ˚C and $133x10^{-3}$ mBar (LABCONO Freeze Dry System/Freezon 4.5).

Microspheres were placed (1.00 g) into BD Falcon 5 mL polystyrene round-bottom tubes (12 x 75 mm style; BD Bioscience Discovery Labware, Bedford, MA) and capped. Samples were sent to Sterile Process Technology (Johnson & Johnson) for radiation exposure. Samples were then analyzed within one week after exposure. A visual assessment for changes in color and texture was immediately performed following each radiation exposure. Material characterization studies were performed in triplicate.

4.1.7. Statistical Analysis

Statistical analysis was performed with Kaleida Graph (Synergy Software, Reading, PA). Differences were considered significant at $p < 0.05$ using ANOVA followed by pairwise comparison with Dunnett's post hoc test.

*Other methods described in Chapter 9.

4.5 References

1. Hersh, E. V.; Moore, P. A.; Ross, G. L., *Clinical Therapeutics* **2000,** *22* (5), 500-548.
2. O'Neil, M.; Heckelman, P.; Koch, C.; Roman, K., *The Merck Inex*. 14th ed.; 2006.
3. Erdmann, L.; Uhrich, K. E., *Biomaterials* **2000,** *21* (19), 1941-1946.
4. Schmeltzer, R. C.; Anastasiou, T. J.; Uhrich, K. E., *Polymer Bulletin* **2003,** *49* (6), 441-448.
5. Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E., *Macromolecules* **2005,** *38* (16), 6895-6901.
6. Carbone, A. L.; Uhrich, K. E., *Macromolecular Rapid Communications* **2009,** *30* (12), 1021-1026.
7. Whitaker-Brothers, K.; Uhrich, K., *Journal of Biomedical Materials Research Part A* **2004,** *70A* (2), 309-318.
8. Whitaker-Brothers, K.; Uhrich, K., *Journal of Biomedical Materials Research Part A* **2006,** *76A* (3), 470-479.
9. Bryers, J. D.; Jarvis, R. A.; Lebo, J.; Prudencio, A.; Kyriakides, T. R.; Uhrich, K., *Biomaterials* **2006,** *27* (29), 5039-5048.
10. Yeagy, B. A.; Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E.; Cook, T. J., *Journal of Microencapsulation* **2006,** *23* (6), 643-653.
11. Schmeltzer, R. C.; Uhrich, K. E., *Journal of Bioactive and Compatible Polymers* **2006,** *21* (2), 123-133.
12. Schmeltzer, R. C.; Johnson, M.; Griffin, J.; Uhrich, K., *Journal of Biomaterials Science, Polymer Edition* **2008,** *19* (10), 1295-1306.
13. Rosenberg, L. E.; Carbone, A. L.; Römling, U.; Uhrich, K. E.; Chikindas, M. L., *Letters in Applied Microbiology* **2008,** *46* (5), 593-599.
14. Johnson, M. L.; Uhrich, K. E., *Journal of Biomedical Materials Research Part A* **2009,** *91A* (3), 671-678.
15. deRonde, B. M.; Carbone, A. L.; Uhrich, K., *Polymer Degradation and Stability* **2010,** *95* (9), 1778-1782.
16. Harten, R. D.; Svach, D. J.; Schmeltzer, R.; Uhrich, K. E., *Journal of Biomedical Materials Research Part A* **2005,** *72A* (4), 354-362.
17. Rosario-Meléndez, R.; Ouimet, M. A.; Uhrich, K. E., *Polymer Bulletin* **2012,** *Manuscript accepted*.
18. Reynolds, M. A.; Prudencio, A.; Aichelmann-Reidy, M. E.; Woodward, K.; Uhrich, K. E., *Current Drug Delivery* **2007,** *4* (3), 233-239.
19. Gorna, K.; Gogolewski, S., *Polymer Degradation and Stability* **2003,** *79* (3), 465-474.
20. Razem, D.; Katusin-Razem, B., *Radiation Physics and Chemistry* **2008,** *77* (3), 288-344.
21. Calis, S.; Bozdag, S.; Kas, H. S.; Tuncay, M.; Hincal, A. A., *Farmaco* **2002,** *57* (1), 55-62.
22. Williams, H. E.; Huxley, J.; Claybourn, M.; Booth, J.; Hobbs, M.; Meehan, E.; Clark, B., *Polymer Degradation and Stability* **2006,** *91* (9), 2171-2181.
23. Edlund, U.; Albertsson, A. C.; Singh, S. K.; Fogelberg, I.; Lundgren, B. O., *Biomaterials* **2000,** *21* (9), 945-955.
24. Odelius, K.; Plikk, P.; Albertsson, A.-C., *Biomaterials* **2008,** *29* (2), 129-140.
25. Gogolewski, S.; Mainil-Varlet, P., *Biomaterials* **1997,** *18* (3), 251-255.

26. Benson, R. S., *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* **2002,** *191* (1,Äì4), 752-757.
27. Igartua, M.; Hernandez, R. M. A.; Rosas, J. E.; Patarroyo, M. E.; Pedraz, J. L., *Eur J Pharm Biopharm* **2008,** *69* (2), 519-26.
28. Nugroho, P.; Mitomo, H.; Yoshii, F.; Kume, T., *Polymer Degradation and Stability* **2001,** *72* (2), 337-343.
29. Loo, S. C. J.; Ooi, C. P.; Boey, Y. C. F., *Polymer Degradation and Stability* **2004,** *83* (2), 259-265.
30. Phong, L.; Han, E. S. C.; Xiong, S.; Pan, J.; Loo, S. C. J., *Polymer Degradation and Stability* **2010,** *95* (5), 771-777.
31. Martinez-Sancho, C.; Herrero-Vanrell, R.; Negro, S., *J Control Release* **2004,** *99* (1), 41-52.
32. Yagoubi, N.; Peron, R.; Legendre, B.; Grossiord, J. L.; Ferrier, D., *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* **1999,** *151* (1,Äì4), 247-254.
33. Fischer, D.; Li, Y.; Ahlemeyer, B.; Krieglstein, J.; Kissel, T., *Biomaterials* **2003,** *24* (7), 1121-1131.

5. MORPHINE-BASED POLY(ANHYDRIDE-ESTER) FOR EXTENDED PAIN RELIEF

5.1. Introduction

Morphine is a potent narcotic analgesic used for the treatment of acute and chronic pain, providing reliable analgesia.[1-6] However, morphine has a half-life in plasma of 2-4 h, requiring repeated administration to maintain the drug at therapeutic levels for an extended time period.[5-7] Repeated administration affects patient comfort because the daily activities of the patient will be interrupted to take the medication, which can lead to low compliance.[6-9] In addition, morphine use is often accompanied by the development of tolerance and dependence, leading to an increase in dosing (i.e., amount and frequency).[1, 10] Other side effects that can result from morphine use are respiratory depression, somnolence, and gastrointestinal effects (e.g., nausea, vomiting, and constipation).[4, 5]

Controlled-release morphine formulations can prolong the analgesic effect of the drug and prevent accidental withdrawals due to missed doses.[4, 7] In recent years, the number of morphine delivery systems for controlled-release has increased. Various delivery systems that use enteral and parenteral administration are commercially available. Among the different administration routes, enteral is the most frequently used. Among commercially available morphine delivery systems (tablets or capsules) are Kadian®,[6, 10] Avinza®,[2, 5] and MS Contin®[7] that can release morphine for 12-24 h. Even though these tablets and capsules are successful at maintaining long-term benefits of the drug without dose escalation, these tablets and capsules are also sensitive to physical

alterations that affect their release mechanism.[10, 11] When the tablet or capsule is crushed, chewed, or dissolved, it increases the risk of administration of a fatal dose.[11] Because these formulations contain a large dose that can be easily separated (by crushing or breaking the tablet/capsule), they also increase the potential for recreational use.[6]

Other formulations have been extensively explored including lipid-based carriers,[9, 12-14] drug encapsulation within polymers,[15-18] and polymer-drug complexes.[19-21] Previously, morphine was chemically incorporated into a polyurethane backbone (as a pendant group); however, polyurethanes are resistant to biodegradation under physiological conditions and are of limited biological potential.[22] The major drawbacks of these formulations are low drug loading and/or rapid drug release, as usually evidenced by a burst release.

The chemical incorporation of drugs into poly(anhydride-ester) (PAE) backbones could solve most of the drawbacks associated with the controlled-release formulations mentioned above. In the last decade, multiple non-steroidal anti-inflammatory drugs (e.g., salicylic acid and other salicylates) and antiseptics/antioxidants (e.g., catechol) have been chemically incorporated into PAE backbones.[23-31] These new classes of polymers are capable of achieving high drug loading (50-80 %) in a reproducible manner. The drug is chemically incorporated in each repeat unit through a "linker" molecule. These PAEs release the drug in a near zero-order fashion without a burst.[32-34] Drug release can be controlled by altering the chemical composition of the polymer (i.e., "linker" molecule or making copolymers).[26, 34-36] These PAEs are also advantageous because they can be formulated into different geometries depending on the intended administration route. For example, they can be formulated into microspheres for injectable administration.[37, 38]

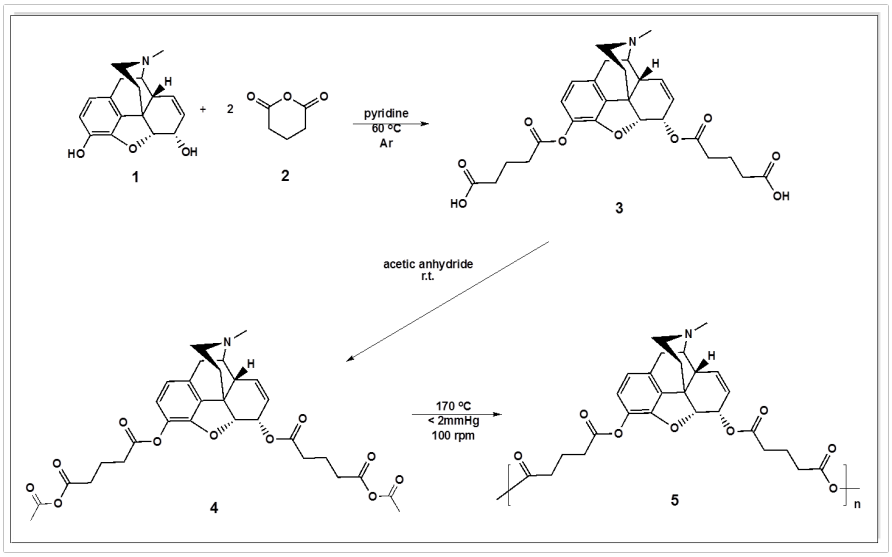
Based upon our previous experience of incorporating drugs into PAE backbones, a morphine-based PAE was designed to control morphine release to achieve prolonged analgesia. This chapter presents the synthesis and characterization of this morphine-based PAE (termed "PolyMorphine"). Furthermore, *in vitro* studies were performed to study polymer degradation and drug release in buffered media mimicking physiological conditions, and cytocompatibility towards fibroblasts. *In vivo* studies of analgesia in mice were performed using tail-flick latency (TFL) tests.

5.2. Results and Discussion

5.2.1. Synthesis and Physicochemical Characterization

To overcome the limitations of commercially available morphine delivery systems and based upon our experience with the chemical incorporation of drugs into biodegradable polymer backbones, a morphine-based PAE, described herein as PolyMorphine (**5** in Scheme 5.1), was developed and evaluated. The synthesis of this polymeric prodrug consists of three steps as outlined in Scheme 5.1: esterification of morphine to yield the diacid (**3**), which is then activated via acetylation to form the monomer (**4**) that undergoes melt-condensation polymerization to yield the polymer (**5**). All compounds synthesized were characterized to assess their physical and chemical properties. Their chemical structures were assessed using proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR), and infrared (IR) spectroscopies. Mass spectrometry (MS) and gel permeation chromatography (GPC) were used to determine

the molecular weight (MW) and weight-average molecular weight (M_w), respectively. The thermal properties were evaluated using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).



Scheme 5.1. Synthesis of PolyMorphine **5** from the reaction of morphine **1** and glutaric anhydride *via* ring-opening, followed by acetylation of the diacid **3** and polymerization of the monomer **4** by melt-condensation.

To synthesize **3,** various reaction conditions were explored by changing the solvent and the base catalyst. Among the conditions tested, the reaction carried out neat in pyridine yielded the best results (i.e., full conversion into product and easy product isolation). Because the allylic hydroxyl group of morphine is less reactive than the phenolic alcohol, the complete conversion of both alcohols takes 3 days at room temperature. When heated to 60 °C, esterification of the phenolic and allylic alcohols is completed within 24 hours. The isolation of the product was performed by azeotropic

removal of pyridine with toluene to reproducibly afford **3** in high yields (95 %). Figure 5.1 shows the ^{13}C-NMR of **1**, **3**, and **5**; the key peaks for the nitrogen-containing ring and the cyclic ether are indicated. As shown in Figure 5.1, the structure of the drug was preserved after synthesizing **3**. The IR spectrum of **3** (Figure 5.2, red) shows the attachment of glutaric linkers by the formation of the ester bonds by the presence of the ester carbonyl (C=O) at 1732 cm^{-1} and the presence of terminal carboxylic acids C=O at 1712 cm^{-1} and O-H at 3350 cm^{-1}. Compared to the IR spectrum of morphine (Figure 5.2, green), the alcohols O-H at 3200 cm^{-1} disappear and the C=O peaks appear. The MW of **3** was determined as M/Z = 514 by MS, which corresponds to the MW of **3** (513.54) plus a proton. The thermal analysis of **3** showed that it decomposes at 227 °C and did not display a melting temperature (T_m).

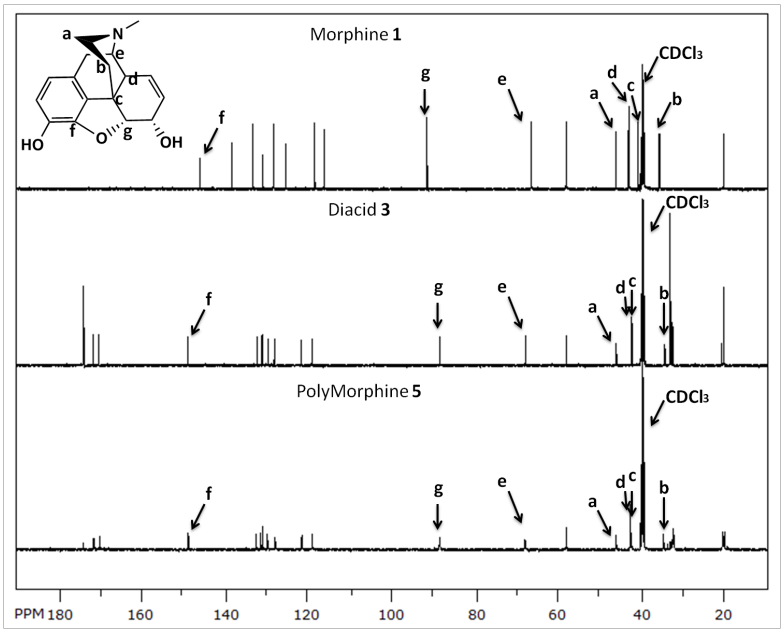


Figure 5.1. ^{13}C-NMR spectra of morphine **1**, diacid **3**, and PolyMorphine **5**, showing the preservation of the chemical integrity of the drug; key peaks for the nitrogen-containing ring and the cyclic ether are indicated.

Two different polymerization methods were investigated to prepare PolyMorphine. Due to the concern that morphine intermediates might be thermally unstable, solution polymerization was first evaluated. This method used triphosgene (which forms phosgene *in situ*) as the coupling agent in the presence of triethylamine.[39] However, this polymerization method not only resulted in low M_w polymer and low yields, but the pure polymer could not be isolated. As a result, melt-condensation polymerization was attempted.[39] Monomer **4** was prepared by the acetylation of **3** in excess acetic anhydride at room temperature. Characterization of **4** was performed with the same methods used to characterize **3**; the NMR and IR spectra confirmed the formation of **4**. Monomer **4** decomposes at 297 °C and melts at 164 °C. This high decomposition temperature (T_d) of **4** and its moderate T_m made melt-condensation polymerization possible because it was thermally stable.

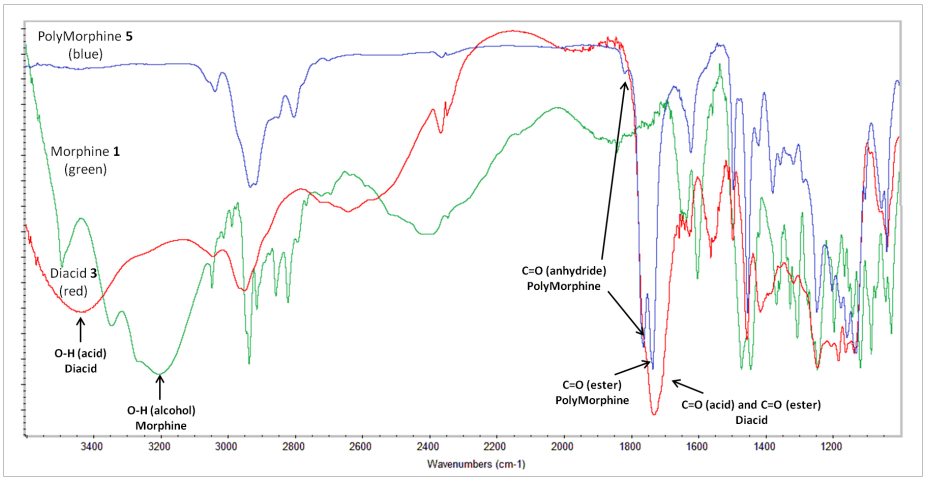


Figure 5.2. Infrared spectra of (blue) PolyMorphine **5**, (red) diacid **3**, and (green) morphine **1**, key stretch bands for OH acid, C=O acid, C=O ester, and C=O anhydride are indicated.

Melt-condensation polymerization of activated **4** at 170 °C *in vacuo* yielded **5** with reasonably high M_w (26,000 Da), low PDI (1.14) and high yields (70 %). Figure 5.1 also shows the ^{13}C-NMR spectrum of **5**, as seen on the figure the structure of the drug was preserved. The IR spectrum of **5** (Figure 5.2, blue) shows the formation of the anhydride bonds by the presence of the anhydride C=O at 1818 and 1761 cm^{-1}, the preservation of the ester bonds by the presence of the ester C=O at 1734 cm^{-1}, and the disappearance of terminal carboxylic acid C=O at 1712 cm^{-1}. PolyMorphine **5** decomposes at 185 °C, does not have a T_m, and its glass transition temperature (T_g is 120 °C. Having such a high T_g is a positive attribute for *in vivo* applications (body temperature is 37 °C) because the polymer will not deform once implanted in the body.

5.2.2. *In Vitro* Degradation and Drug Release

Given that **5** was designed to degrade and release free morphine, *in vitro* hydrolysis studies were performed to characterize polymer degradation (Figure 5.3). Since the hydrolytic cleavage of the anhydride bonds is faster than the ester bonds,[40, 41] the degradation of **3** was expected to be the rate-determining step in the degradation of **5**. In addition, the two ester bonds in compound **3** are not equivalent and would likely degrade at different rates. Diacid **3** is an important intermediate; if it does not degrade to release free morphine, then polymer **5** will not degrade into free morphine.

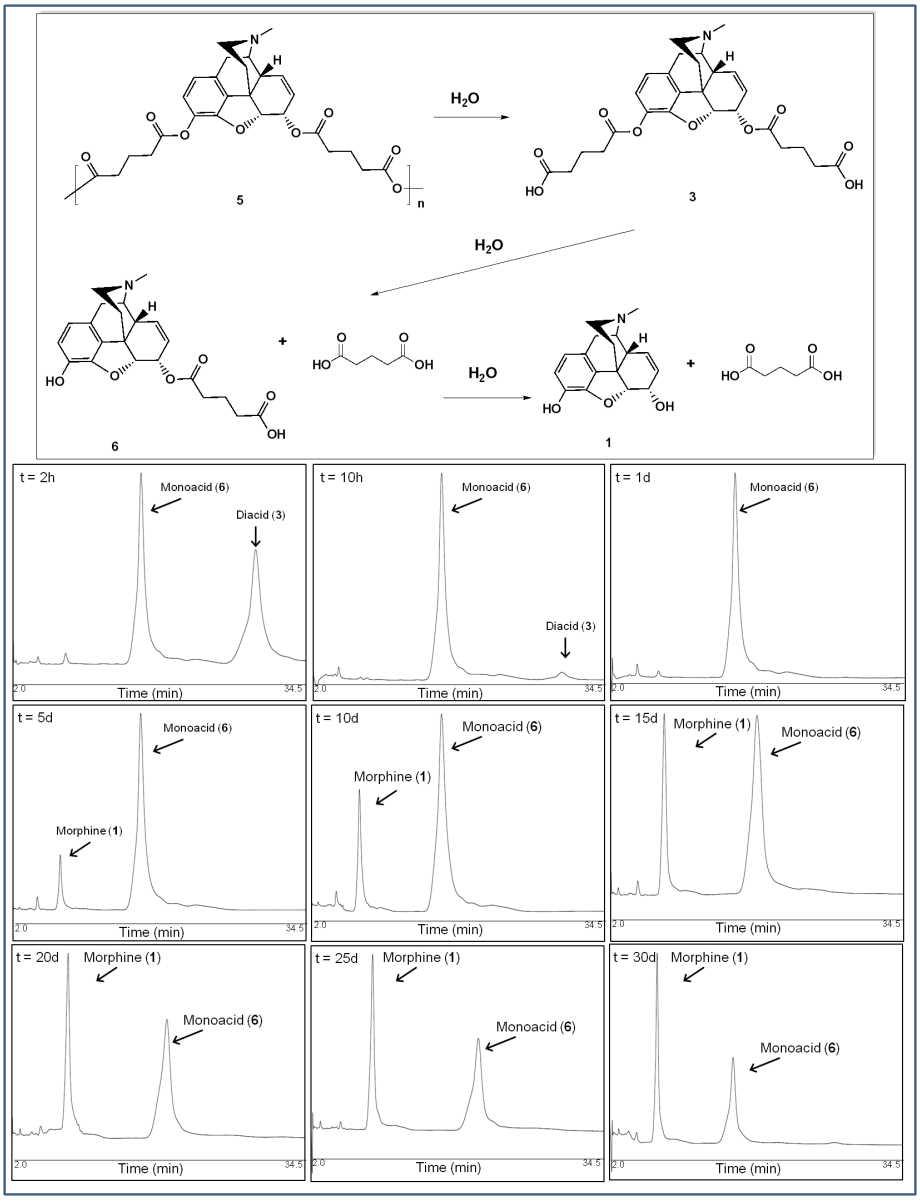


Figure 5.3. Hydrolytic degradation scheme of PolyMorphine (**5**). (Bottom) Chromatograms showing the *in vitro* degradation of diacid (**3**) into monoacid (**6**) and free morphine (**1**) at different time points (2 h, 10 h, 1 d, 5 d, 10 d, 15 d, 20 d, 25 d, and 30 d).

Mimicking physiological conditions (37 °C and pH 7.4 buffer), the hydrolytic degradation of **3** was analyzed by HPLC where three distinctive peaks were detected

throughout the experiment: **3** (R_t = 28.5 min), **6** (R_t = 16.2 min), and **1** (R_t = 6.5 min). Figure 5.3 (bottom) shows representative chromatograms for the degradation of **3** into the intermediate **6** and **1**. Diacid **3** completely hydrolyzes into a monoacid (Figure 5.3 top, **6**) during the first day. The monoacid then hydrolyzed into free morphine (that started to be detected on day 2) and was still present after 30 days (Figure 5.3 bottom). The formation of **6** during degradation was confirmed by the analysis of the chemically synthesized monoacid **7** (Figure 5.4). The retention time of **7** was 18.1 min, which is different from that of **6**. When both monoacids were analyzed simultaneously, a peak with two maximums was observed; the low resolution suggests the presence of two similar compounds. This degradation pathway is supported by previous studies on the hydrolysis of heroin into 6-monoacetylmorphine and ultimately into morphine.[42]

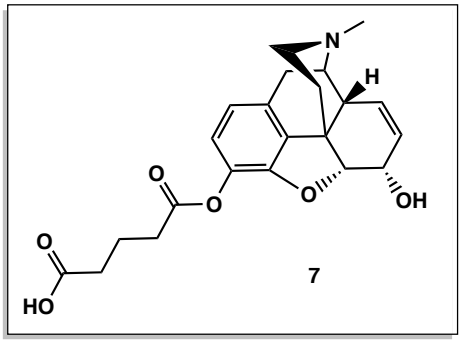


Figure 5.4. Chemical structure of compound **7**.

Following analysis of **3**, the hydrolytic degradation of **5** was studied under similar conditions. The HPLC results indicated that the polymer degrades via hydrolytic cleavage of the anhydride bonds to generate **3**, which is then hydrolyzed into **6,** which further hydrolyzes into **1** (Figure 5.3 top).

5.2.3. *In Vitro* Cytocompatibility

Investigating the potential toxicity of these novel materials is critical to understanding the potential *in vivo* use of this prodrug. The cytotoxicity of **3** and **5** towards fibroblasts was studied in vitro (by Roberto Delgado-Rivera, Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ). Fibroblasts were used for this study because they are the most frequently used cells for initial cytotoxicity testing of biomaterials.[43] Cytocompatibility was evaluated by culturing 3T3 fibroblasts cells in medium containing **3** and **5** (separately) at concentrations of 0.10 and 0.01 mg/mL. These concentrations were chosen because they are well above the concentrations seen in vitro (10-100 times higher) and can be used to determine a possible dose dependent toxicity. Studies were performed evaluating cell viability at 24, 48, and 72 h, to evaluate early and late degradation stages. To quantify cell viability, representative fluorescence microscopy images of each condition were taken to determine the total number of cells (live and dead). Statistical analysis showed no significant differences with a 95 % confidence level between the samples containing **3** and **5** and the positive control for both concentrations used at all time points. Comparison between the diacid- and polymer-containing samples and the media control indicate normal to higher cell viability, suggesting that both **3** and **5** are non-cytotoxic (Figure 5.5A). Figure 5.5 (B-D) shows representative fluorescence microscopy images of the positive control (fibroblasts with cell culture media), the negative control (fibroblasts with cell culture

media and 5 % ethanol), cell culture media containing **3** (0.10 mg/mL at 48 h), and cell culture media containing **5** (0.10 mg/mL at 48 h). Green fluorescence indicates viable cells whereas red indicates dead cells. These results show no significant cytotoxicity caused by **5** or **3**.

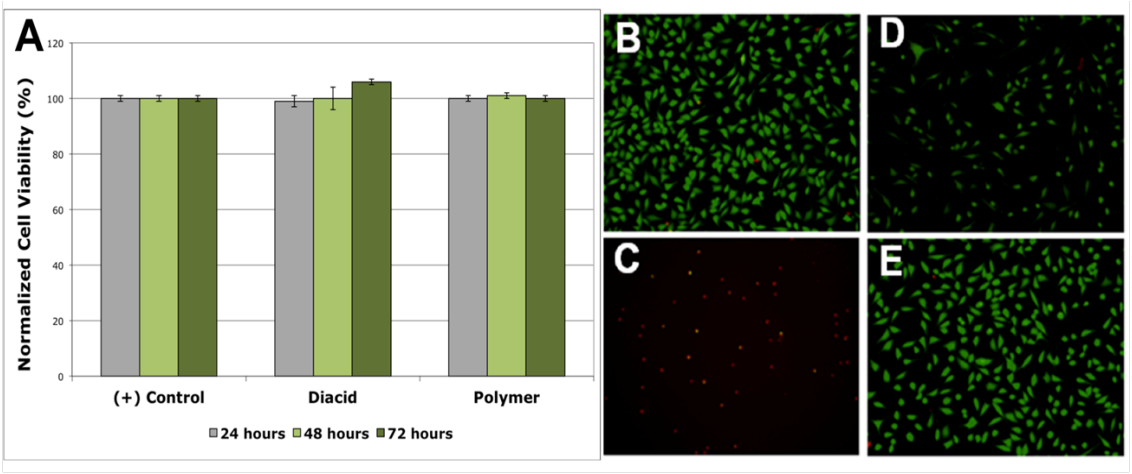


Figure 5.5. *In vitro* cell cytocompatibility of diacid (**3**) and PolyMorphine (**5**). (A) Cell viability of the positive control (fibroblasts with cell culture media only), **3** (at 0.10 mg/mL), and **5** (at 0.10 mg/mL), no statistical differences at 95 % confidence level between the samples containing **3** and **5** and the positive control; Fluorescent microscopy images (green = viable cell and red = dead cells) of: (B) positive control, (C) negative control (fibroblasts with cell culture media and 5 % ethanol), (D) diacid **3**, and (E) **5**.

5.2.4. *In Vivo* Evaluation of Analgesic Effect

As indicated above, a key impetus of this work was to develop a prodrug form of morphine (PolyMorphine), which, when administered *in vivo*, would hydrolytically

degrade in a controlled fashion to provide extended analgesia. To determine whether **5** would meet this objective, mice were administered systemically with a drug or control solution by i.p. injection, and their nociception was measured using the TFL test (Figure 5.6). TFL test measures the animal's response to a thermal stimulus (i.e., hot water) and the main end point is a withdrawal response (i.e., tail withdrawn form the hot water).

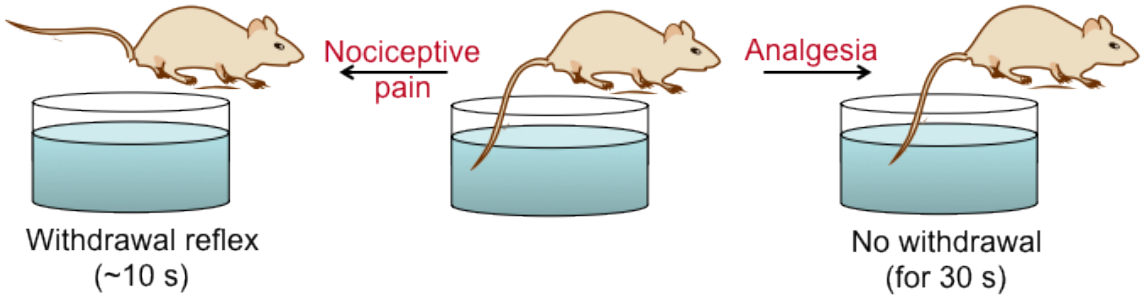


Figure 5.6. Representation of the TFL test used to assess nociceptive behavior and morphine sensitivity. When the animal experiences nociceptive pain, it withdraws the tail by flicking it out of the water in ~ 10 s (left). If the animal experiences analgesia, it would not feel the painful sensation and no withdrawal reflex occurs (right). For animals experiencing analgesia, the tail was removed from the water at the cutoff time (30 s) to avoid tissue damage.

TFL test was performed by immersing the distal third of the animal's tail in a water bath at 49 °C and measuring pain threshold by the time it takes for the animal to flick its tail out of the water bath. Four treatment groups were used: vehicle control, free morphine (at 10 mg/kg), **3** (at 50 mg/kg), and **5** (at 200 mg/kg). Doses were chosen after a pilot dose-response experiment. Doses do not contain the same amount of morphine, however,

higher concentration of morphine after a single administration does not result in an extended analgesic effect.[44] At various time points post administration (starting after 30 min), TFL was measured.

As shown in Figure 5.7, free morphine provided strong analgesia, peaking at 30 min post-administration (Figure 5.7A, filled diamonds). The analgesic effect of free morphine diminished with time; by the 4 h time point, the analgesic effect was completely gone. This time course of analgesia has been well-established for free morphine, as the drug is metabolized *in vivo* and plasma drug level drops off.[8] Diacid (**3**) showed a similar time course of analgesic effect as free morphine (Figure 5.7A, filled triangles).

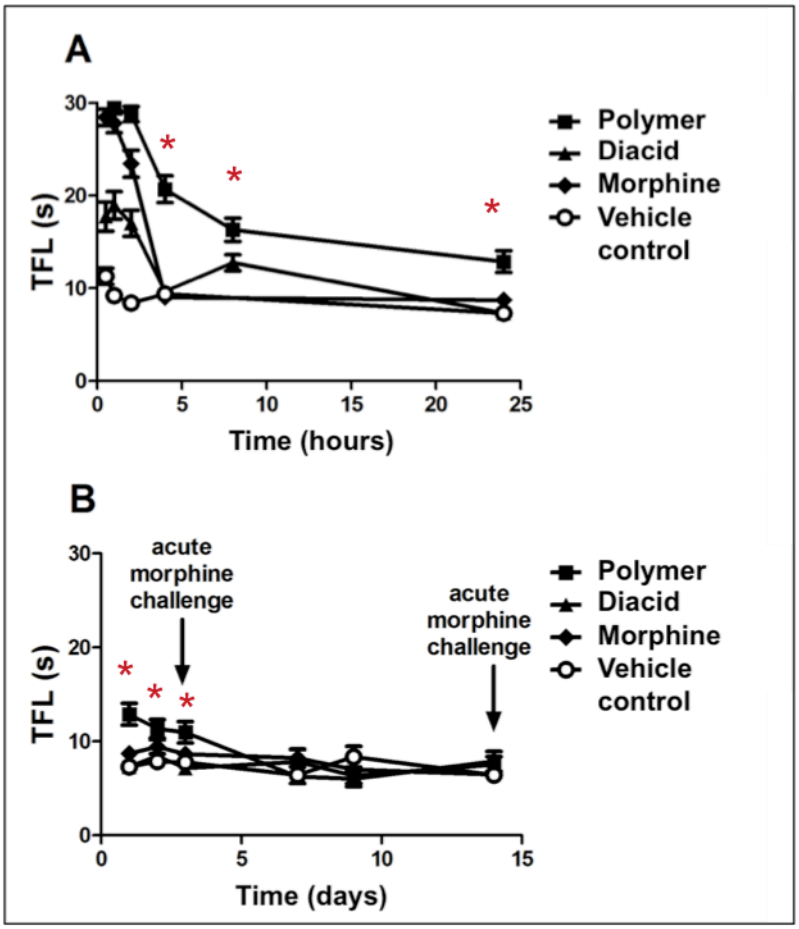


Figure 5.7. PolyMorphine provided extended analgesia in mice. (A) TFL test results at 0.5-24 h post-administration. (B) TFL results from day 1 through day 14 (vertical arrows indicate the days that animals received acute morphine challenge to evaluate morphine tolerance development). PolyMorphine provides extended analgesia compared with free morphine. Data are shown as mean ± standard error of mean. N = 30 for each time point prior to and including day 3. N = 15 after day 3. * Indicates that PolyMorphine data are significantly different from either free morphine or vehicle control.

Systemic administration of PolyMorphine (**5**) also resulted in strong analgesia, reaching a peak effect at the 1 h time point (Figure 5.7A, filled squares). Different from

free morphine, however, is the noticeably extended time course of the analgesic effect from PolyMorphine. Analgesia was sustained throughout the 24 h time frame post drug administration with gradual decline (Figure 5.7A), with the analgesic effect still significant 3 days post-administration (Figure 5.7B). These results clearly indicate that PolyMorphine, when administered *in vivo*, provides extended pain relief. The fact that analgesia was still significant 3 days post-administration was note-worthy; this study is the first example of a single dose, systemically administered morphine formulation that displayed analgesia for over 24 h.

Compared to the *in vitro* drug release studies, hydrolysis of the polymer seems to be faster *in vivo*. As morphine, monoacid **6**, and diacid **3** are detected during *in vitro* degradation studies, it is possible that the analgesic effect comes from all compounds. It was already shown that administration of **3** results in analgesia. Therefore, further studies are needed to determine the concentration in blood of each degradation product at each time point.

In opioid biology, a well-known effect of the extended use of morphine (and related opioid alkaloids with strong analgesic properties), both in rodent and human, is tolerance development with repeated exposure.[45-47] As a preliminary evaluation of animals' sensitivity to acute morphine, two time points were chosen at which the animals' responsiveness to an acute morphine challenge was tested. If animals became morphine-tolerant, they would be less responsive to an acute morphine challenge (administration of 10 mg/kg of morphine). The first time point was 3 days post-drug administration, as this was the time when PolyMorphine's analgesic effect has decreased substantially toward the baseline level. Half of the mice from each drug group were

subjected to acute morphine challenge on day 3. The second time point was on day 14, when the remaining half of the mice from each experimental group were subjected to acute morphine challenges. As shown in Figure 5.8 mice in every group showed full responsiveness to acute morphine challenge, at both day 3 (Figure 5.8A) and day 14 (Figure 5.8B), reaching the 30 s cutoff time in TFL test. It should be noted that, although this preliminary assessment suggested an absence of overt morphine tolerance, more extensive work is needed to fully evaluate the issue of morphine tolerance.

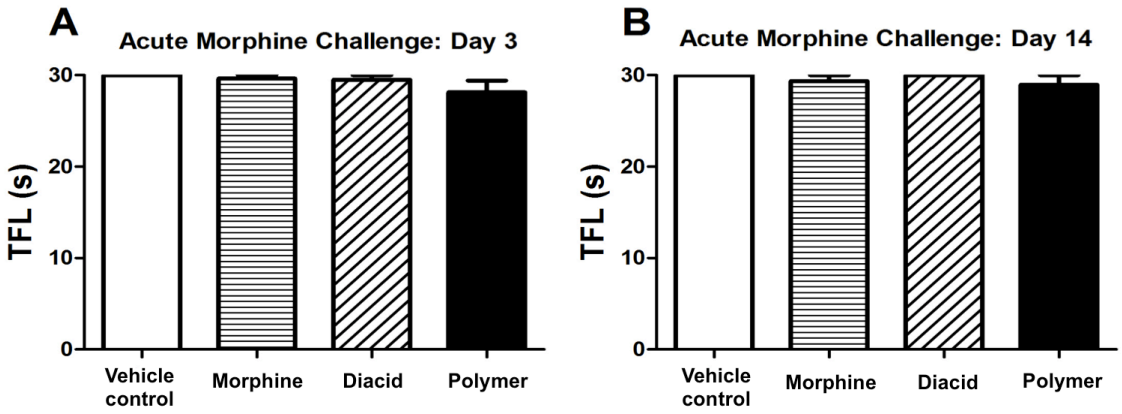


Figure 5.8. Mice retained responsiveness to acute morphine challenge (10 mg/kg, i.p.) (A) 3 d (B) 14 d after the initial administration. N = 15 for all groups. Animals retain full responsiveness to acute morphine challenge, regardless of whether they received free morphine or PolyMorphine. If the animals become tolerant to morphine, it is expected that they would be non-responsive or would flick their tails in less than 30 s (cutoff time) when their tails are immersed in the hot water.

5.3. Conclusion

This study reports the preparation and evaluation of PolyMorphine, a polymer version of morphine that provides extended analgesia while potentially reducing tolerance development. PolyMorphine was synthesized via melt-condensation polymerization and its physicochemical properties were fully characterized to confirm the preservation of morphine's structural integrity. *In vitro* studies were performed to determine the degradation pathway of the polymer and a key intermediate, showing that PolyMorphine hydrolyzes into free morphine. *In vitro* cytocompatibility studies showed that PolyMorphine is non-cytotoxic towards fibroblasts. When administered *in vivo*, PolyMorphine provided sustained pain relief for up to 3 days, more than 20 times the analgesic time window of free morphine. These results demonstrated, for the first time, a systemically administered prodrug that yields a long-lasting analgesic effect. Furthermore, based on a preliminary test of sensitivity to an acute morphine challenge, no overt signs of morphine tolerance development were observed in PolyMorphine-administered animals. In consideration of the abuse liability of many controlled release formulations of opioid analgesics, PolyMorphine may offer a desirable option as a long-acting, low abuse liability alternative to conventional opioid analgesics. Clearly, these potential promises warrant further investigation.

5.4. Experimental

5.4.1. Materials

Morphine was kindly provided by Noramco Inc. (Athens, GA). Unless otherwise specified, all other chemicals and reagents were purchased from Sigma-Aldrich (Milwaukee, WI).

5.4.2. Polymer Synthesis

5.4.2.1. Diacid Synthesis (3)

Morphine (**1** in Scheme 1, 1.00 g, 1 eq) was dissolved in anhydrous pyridine under argon and stirred for 5 min. Glutaric anhydride (**2**, 3.97 g, 10 eq) was slowly added manually. The reaction mixture was heated to 60 °C and stirred overnight. Pyridine was azeotropically removed using toluene. The brown paste obtained was washed 10 x 50 mL with DCM to remove the excess glutaric acid. The final product was dried under vacuum at room temperature. Yield: 0.95 g (95 %) beige foam. ^{1}H-NMR (500 MHz, DMSO-d_6, δ): 6.73 (d, 1H, ArH), 6.58 (d, 1H, ArH), 5.50 (dq, 2H, CH and CH), 5.15 (s, 1H, CH), 5.05 (d, 1H, CH), 3.37 (s, 1H, CH_2), 2.98 (d, 1H, CH), 2.75 (s, 1H, CH), 2.40-2.15 (comp, 14H, CH_2, CH_2, CH_2, CH_2, CH_2, and CH_3), 2.08 (t, 1H, CH_2), 1.86-1.68 (comp, 4H, CH_2 and CH_2), 1.65 (d, 1H, CH_2). ^{13}C-NMR (500 MHz, DMSO-d_6, δ): 174.1 (2C), 171.9 (1C), 170.5 (1C), 149.1 (1C), 131.5 (1C), 130.5 (1C), 130.3 (1C), 129.2 (1C), 127.8 (1C), 122.5 (1C), 119.7 (1C), 87.9 (1C), 67.4 (1C), 58.8 (1C), 45.8 (1C), 41.4 (1C), 40.8 (1C), 36.6 (1C), 32.9 (1C), 32.8 (1C), 32.6 (3C), 32.3 (1C), 32.9 (1C), 20.0 (1C). IR

(KBr pellet): 3550 cm^{-1} (OH, acid), 1732 cm^{-1} (C=O, ester), 1712 cm^{-1} (C=O, acid). MS: M/Z = 514 [M + 1]. T_d = 227 °C.

5.4.2.2. Monomer Synthesis (4)

Morphine-based diacid (**3**, 0.18 g) was acetylated by reacting with an excess of acetic anhydride (36 mL, Fisher, Fair Lawn, NJ). The reaction mixture was stirred overnight at room temperature. The excess acetic anhydride was removed under reduced pressure. Yield: 0.16 g (89 %), orange paste. ^{1}H-NMR (500 MHz, DMSO-d_6, δ): 6.74 (d, 1H, ArH), 6.59 (d, 1H, ArH), 5.50 (dq, 2H, CH and CH), 5.18 (s, 1H, CH), 5.05 (1H, CH), 5.05 (d, 1H, CH), 3.30 (s, 1H, CH_2), 2.97 (d, 1H, CH), 2.78-2.12 (comp, 20H, CH, 5CH_2 and 3CH_3), 2.05 (t, 1H, CH_2), 1.96-1.77 (comp, 4H, CH_2 and CH_2), 1.62 (d, 1H, CH_2). ^{13}C-NMR (500 MHz, DMSO-d_6, δ): 172.2 (2C), 170.8 (2C), 169.2 (1C), 168.8 (1C), 145.0 (1C), 132.5 (1C), 132.3 (1C), 131.2 (1C), 131.1 (1C), 128.4 (1C), 122.3 (1C), 119.8 (1C), 89.7 (1C), 69.2 (1C), 58.4 (1C), 46.5 (1C), 43.4 (1C), 43.3 (1C), 35.4 (1C), 34.3 (1C), 34.2 (1C), 32.9 (3C), 32.5 (1C), 32.0 (1C), 30.0 (2C), 20.0 (1C). IR (solvent-casted DCM): 1809 cm^{-1} and 1761 cm^{-1} (C=O, anhydride), 1732 cm^{-1} (C=O, ester). MS: M/Z = 598 [M + 1]. T_m = 164 °C. T_d = 297 °C.

5.1.1.1. Polymer Synthesis (5)

Morphine-based monomer (**4**, 1.0 g) was polymerized by melt-condensation polymerization at 170 °C, under constant vacuum (< 2 mmHg), and constant stirring (100 rpm) using an overhead mechanical stirrer (T-line laboratory stirrer, Talboys Engineering Corp., Montrose, PA). Polymerization continued until the mixture solidified (~ 30 min). The product was cooled to room temperature and dissolved in DCM (2 mL). The polymer was precipitated dropwise over excess diethyl ether (50 mL) and isolated by vacuum filtration. The product was dried under vacuum at room temperature overnight. Yield: 0.70 g (70 %), tan solid. ^{1}H-NMR (500 MHz, DMSO-d_6, δ): 6.71 (br, 1H, ArH), 6.55 (br, 1H, ArH), 5.50 (br, 2H, CH and CH), 5.15 (br, 1H, CH), 5.05 (br, 1H, CH), 3.29 (br, 1H, CH_2), 2.93 (br, 1H, CH), 2.76-2.17 (br, 15H, CH_2, CH_2, CH_2, CH_2, CH_2, CH_2, and CH_3), 2.00 (br, 1H, CH_2), 1.93-1.67 (br, 4H, CH_2 and CH_2), 1.68 (br, 1H, CH_2). ^{13}C-NMR (500 MHz, DMSO-d_6, δ): 175.1 (1C), 172.8 (1C), 172.6 (1C), 171.2 (1C), 149.9 (1C), 133.5 (1C), 132.4 (1C), 131.9 (1C), 130.8 (1C), 129.0 (1C), 122.5 (1C), 120.0 (1C), 89.3 (1C), 68.8 (1C), 58.8 (1C), 46.8 (1C), 43.5 (1C), 43.1 (1C), 35.4 (1C), 33.8 (1C), 33.5 (1C), 33.1 (3C), 32.9 (1C), 20.9 (1C). IR (solvent-casted DCM): 1818 cm^{-1} and 1761 cm^{-1} (C=O, anhydride), 1734 cm^{-1} (C=O, ester). M_w = 26,100 Da, PDI = 1.14. T_g = 120 °C. T_d = 185 °C.

5.1.1.1. Monoacid Synthesis

Morphine (**1** in Scheme 1, 0.10 g, 1 eq) was dissolved in anhydrous pyridine under argon and stirred for 5 min. Glutaric anhydride (**2**, 0.18 g, 2 eq) was slowly added manually. The reaction mixture was stirred at room temperature overnight. Pyridine was

azeotropically removed using toluene. The light yellow oil obtained was dried under vacuum at room temperature. Yield: 0.12 g (86 %) light yellow foam. ^{1}H-NMR (500 MHz, DMSO-d_6, δ): 6.70 (d, 1H, ArH), 6.54 (d, 1H, ArH), 5.52 (d, 1H, CH), 5.26 (s, 1H, CH), 4.75 (d, 1H, CH), 4.12 (s, 1H, CH), 3.29 (s, 1H, CH_2), 2.96 (d, 1H, CH), 2.38-2.17 (comp, 9H, CH_2, CH_2, CH_2, and CH_3), 2.02 (t, 1H, CH_2), 1.86-1.68 (comp, 4H, CH_2 and CH_2). MS: M/Z = 400 [M + 1].

5.4.3. High Performance Liquid Chromatography (HPLC)

Quantitative analysis of the *in vitro* degradation products was performed via HPLC using an XTerra® RP18 5 μm 4.6 x 150 mm column (Waters, Milford, MA) on a Waters 2695 Separations Module equipped with a Waters 2487 Dual λ Absorbance Detector. The system was connected to a Dell computer running Empower software. Samples were filtered using 0.22 μm poly(vinylidine fluoride) syringe filters (Fisher). The HPLC method was adapted from previously published methods.[50, 51] The mobile phase used was composed of 50 mM KH_2PO_4, 2.5 mM sodium dodecyl sulfate, 25 % acetonitrile, and 75% water at pH 3. Samples (20 μL) were run at 35 °C at a flow rate of 1 mL/min. Absorbance was monitored at λ = 210 nm. The instrument was calibrated using standard morphine **1** and diacid **3** solutions of known concentrations.

5.4.4. *In Vitro* Drug Release

Diacid **3** (5.0 mg, triplicate) was placed into scintillation vials and 20.00 mL phosphate buffered saline (PBS) pH 7.4 added. Samples were incubated at 37 °C under constant shaking (60 rpm) in an Excella E25 Incubator Shaker (New Brunswick Scientific). PBS (1.00 mL) was removed at predetermined time points (2 h, 5 h, 10 h, and daily starting on day 1 for 30 days) and replaced with fresh PBS (1.00 mL). The pH was checked using an Accumet® Research AR15 pH meter (Fisher Scientific) and adjusted to 7.4 using 0.50 M NaOH when needed. Samples were immediately analyzed by HPLC.

For the polymer degradation studies, polymer **5** (5.0 mg, triplicate) was placed into scintillation vials and 20.00 mL phosphate buffered saline (PBS) pH 7.4 added. Samples were incubated at 37 °C under constant shaking (60 rpm) in an Excella E25 Incubator Shaker (New Brunswick Scientific). PBS (20.00 mL) was removed daily and replaced with fresh PBS. The pH was checked using an Accumet® Research AR15 pH meter (Fisher Scientific). Samples were immediately analyzed by HPLC.

5.4.5. *In Vitro* Cytocompatibility

*[*In vitro *studies were performed by Roberto Delgado-Rivera, Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ]*

Cytocompatibility was evaluated by culturing 3T3 fibroblasts cells (NIH 3T3 fibroblast cell line) in diacid- and/or polymer-containing medium at concentrations of 0.10 and 0.01 mg/mL. Cell culture medium consisted of Dulbecco's modified Eagle's medium (DMEM), 10 vol% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA),

1% l-glutamate, and 1% penicillin/streptomycin. Fibroblasts were seeded at a density of 2,000 cells/well in 96 well plates containing 150 µL of culture medium. The positive control consisted of fibroblasts with cell culture media only and the negative control consisted of fibroblasts with cell culture media and 5% 200-proof ethanol (PHARMCO-AAPER). Cells were incubated at 37 °C and 5% CO_2 for 24, 48 and 72 h. Cell viability was determined using Calcein AM and ethidium homodimer-1 staining (Molecular Probes) according to the manufacturer's protocol and the results normalized to the positive control. For each of the three time points (24, 48 and 72 h), a student's t-test was performed to assess for statistical significance between the positive control and experimental conditions. Experiments were performed in quadruplicate.

5.4.6. *In Vivo* Analgesic Effect

*[*In vivo *studies were performed by Carolyn L. Harris and Dr. Lei Yu, Department of Genetics and Alcohol Studies, Rutgers University, Piscataway, NJ]*

Adult male C57Bl/6J mice were obtained from Charles River (Kingston, NY). Animals were approximately 10 weeks old and weighed between 19.5 – 27.7 g at the beginning of the study. Animals were housed in climate-controlled rooms with a 12:12 hour light/dark cycle, with food and water available ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Rutgers University, and consistent with the Guide for the Care and Use of Laboratory Animals

(National Institutes of Health, 2011). Animals were pre-handled twice a day for 3 days prior to the experiment.

Polymer **5** (200.0 mg powder) was suspended in 10 mL of 5 % Cremophor EL in saline by vortex and stirred for 15 min. Diacid **3** (50.0 mg foam) and morphine HCl (10 mg) were each dissolved in 10 mL of 5 % Cremophor EL in saline. A 5 % Cremophor EL saline solution was used as the vehicle control. All administrations were by intraperitoneal (i.p.) injection. Drug dosing was as follows: free morphine (morphine HCl) at 10 mg/kg, **3** at 50 mg/kg, and **5** at 200 mg/kg.

Nociception in mice was measured with the TFL test. Animals were wrapped loosely in soft cloth, where each cage of animals had its own cloth to minimize cross-cage olfactory sensory stimulation. TFL was tested by immersing the distal third of the animal's tail in a water bath at 49 °C, and the TFL time was recorded with a 30 s cutoff time to avoid tissue damage. Animals were only tested one time at each time point.

5.4.7. *In Vivo* Morphine Sensitivity

*[*In vivo *studies were performed by Carolyn L. Harris and Dr. Lei Yu, Department of Genetics and Alcohol Studies, Rutgers University, Piscataway, NJ]*

There were 30 animals in each group at the beginning of the study. TFL was measured at the following time points after the drug administration: 30 min, 1 h, 2 h, 4 h, 8 h, 1 d, 2 d, 3 d, 7 d, 9 d, and 14 d. On day 3, 15 animals from each group (including the vehicle control group) were tested for morphine sensitivity using the TFL test after by

being subjected to an acute morphine dose (10 mg/kg of free morphine in 5 % Cremphor EL in saline). The remaining 15 animals continued to be tested as scheduled. On day 14, after being tested for TFL, all animals received an acute dose of morphine (10 mg/kg of free morphine) and tested for morphine sensitivity using the TFL test.

*Other methods described in Chapter 9.

5.5. References

1. Sakurada, T.; Komatsu, T.; Sakurada, S., *NeuroToxicology* **2005,** *26* (5), 801-809.
2. Portenoy, R. K.; Sciberras, A.; Eliot, L.; Loewen, G.; Butler, J.; Devane, J., *Journal of Pain and Symptom Management* **2002,** *23* (4), 292-300.
3. Vermeire, A.; Remon, J. P., *International Journal of Pharmaceutics* **1999,** *187* (1), 17-51.
4. Davis, M. P., *American Journal of Cancer* **2006,** *5* (3), 171-182.
5. King, C. R.; Khabazian, A., *Clinical Journal of Oncology Nursing* **2003,** *7* (4), 458.
6. Ross, E. L.; Hahn, K., *International Journal of Clinical Practice* **2008,** *62* (3), 471-479.
7. Hagen, N. A.; Thirlwell, M.; Eisenhoffer, J.; Quigley, P.; Harsanyi, Z.; Darke, A., *Journal of Pain and Symptom Management* **2005,** *29* (1), 80-90.
8. Olsson, B.; Wagner, Z. G.; Mansson, P.; Ragnarsson, G., *International Journal of Pharmaceutics* **1995,** *119* (2), 223-229.
9. Kim, T.; Kim, J.; Kim, S., *Cancer Chemotherapy and Pharmacology* **1993,** *33* (3), 187-190.
10. Chao, J., *Pain Medicine* **2005,** *6* (3), 262-265.
11. Smith, H. S., *Pain Medicine* **2009,** *10*, S30-S38.
12. Grant, G. J.; Vermeulen, K.; Zakowski, M. I.; Stenner, M.; Turndorf, H.; Langerman, L., *Anesthesia & Analgesia* **1994,** *79* (4), 706-709.
13. Wang, J.-J.; Sung, K. C.; Yeh, C.-H.; Fang, J.-Y., *International Journal of Pharmaceutics* **2008,** *353* (1-2), 95-104.
14. Küchler, S.; Wolf, N. B.; Heilmann, S.; Weindl, G.; Helfmann, J.; Yahya, M. M.; Stein, C.; Schäfer-Korting, M., *Journal of Biotechnology* **2010,** *148* (1), 24-30.
15. Polard, E.; Le Corre, P.; Chevanne, F.; Le Verge, R., *International Journal of Pharmaceutics* **1996,** *134* (1-2), 37-46.
16. Morales, M. E.; López, G.; Gallardo, V.; Ruiz, M. A., *Molecular Pharmaceutics* **2011,** *8* (2), 629-634.

17. Morales, M. E.; Gallardo Lara, V.; Calpena, A. C.; Doménech, J.; Ruiz, M. A., *Journal of Controlled Release* **2004,** *95* (1), 75-81.
18. Arias, J. L.; Gómez-Gallo, A.; Delgado, Á. V.; Ruiz, M. A., *Colloids and Surfaces B: Biointerfaces* **2009,** *70* (2), 207-212.
19. Fernández-Arévalo, M.; Alvarez-Fuentes, J.; Iruin, A.; Holgado, M. A., *AAPS PharmSciTech* **2004,** *5* (3), e39.
20. Holgado, M. A.; Iruin, A.; Alvarez-Fuentes, J.; Fernández-Arévalo, M., *European Journal of Pharmaceutics and Biopharmaceutics* **2008,** *70* (2), 544-549.
21. Alvarez-Fuentes, J.; Fernández-Arévalo, M.; Holgado, M. A.; Caraballo, I.; Rabasco, A. M.; Micó, J. A.; Rojas, O.; Ortega-Alvaro, A., *International Journal of Pharmaceutics* **1996,** *139* (1-2), 237-241.
22. Mahkam, M.; Sharifi-Sanjani, N., *Polymer Degradation and Stability* **2003,** *80* (2), 199-202.
23. Erdmann, L.; Uhrich, K. E., *Biomaterials* **2000,** *21* (19), 1941-1946.
24. Schmeltzer, R. C.; Anastasiou, T. J.; Uhrich, K. E., *Polymer Bulletin* **2003,** *49* (6), 441-448.
25. Schmeltzer, R. C.; Schmalenberg, K. E.; Uhrich, K. E., *Biomacromolecules* **2004,** *6* (1), 359-367.
26. Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E., *Macromolecules* **2005,** *38* (16), 6895-6901.
27. Schmeltzer, R. C.; Uhrich, K. E., *Polymer Bulletin* **2006,** *57* (3), 281-291.
28. Prudencio, A.; Carbone, A. L.; Griffin, J.; Uhrich, K. E., *Macromolecular Rapid Communications* **2009,** *30* (13), 1101-1108.
29. Anastasiou, T. J.; Uhrich, K. E., *Journal of Polymer Science Part A: Polymer Chemistry* **2003,** *41* (22), 3667-3679.
30. Carbone, A. L.; Song, M.; Uhrich, K. E., *Biomacromolecules* **2008,** *9* (6), 1604-1612.
31. Kim, Y.; Uhrich, K. E., *Journal of Polymer Science Part A: Polymer Chemistry* **2010,** *48* (24), 6003-6008.
32. Whitaker-Brothers, K.; Uhrich, K., *Journal of Biomedical Materials Research Part A* **2006,** *76A* (3), 470-479.
33. Johnson, M. L.; Uhrich, K. E., *Journal of Biomedical Materials Research Part A* **2009,** *91A* (3), 671-678.
34. Whitaker-Brothers, K.; Uhrich, K., *Journal of Biomedical Materials Research Part A* **2004,** *70A* (2), 309-318.
35. Schmeltzer, R. C.; Uhrich, K. E., *Journal of Bioactive and Compatible Polymers* **2006,** *21* (2), 123-133.
36. Carbone, A. L.; Uhrich, K. E., *Macromolecular Rapid Communications* **2009,** *30* (12), 1021-1026.
37. Yeagy, B. A.; Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E.; Cook, T. J., *Journal of Microencapsulation* **2006,** *23* (6), 643-653.
38. Rosario-Meléndez, R.; Ouimet, M. A.; Uhrich, K. E., *Polymer Bulletin* **2012,** *Manuscript accepted*.
39. Schmeltzer, R. C.; Johnson, M.; Griffin, J.; Uhrich, K., *Journal of Biomaterials Science, Polymer Edition* **2008,** *19* (10), 1295-1306.
40. Achim, G., *Biomaterials* **1996,** *17* (2), 103-114.

41. Siepmann, J.; Gopferich, A., *Advanced Drug Delivery Reviews* **2001,** *48* (2,Äì3), 229-247.
42. Kamendulis, L. M.; Brzezinski, M. R.; Pindel, E. V.; Bosron, W. F.; Dean, R. A., *Journal of Pharmacology and Experimental Therapeutics* **1996,** *279* (2), 713-717.
43. Dumitriu, S., *Polymeric Biomaterials*. Second ed.; Mercel Dekker Inc.: New York, NY, 2002.
44. Razem, D.; Katusin-Razem, B., *Radiation Physics and Chemistry* **2008,** *77* (3), 288-344.
45. Harrison, L. M.; Kastin, A. J.; Zadina, J. E., *Peptides* **1998,** *19* (9), 1603-1630.
46. Williams, J. T.; Christie, M. J.; Manzoni, O., *Physiological Reviews* **2001,** *81* (1), 299-343.
47. Ueda, H.; Ueda, M., *Front Biosci* **2009,** *14*, 5260-72.
48. Mathiowitz, E.; Jacob, J.; Jong, Y.; Carino, G.; Chickering, D.; Chaturvedi, P.; Santos, C.; Vijayaraghavan, K.; Montgomery, S.; Bassett, M.; C, M., *Nature* **1997,** *386* (6623), 410-414.
49. Allen, T.; Cullis, P., *Science* **2004,** *303* (5665), 1818-1822.
50. Gerostamoulos, J.; Drummer, O. H., *Forensic Science International* **1996,** *77* (1,Äì2), 53-63.
51. Meng, Q. C.; Soleded Cepeda, M.; Kramer, T.; Zou, H.; Matoka, D. J.; Farrar, J., *Journal of Chromatography B: Biomedical Sciences and Applications* **2000,** *742* (1), 115-123.

6. BIODEGRADABLE IBUPROFEN- AND NAPROXEN-BASED POLYESTERS

6.1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have analgesic, antipyretic, and anti-inflammatory activity.[1, 2] Although effective in treating pain, fever, and inflammation, their use is limited by the side effects.[1, 2] They are generally administered orally and distributed throughout the body to both target and non-target sites.[3] This results in increased side effects and required frequent dosing to maintain the drug within therapeutic levels. Ibuprofen (**1**) and naproxen (**2**), Figure 6.1, are propionic acid-derivative NSAIDs commonly used to treat pain and swelling associated with rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and Ankylosing spondylitis, among other uses.[4-6] Administration of high systemic doses is often required to treat these long-lasting symptoms because both **1** and **2** have relatively short half-life in plasma (2.1 and 14 hours, respectively).[1, 7] When repeatedly administered, severe gastrointestinal (GI) side effects such as stomach ulceration, bleeding, and perforation occur.[8, 9] To exploit the therapeutic potential of **1** and **2**, a controlled drug delivery system is needed.

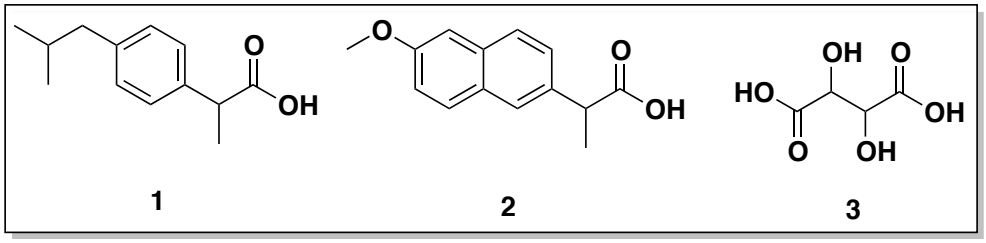


Figure 6.1. Chemical structures of ibuprofen (**1**), naproxen (**2**), and tartaric acid (**3**).

Drug delivery systems have been developed to localize drug release and prolong the duration of the drug effect. The preparation of polymer microparticles encapsulating **1** or **2** has been studied.[10-16] The major issues associated with this type of drug delivery systems are low drug loading (less than 30 %) and short-term (rapid) drug release after a burst. Acrylic and vinyl polymers have been widely studied to conjugate **1** or **2** into the polymer backbone.[8, 9, 17-21] Although these polymers are biocompatible, they are not biodegradable. Therefore, when the entire drug is released, the polymer could remain in the body which could cause patient discomfort and adverse effects.[3] Despite the limitations, these drug delivery systems have been shown to lower the side effects associated with the systemic administration of the drug and to increase the duration of the drug anti-inflammatory effects.[8]

To improve the controlled delivery of **1** and **2**, we have designed polymers containing 65-67 wt.% of drug chemically incorporated into the polymer that upon hydrolytic degradation is released without a burst. The chemical incorporation of bioactive molecules into biodegradable polymer backbones [poly(anhydride-esters)] has been studied as a novel drug delivery method. For example, salicylic acid, salicylate derivatives, and morphine have been chemically incorporated into polymer backbones achieving more than 50 wt.% drug loading.[22-25] The drug is released in a controlled manner via hydrolytic degradation of the polymer.[23, 25, 26] These drugs possess two reactive functional groups that allow their chemical incorporation into a polymer backbone. However, not all drugs possess two reactive functional groups for polymerization, such and **1** and **2**. Drugs containing only one reactive functional group

can be incorporated into a polymer as pendant groups. We have already explored this type of chemical incorporation with phenolic antiseptics, also achieving high drug loading (48-58 wt.%).[24] The incorporation of **1** and **2** into biodegradable polyester backbones was done as pendant groups as they only have one reactive functional group.

This work presents the synthesis and characterization of biodegradable ibuprofen- and naproxen-based polyesters. Tartaric acid (Figure 6.1, **3**), a naturally occurring and biocompatible compound that has antioxidant properties,[27] was used as "backbone" for this polymer. The polymers were synthesized at 130 °C catalyzed by tin (II) 2-ethylhexanoate. Chemical structures validation and physical properties characterization of all compounds were performed. Also, *in vitro* studies were performed to study drug release from the polymers in phosphate buffered saline (PBS) mimicking physiological conditions. Cytocompatibility towards mouse fibroblasts and human blood-derived macrophages was tested and released drug bioactivity *in vitro*.

6.2. Results and Discussion

Current systems to deliver **1** or **2** have been shown to decrease the side effects associated with the drug and prolong the drug effects. However, they achieve low drug loading (less than 30 %) with a burst drug release, or use a non-biodegradable polymer. In an attempt to develop a biodegradable drug delivery system that can achieve higher drug loading and more controlled release, we have synthesized and characterize polyesters containing **1** and **2** as pendant groups.

6.2.1. Synthesis and Characterization

A published procedure[28] for the synthesis of chicoric acid was adapted to synthesize the polymer precursors ibuprofen- and naproxen-based diacids **6a** and **6b**, respectively (Scheme 6.1).

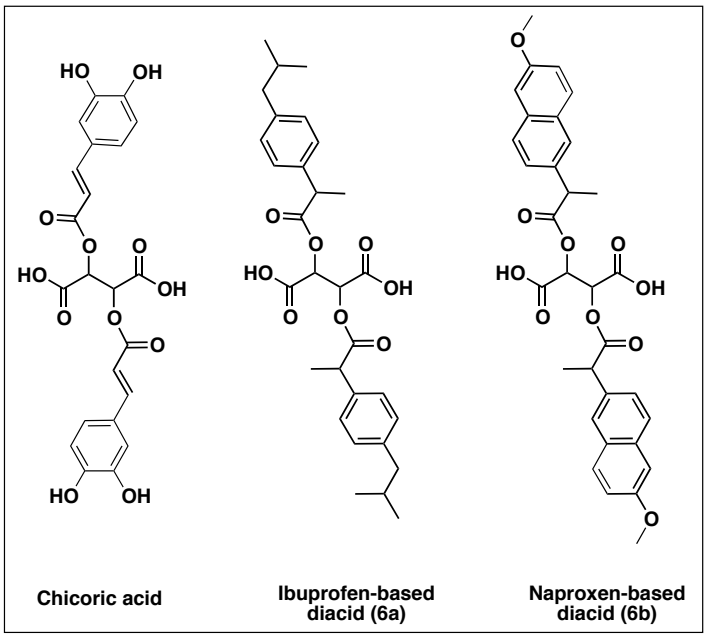
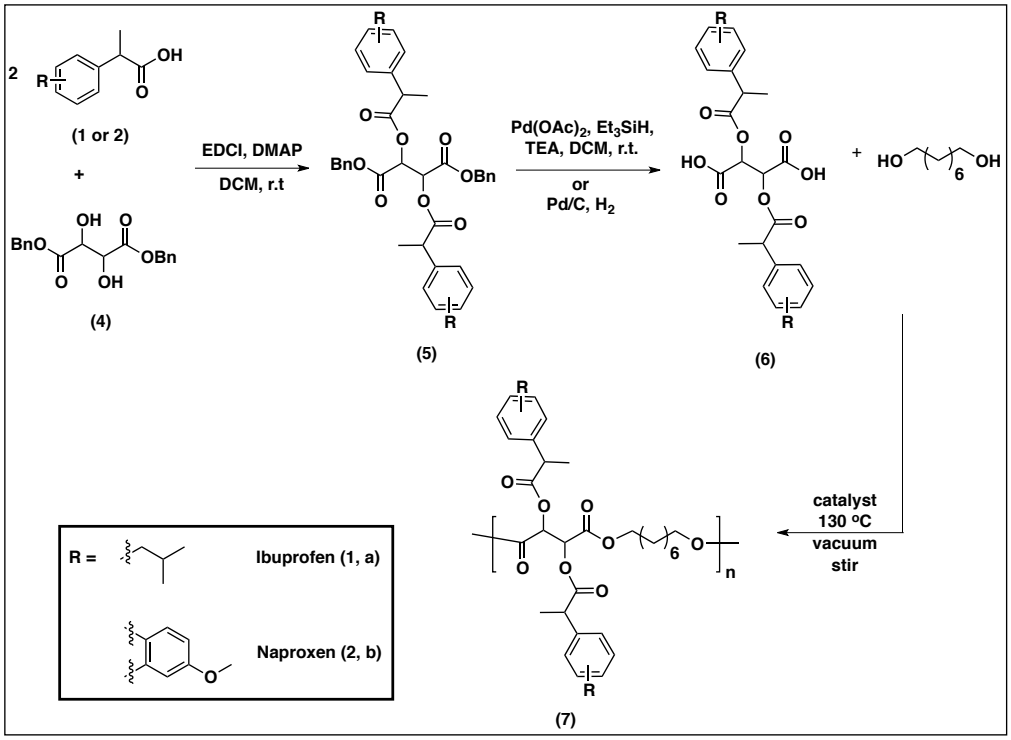


Figure 6.2. Chemical structures of chicoric acid (left), ibuprofen-based diacid (**6a**), and naproxen-based diacid (**6b**).

This synthetic procedure was chosen because of the structural similarities between chicoric acid and the diacids **6a** and **6b** (Figure 6.2). Dibenzyl-protected tartaric acid (**4**) was used for the synthesis of ibuprofen- and naproxen-protected diacids (**5a** and **5b**, respectively), to couple the NSAID (**1** or **2**) to the hydroxyl groups of the tartrate

backbone using EDCI (first step Scheme 6.1). Selective deprotection to obtain the diacids **6a** and **6b** was performed using silane-promoted palladium-mediated hydrogenation (second step Scheme 6.1). This debenzylation method is known to preserve sensitive functional groups and the newly formed ester linkages. As expected, compounds **6a** and **6b** were successfully synthesized using this method. However, the product isolation process was complicated and tedious (comprised of multiple extractions). Therefore, the use of H_2 and Pd/C was explored. This common hydrogenation method yielded the pure products (**6a** and **6b**) after an easy isolation comprised of filtration of the Pd/C and evaporation of the solvent and byproducts. All compounds were obtained in high yields (i.e., more than 77 %).



Scheme 6.1. Synthesis of ibuprofen- and naproxen-protected diacids (**5a** and **5b**, respectively) by coupling of the drug's (**1** or **2**) carboxylic acids to the hydroxyl groups of

the dibenzyl protected tartaric acid (**4**). Deprotection to yield the diacids (**6a** and **6b**) was performed using two different hydrogenation methods. Synthesis of ibuprofen- and naproxen-tartaric polymers (**7a** and **7b**) was performed using tin (II) 2-ethylhexanoate as catalyst at 130 °C.

The chemical structures of the compounds were confirmed by proton and cabon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) and infrared (IR) spectroscopies and the molecular weights (MWs) by mass spectrometry (MS). Figure 6.3 shows the ^{1}H-NMR spectra of the ibuprofen-containing compounds **5a** and **6a**. All the expected peaks are shown in the spectra (Figure 6.3 and 6.4) and no unexpected peaks were found. This data indicates the successful coupling of the drug to the tartrate backbone and that the deprotection did not break any other bonds. When the two spectra are compared (Figure 6.3 top and center), it is observed that the debenzylation was successful as demonstrated by the disappearance of the benzylic protons (i-k, Figure 6.3 top). In the case of the naproxen-containing compounds **5b** and **6b**, the debenzylation was also demonstrated by ^{1}H-NMR (Figure 6.4 top and center). The ^{13}C-NMR (not shown) showed the presence of all carbons and no extra peaks were observed, therefore supporting that the deprotection was successful. For further characterization, the IR spectra of **5a** and **5b** show the formation of the ester bonds by the presence of the ester carbonyls (C=O) at ~ 1770 and 1750 cm^{-1}. The IR spectra of **6a** and **6b** show that the deprotection was successful by the presence of the ester carbonyls (C=O) at ~ 1760 and the presence of terminal carboxylic acid C=O at ~ 1730 cm^{-1} (Figures 6.5 and 6.6, top). The MW the compounds were 724, 777, 549, and 597, which correspond to [M + Na] of **5a**, **5b**, **6a**, **6b**, respectively. All

compounds were viscous oils or foams and did not display melting points (T_m); the decomposition temperatures (T_d) ranged between 224-294 °C. These high T_d values are important when polymerizing at high temperatures.

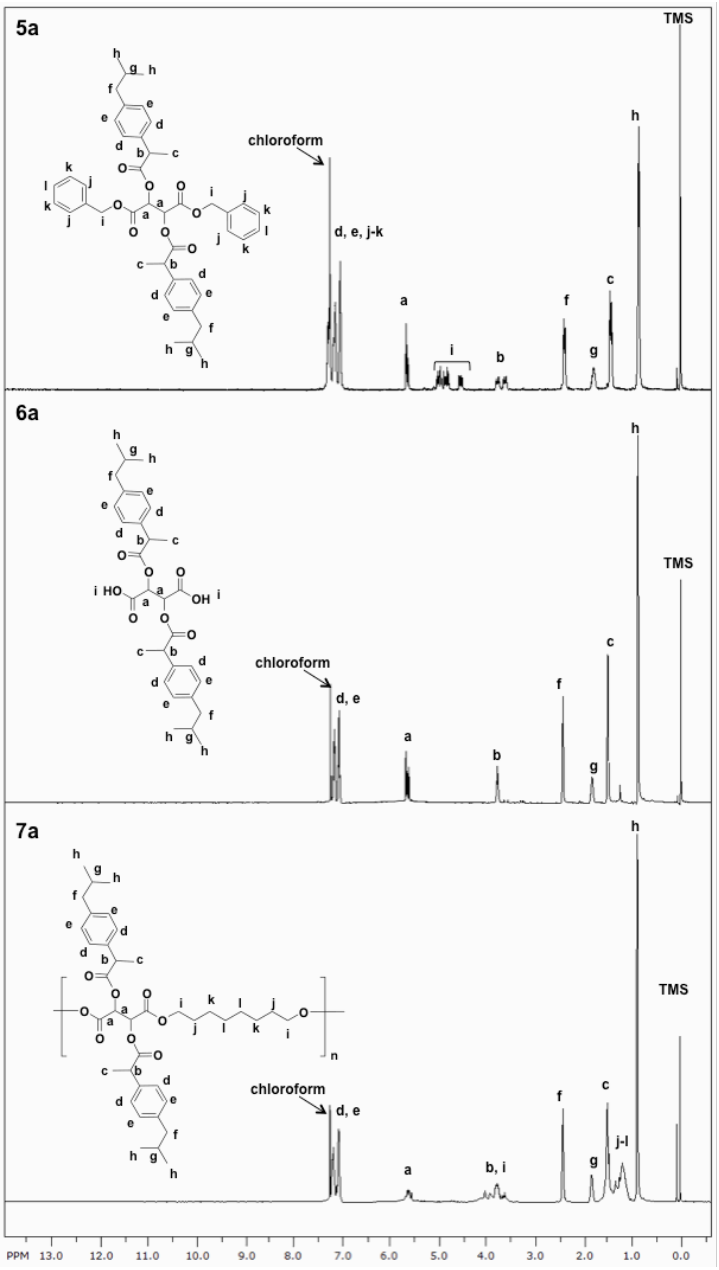


Figure 6.3. ^{1}H-NMR spectra of compounds **5a** and **6a** showing the presence and disappearance of the benzyl protecting groups and polymer **7a**.

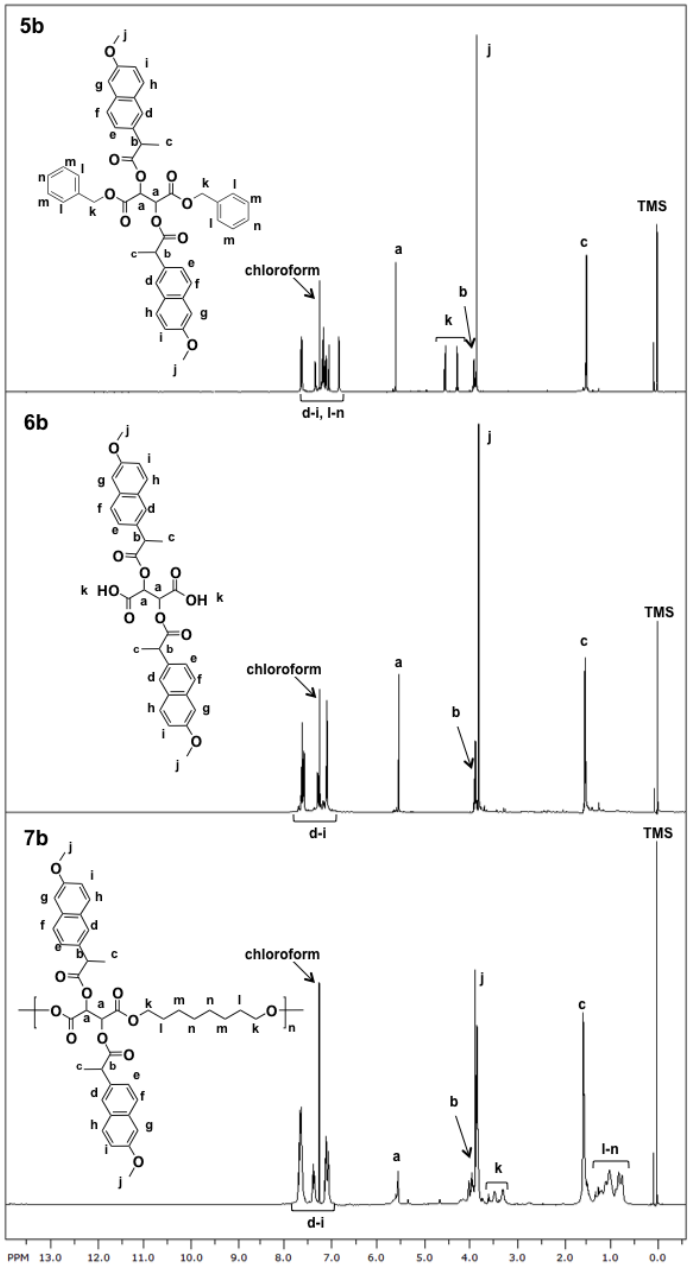


Figure 6.4. ^{1}H-NMR spectra of compounds **5b** and **6b** showing the presence and disappearance of the benzyl protecting groups and polymer **7b**.

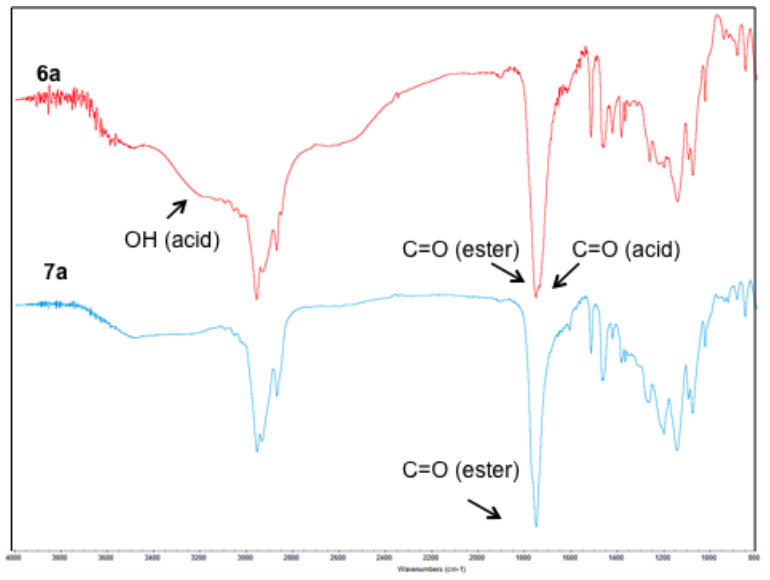


Figure 6.5. Infrared spectra of ibuprofen-based diacid (**6a**) and ibuprofen-based polyester (**7a**); key absorption bands for OH acid, C=O acid, and C=O ester are indicated.

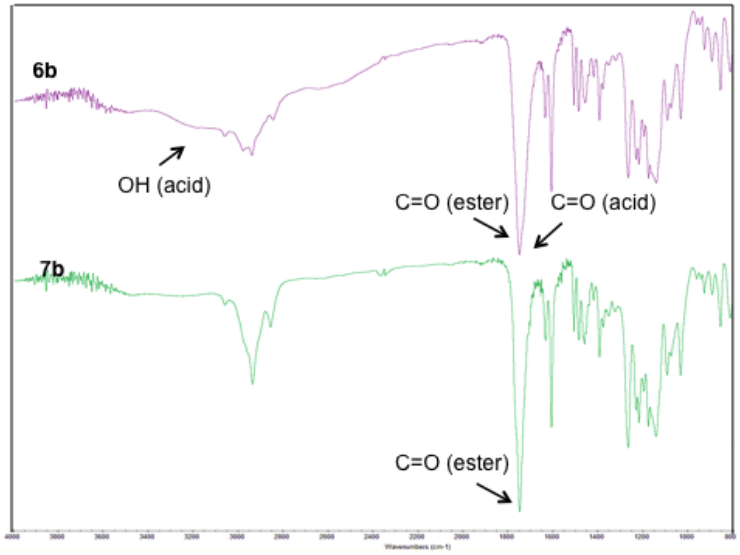


Figure 6.6. Infrared spectra of naproxen-based diacid (**6b**) and naproxen-based polyester (**7b**); key absorption bands for OH acid, C=O acid, and C=O ester are indicated.

The synthesis of the biodegradable polyesters was performed reacting the diacids **6a** and **6b** (respectively) with 1,8-octainediol using tin (II) 2-ethylhexanoate as catalyst at 130 °C (step 3, Scheme 6.1). Polyesters containing tartaric acid and 1,8-octainediol have been previously reported with tin (II) 2-ethylhexanoate as catalyst,[30] which sets a precedent for this type of reaction. Polyesters were prepared using 1,8-octanediol because it is a material generally regarded as safe that has bacteriostatic, bactericidal, and preservative properties.[31] In addition, tin (II) 2-ethylhexanoate the catalyst of choice for many polymerizations due to its low cost, low toxicity, and high efficiency.[32-34] The ^{1}H-NMR spectra for the polymers (**7a** and **7b**) show broadening of the peaks and the presence of all the peaks expected (Figure 6.3 and 6.4 bottom). The IR spectra of **7a** and **7b** show the presence of the ester C=O at ~ 1770 and 1750 cm^{-1} (Figures 6.5 and 6.6 bottom, respectively). Polymers with moderate weight-average molecular weight (M_w) (11,200 and 6,000 Da) and low polydispersity index (PDI) were obtained (1.2-1.4). These polymers decomposed at temperatures above 250 °C and have low T_g values (-17 °C for **7a** and 23 °C for **7b**).

6.2.2. *In Vitro* Drug Release

After successfully synthesizing the polymers, the ability to release the free drug was studied *in vitro*. Polymer samples (**7a** and **7b**, in triplicate) were incubated in phosphate buffered saline PBS at pH 7.4 mimicking physiological conditions (37 °C and 60 rpm). At predetermined time points, the media was collected and analyzed using HPLC. The retention time (R_t) for **1** was 3.08 min and for **2** 2.40 min, the diacids **6a** and

6b had R_t of 4.61 and 3.17 min, respectively. During the studies, no peaks for high M_w oligomers were detected and diacid peaks were detected in trace amounts. Figure 6.7 shows the *in vitro* drug release profiles for **1** and **2**. No burst release was observed and the drugs were released in a controlled manner. Both drugs were released at approximately the same rate, which was expected due to the structural similarities between the two polymers. After 30 days, polymer **7a** released ~ 14 % of **1** and **7b** released ~ 8 % of **2** (based on calculated theoretical values). At this rate we expect 100 % release in 7 to 8 months.

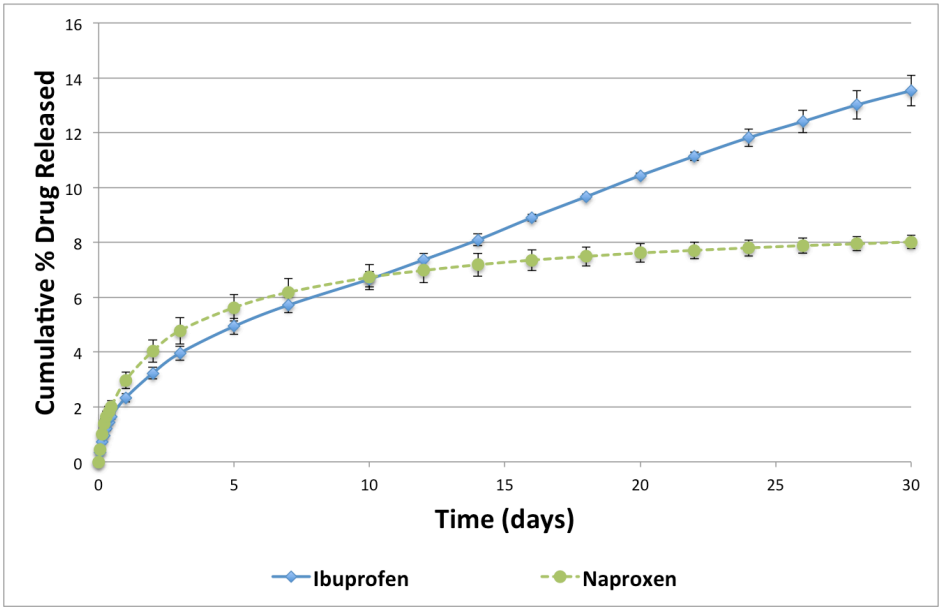


Figure 6.7. *In vitro* ibuprofen (**1**, filled diamonds) and naproxen (**2**, filled circles) release profiles from polymers **7a** and **7b** (± standard error).

6.2.3. *In Vitro* Cytocompatibility

Cytocompatibility of the diacids and polymers was evaluated by culturing L929 mouse fibroblasts, a commonly used cell type to test toxicity of new biomaterials (performed by Weiling Yu, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ).[35] The diacids (**6a** and **6b**) and the polymers (**7a** and **7b**), separately, were dissolved in DMSO and then diluted with cell culture media to concentrations of 0.10 and 0.05 mg/mL to mimic late and early stage polymer degradation. The study was performed over 72 h and cell viability evaluated at 24, 48, and 72 h. Figure 6.8 shows cell viability for all samples and the DMSO-containing media control, all samples were normalized with the control. Comparison between the diacids- and polymers-containing samples and the control showed that only polymer **7a** at 0.10 mg/mL resulted in a consistent decrease in normalized cell viability with time. All other samples resulted in normal cell viability.

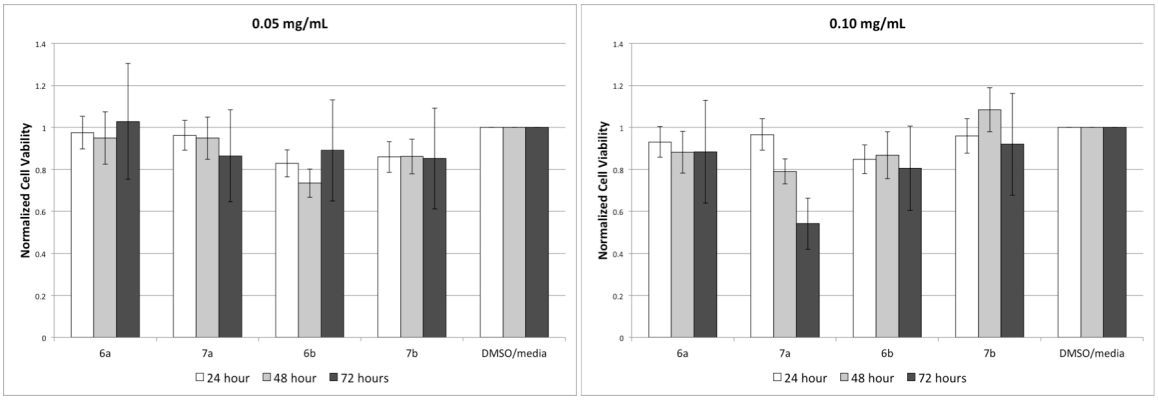


Figure 6.8. Normalized L929 cell viability in culture media containing polymers and diacids (left: 0.05 mg/mL; right: 0.10 mg/mL) at 24, 48, and 72 h. Data represent mean and standard deviation of six samples.

6.2.4. *In Vitro* TNF-α Inhibition

To assess the bioactivity of the drugs released from the polymers, *in vitro* TNF-α inhibition was tested (performed by Weiling Yu, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ). TNF-α is a cytokine involved in inflammation and secreted by macrophages. Therefore, human blood-derived macrophages were stimulated with LPS and exposed to the free drugs (**1** and **2**) and polymers degradation media at day 5 (0.5 μg/mL). Cells incubated in media alone were used a negative control and cells with media and LPS as positive control. The other components of the polymers (i.e., **3** and 1,8-octanediol) were tested as controls, none of them showed TNF-α inhibition. At the two concentrations used, the free drugs and the respective degradation media behaved similarly without significant differences between them (Figure 6.9). These results suggest that the drugs preserved their bioactivity after being released from the polymers.

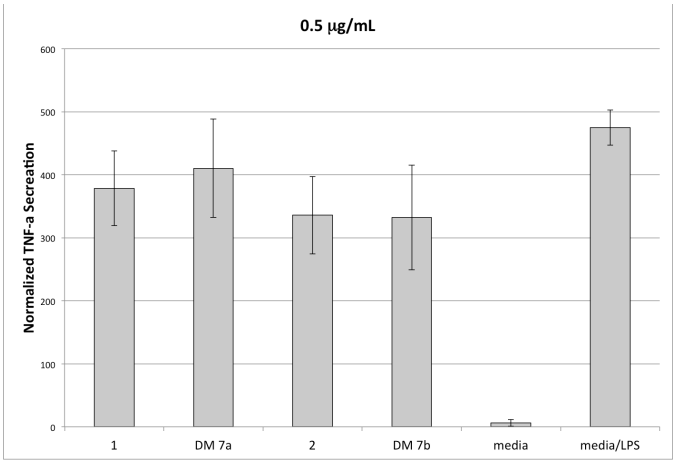


Figure 6.9. *In vitro* TNF-α inhibition after 24 h incubation of LPS stimulated human blood-derived macrophages in cell media containing the free drugs (**1** and **2**) and the degradation media (DM) from the polymers (**7a** and **7b**) at drug concentration of 0.5

μg/mL. Media was used as negative control and media containing LPS as positive control. Data represent mean and standard deviation of five samples.

Macrophage viability after incubating with free drugs and polymer degradation media was also investigated. All samples showed no cytotoxicity supporting that these biomaterials are cytocompatible (Figure 6.10).

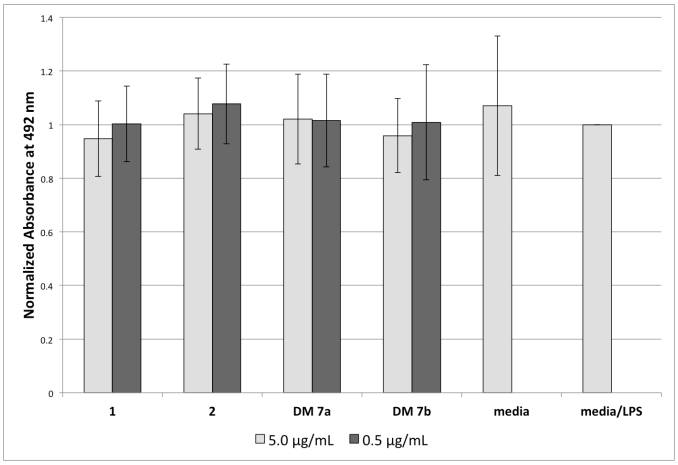


Figure 6.10. Normalized human blood-derived macrophages cell viability in culture media containing polymers and diacids (at 5.0 and 0.5μg/mL) after 24 h of incubation. Either free drugs dissolved in PBS or polymer degradation media (DM) on day 5 were used to make the solutions. Data represent mean and standard deviation of five samples.

6.3. Conclusion

In this work, we presented the synthesis and characterization of novel biodegradable polyester, comprised of all biocompatible elements: tartaric acid, 1,8-octanediol, and an NSAID. With these polymers, the duration of the drug effect can be prolonged (more than 1 month), and no surgical removal of the polymer is anticipated after complete drug release (i.e., biodegradable). These biomaterials are not cytotoxic towards fibroblasts. These polymers that can be used to deliver **1** and **2** in a controlled manner, and thus, have potential to treat inflammatory diseases such as arthritis. Our future work includes the incorporation of other propionic acid-derivative NSAIDs as pendant groups to polyesters and the *in vivo* anti-inflammatory activity testing.

6.4. Experimental

6.4.1. Materials

Naproxen and 1-[-3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) were purchased from Fisher Scientific (Pittsburg, PA). Human blood was obtained from the New Jersey Blood Center and the NCTC clone 929 (strain L) mouse areolar fibroblast cells form ATCC (Manassas, VA). Ficoll-Paque Premium was purchased from GE Healthcare. Human cluster of differentiation 14 (CD14+) beads and the magnetic-activated cell sorting (MACS) cell separator were purchased from Miltenyi Biotec. Advanced Roswell Park Memorial Institute (RPMI)-1640 media, fetal bovine serum (FBS), Penicillin/streptomycin (pen/strep), L-Glutamine, trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA), and Dulbecco's modified eagle medium

(DMEM) were obtained from GIBCO BRL (Rockville, MD). Granulocyte-macrophage colony-stimulating factor (GM-CSF) was purchased from R&D Systems (Minnneapolis, MN). CellTiter 96® Aqueous One Solution Cell Proliferation Assay was obtained from Promega (Madison, WI). Human TNF-α enzyme-linked immunosorbent assay (ELISA) kit was purchased from Biolegend. Unless otherwise specified, all other chemicals and reagents were purchased from Sigma-Aldrich (Milwaukee, WI) and used as received.

6.4.2. Protected Diacids Synthesis

6.4.2.1. Ibuprofen-tartrate Protected Diacid (5a)

Ibuprofen (**1**, 3.21 g, 2.2 eq) was dissolved in anhydrous DCM and stirred under argon. Then 4-(dimethylamino)pyridine (DMAP, 1.90 g, 2.2 eq) was added. Dibenzyl-L-tartrate (**4**, 2.34 g, 1 eq) was dissolved in anhydrous DCM and added to the reaction mixture which was followed by the addition of EDCI (5.96 g, 4.4 eq). The resulting yellowish solution was left stirring for 2 h. The reaction mixture was diluted with ethyl acetate (EtOAc) and extracted with 10% $KHSO_4$ and saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and solvent evaporated under reduced pressure to give a brown viscous oil that was dried *in vacuo* at room temperature overnight. Yield: 2.99 g (93 %). ^{1}H-NMR ($CDCl_3$, 500MHz, δ): 7.30 (6H, m, ArH), 7.16 (6H, m, ArH), 7.06 (6H, m, ArH), 5.67 (2H, split, CH), 5.05-4.53 (4H, split, CH2), 3.80-3.60 (2H, dm, CH), 2.41 (4H, m, CH_2), 1.79 (2H, m, CH), 1.45 (6H, t, CH_3), 0.86 (12H, d, CH_3). ^{13}C-NMR ($CDCl_3$, 500MHz, δ): 173.5 (1C), 173.2 (1C), 165.7 (1C), 165.3 (1C), 140.9 (2C), 136.9 (2C), 135.0 (2C), 129.5 (6C), 128.6 (6C), 127.7 (6C), 71.1 (2C), 67.7 (2C), 45.1 (2C),

44.7 (2C), 30.4 (2C), 22.7 (4C), 18.5 (2C). IR: 1769 cm^{-1} (C=O ester) and 1751 cm^{-1} (C=O ester). MS: M/Z = 729 [M + Na]. T_d = 237 ^oC.

6.4.2.2. Naproxen-tartrate Protected Diacid (5b)

Synthesis of the **5b** was performed using the procedure described for **5a** using 2.2 eq of naproxen (**2**) instead of **1**. Yield: 0.81 g (81 %), green foam. ^1H-NMR ($CDCl_3$, 500MHz, δ): 7.64 (6H, t, ArH), 7.37 (2H, d, ArH), 7.18 (6H, m, ArH), 7.10 (2H, d, ArH), 7.03 (2H, d, ArH), 6.83 (4H, d, ArH), 5.62 (2H, s, CH), 4.57-4.29 (4H, dd, CH_2), 3.93 (2H, m, CH), 3.88 (6H, s, OCH_3), 1.52 (6H, d, CH_3). ^{13}C-NMR ($CDCl_3$, 500MHz, δ): 173.5 (2C), 165.4 (2C), 158.0 (2C), 135.1 (2C), 134.6 (2C), 134.0 (2C), 129.6 (2C), 129.1 (2C), 128.6 (4C), 128.5 (2C), 128.0 (6C), 127.4 (2C), 126.5 (2C), 119.3 (2C), 105.8 (2C), 71.2 (2C), 67.6 (2C), 55.5 (2C), 45.0 (2C), 18.4 (2C). IR: 1767 cm^{-1} (C=O ester) and 1748 cm^{-1} (C=O ester). MS: M/Z = 777 [M + Na]. T_d = 294 ^oC.

6.4.3. Diacids Synthesis

6.4.3.1. Ibuprofen-based Diacid (6a)

To palladium (II) acetate [$Pd(OAc)_2$, 4.23 g, 2.5 eq], anhydrous DCM and triethylamine (TEA, 2.96 mL, 2.5 eq) were added and the mixture stirred under argon. Ibuprofen-tartrate protected diacid (**5a**, 6.00 g, 1 eq) was dissolved in DCM and added dropwise to the reaction mixture. The solution was left stirring for 5 min and

triethylsilane (Et_3SiH, 34 mL, 25 eq) was added dropwise via syringe pump (over 1 h). Reaction was left stirring at room temperature under argon overnight. MeOH (3 mL) was added and the mixture was filtered over celite to remove Pd. The filtrate was concentrated under reduced pressure and the orange residue obtained was diluted in EtOAc. The precipitate formed was removed via vacuum filtration. The filtrate was concentrated under reduced pressure; the orange liquid obtained was diluted in acetonitrile and extracted with hexanes. The acetonitrile layer was dried under reduced pressure. The orange residue obtained was diluted in EtOAc and extracted with water. The organic layer was dried over $MgSO_4$ and the solvent evaporated under reduced pressure to give a yellow foam that was dried *in vacuo* at room temperature overnight. Yield: 4.62g (77 %). ^{1}H-NMR ($CDCl_3$, 500MHz, δ): 7.18 (4H, d, ArH), 7.08 (4H, d, ArH), 5.68 (2H, split, CH), 3.79 (2H, t, CH), 2.5 (4H, m, CH_2), 1.84 (2H, m, CH), 1.51 (6H, t, CH_3), 0.88 (12H, d, CH_3). ^{13}C-NMR ($CDCl_3$, 500MHz, δ): 173.6 (1C), 173.3 (1C), 170.7 (1C), 170.2 (1C), 141.0 (2C), 136.7 (2C), 129.5 (4C), 127.6 (4C), 70.5 (2C), 45.1 (2C), 44.8 (2C), 30.4 (2C), 22.6 (4C), 18.4 (2C). IR: 1751 cm^{-1} (C=O ester), 1733 cm^{-1} (C=O acid), and 3231 cm^{-1} (OH acid). MS: M/Z = 549 [M + Na]. T_d = 224 ^{o}C.

6.4.3.2. Naproxen-based Diacid (6b)

Synthesis was performed using the procedure described for **6a** in Section 2.5.1. Yield: 0.90 g (90 %), orange foam. ^{1}H-NMR ($CDCl_3$, 500MHz, δ): 7.70 (4H, t, ArH), 7.38 (4H, d, ArH), 7.15 (4H, d, ArH), 5.57 (2H, s, CH), 4.00 (2H, m, CH), 3.91(6H, s, OCH_3), 1.60 (6H, d, CH_3). ^{13}C-NMR ($CDCl_3$, 500MHz, δ): 173.5 (2C), 160.0 (2C), 157.9

(2C), 135.0 (2C), 134.0 (2C), 129.5 (2C), 127.4 (2C), 126.4 (4C), 126.3 (2C), 119.3 (2C), 105.7 (2C), 71.0 (2C), 55.5 (2C), 44.9 (2C), 18.3 (2C). IR: 1748 cm^{-1} (C=O ester), 1733 cm^{-1} (C=O acid), and 3447 cm^{-1} (OH acid). MS: M/Z = 597 [M + Na]. T_d = 235 ^oC.

6.4.4. Optimized Diacids Synthesis

Ibuprofen- or naproxen-tartrate protected diacid (**5a** or **5b**, 1 eq) was dissolved in anhydrous DCM (10 mL/g of protected diacid) and 10% palladium on carbon (Pd/C, catalytic amount) added. The reaction flask was evacuated by vacuum and purged with hydrogen gas (3x). The reaction was stirred at room temperature under hydrogen overnight. The mixture was filtered over celite to remove Pd/C. The filtrate was dried under reduced pressure to give a yellow or orange foam that was dried *in vacuo* at room temperature overnight. Yield: > 90 %. Characterization described in sections 6.4.3.1 and 6.4.3.2.

6.4.5. Polymers Synthesis

6.4.5.1. Ibuprofen-based Polyester (7a)

Ibuprofen-tartaric diacid (0.51 g, 1 eq), 1,8-octanediol (0.14 g, 1eq), and tin (II) 2-ethylhexanoate (26.4 μL, 5 wt.%) were added to a double-neck round-bottom flask and degassed through vacuum/argon cycles (3x). The mixture was heated to 130°C under vacuum and stir for 6 h. The product was cooled down and dissolved in DCM (minimal amount). The product was isolated by removing the DCM under reduced pressure and

dried under vacuum at room temperature overnight. Yield: 0.42 g (82 %), orange paste. ^{1}H-NMR ($CDCl_3$, 500MHz, δ): 7.20 (4H, b, ArH), 7.08 (4H, b, ArH), 5.60 (2H, b, CH), 4.10-3.6 (6H, b, CH, CH_2), 2.44 (4H, b, CH_2), 1.85 (2H, b, CH), 1.60-1.00 (18H, b, CH_3, $3CH_2$), 0.89 (12H, b, CH_3). ^{13}C-NMR ($CDCl_3$, 500MHz, δ): 173.6 (2C), 165.3 (2C), 140.9 (2C), 136.9 (2C), 129.5 (4C), 127.6 (4C), 71.0 (2C), 66.3 (2C), 45.3 (2C), 44.9 (2C), 30.6 (2C), 29.3 (2C), 28.4 (2C), 25.7 (2C), 22.4 (4C), 18.3 (2C). IR: 1768 and 1750 cm^{-1} (C=O, ester). M_w = 11,200 Da, PDI = 1.4. T_g = -17 °C. T_d = 289 °C.

6.4.5.2. Naproxen-based Polyester (7b)

Synthesis was performed using the procedure described in Section 2.7.1. Yield: 0.19 g (95 %), yellow foam. ^{1}H-NMR ($CDCl_3$, 500MHz, δ): 7.70 (4H, b, ArH), 7.39 (4H, b, ArH), 7.13 (4H, b, ArH), 5.56 (2H, b, CH), 3.97 (2H, b, CH), 3.91(6H, b, OCH_3), 3.60-3.16 (4H, b, CH_2) 1.58 (6H, b, CH_3), 1.57-0.64 (12H, b, $3CH_2$). ^{13}C-NMR ($CDCl_3$, 500MHz, δ): 173.5 (2C), 165.6 (2C), 157.9 (2C), 135.0 (2C), 134.0 (2C), 129.6 (2C), 127.4 (2C), 126.4 (4C), 126.3 (2C), 119.3 (2C), 105.7 (2C), 71.2 (2C), 66.2 (2C), 55.4 (2C), 44.9 (2C), 29.1 (2C), 28.1 (2C), 25.4 (2C), 18.3 (2C). IR: 1768 and 1747 cm^{-1} (C=O, ester). M_w = 6,000 Da, PDI = 1.2. T_g = 23 °C. T_d = 260 °C.

6.4.6. Gel Permeation Chromatography (GPC)

GPC was used to determine M_w and polydispersity index (PDI) of the polymers. A Waters system consisting of a 515 HPLC pump, a 717plus autosampler, and a 410 RI detector was used. Waters Empower 2 software was used for data collection and analysis. Samples were dissolved in tetrahydrofuran (10 mg/mL), 20 μL was injected, and eluted through two PL gel columns 10^3 and 10^5 Å (Polymer Laboratories) used in series at a flow rate of 1 mL/min. The. M_w was calculated relative to narrow M_w polystyrene standards.

6.4.7. High Performance Liquid Chromtography (HPLC)

Quantitative analysis of the *in vitro* degradation products was performed via HPLC using an XTerra® RP18 5 μm 4.6 x 150 mm column (Waters, Milford, MA) on a Waters 2695 Separations Module equipped with a Waters 2487 Dual λ Absorbance Detector. The system was connected to a Dell computer running Empower software. Samples were filtered using 0.22 μm poly(vinylidine fluoride) syringe filters (Fisher). The HPLC method was adapted from previously published methods.[36, 37] The mobile phase used was composed of 10 mM KH_2PO_4, 70 % acetonitrile, and 30% water at pH 3.5. Samples (20 μL) were run at 25 °C at a flow rate of 1 mL/min. Absorbance was monitored at λ = 265 nm for both drugs. The instrument was calibrated using standard ibuprofen **1** and naproxen **2** solutions of known concentrations.

6.4.8. *In Vitro* Drug Release

Ibuprofen and naproxen release from their respective polymer was studied at 37 ˚C in PBS at pH 7.4 with agitation (60 rpm) to mimic physiological conditions. Triplicate samples of each polymer (50.0 mg) were placed in 20 mL scintillation vials (Fisher, Fair Lawn, NJ) with 15 mL of PBS. At predetermined time points, PBS (15 mL) was collected and replaced with fresh PBS (15 mL). Samples were immediately analyzed.

6.4.9. *In Vitro* Cytocompatibility

*[*In vitro *cytocompatibility studies were performed by Weiling Yu, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ]*

In vitro cytocompatibility studies were performed by culturing NCTC clone 929 (strain L) mouse areolar fibroblast cells (L929 cells) in cell media (DMEM supplemented with 10% FBS, 1% pen/strep) containing the dissolved diacids (**6a** and **6b**) or polymers (**7a** and **7b**). Polymers and diacids were dissolved in dimethyl sulfoxide (DMSO) and diluted with cell media to reach concentrations of 0.10 and 0.05 mg/mL. These solutions were added to allocated wells in a 96-well plate with 2000 L929 cells/well. DMSO (0.5%) in cell media was used as negative control.

Cell viability was determined using CellTiter 96® Aqueous One Solution Cell Proliferation Assay. After 24 h, 48 h, and 72 h incubation with polymers or diacids, 20 μL (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 2 h at 37 ˚C. The absorbance was then recorded with a microplate reader (Coulter) at 492 nm.

6.4.10. *In Vitro* TNF-α Inhbition

*[*In vitro *bioactivity studies were performed by Weiling Yu, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ]*

Human blood-derived monocytes were used to study the anti-inflammatory activity of the drugs released from the polymers. Cell isolation and purification methods used were previously described.[38] Briefly, peripheral blood mononuclear cells were collected from healthy donors by density gradient separation using Ficoll at a density of 1.077 ± 0.001 g/cm^3. CD14+ monocytes were isolated by magnetic-activated cell sorting using human CD14 beads and MACS cell separator following manufacturer's protocol. Isolated monocytes were cultured in T175 flasks at a concentration of 1×10^7 cells/flast in RPMI-1640 media (supplemented with 10% FBS, 1% pen/strep, and 4 mM L-glutamine). Monocytes were allowed to adhere for at least 2 h and media was aspirated to remove non-adherent cells. Monocytes were then cultured in RPMI supplemented with 5.0 ng/mL GM-CSF for 7 days at 37 °C and 5% CO_2 to allow macrophages differentiation. After 7 days of culture, macrophages were detached with trypsin-EDTA, re-suspended in RPMI media, counted, re-plated at $1x10^4$ cells/well of a 96 well plate and allowed to attach overnight. The following day cells were used for experiments.

Polymers degradation media of day 5 were diluted with RPMI media to concentration of 0.5 μg/mL. Free drugs (**1** and **2**) were dissolved in PBS and diluted with RPMI media to concentration of 0.5 μg/mL. These solutions were added to allocated wells with 1 X 10^5 cells/well. Lipopolysaccharides (LPS), 1 μg/mL, was added to stimulate TNF-α secretion. Cells were incubated at 37 °C and 5% CO_2 for 24 h. Cells incubated in media alone were used as negative control and LPS-activated cells as positive control. After 24 h, media was collected and the amount of TNF-α was determined using an ELISA kit against human TNF-α according to manufacturer's protocol. Cell viability was determined using the method described in section 6.4.15.

*Other methods described in Chapter 9.

6.5. References

1. Brooks, P. M.; Day, R. O., *New England Journal of Medicine* **1991,** *324* (24), 1716-1725.
2. Laine, L.; Smith, R.; Min, K.; Chen, C.; Dubois, R. W., *Alimentary Pharmacology & Therapeutics* **2006,** *24* (5), 751-767.
3. Jain, J. P.; Modi, S.; Domb, A. J.; Kumar, N., *Journal of Controlled Release* **2005,** *103* (3), 541-563.
4. Crielaard, B. J.; Lammers, T.; Schiffelers, R. M.; Storm, G., *Journal of Controlled Release* **2012,** *161* (2), 225-234.
5. Sieper, J.; Braun, J.; Rudwaleit, M.; Boonen, A.; Zink, A., *Annals of the Rheumatic Diseases* **2002,** *61* (suppl 3), iii8-iii18.
6. Braun, J.; Sieper, J., *The Lancet* **2007,** *369* (9570), 1379-1390.
7. Orme, M., Anti-rheumatic Drugs. In *International Encyclopedia of Pharmacology and Therapeutics*, Beckenridge, A. M., Ed. Pergamon Press: 1990.
8. Khan, M. S. Y.; Akhter, M., *European Journal of Medicinal Chemistry* **2005,** *40* (4), 371-376.
9. Mirzaagha, B., *International Journal of Pharmaceutics* **2008,** *356*, 167-173.
10. Arica, B.; Ccedil; ali, S.; Atlla, P.; Durlu, N. T.; akar, N.; Ka, H. S.; Hincal, A. A., *Journal of Microencapsulation* **2005,** *22* (2), 153-165.

11. Thompson, C. J.; Hansford, D.; Higgins, S.; Rostron, C.; Hutcheon, G. A.; Munday, D. L., *Journal of Microencapsulation* **2009,** *26* (8), 676-683.
12. Fernández-Carballido, A.; Herrero-Vanrell, R.; Molina-Martínez, I. T.; Pastoriza, P., *International Journal of Pharmaceutics* **2004,** *279*, 33-41.
13. S. Bozdag, S. C., H. S. Kas, M. T. Ercan, I. Peksoy, A. A. Hincal, *Journal of Microencapsulation* **2001,** *18* (4), 443-456.
14. Castelli, F.; Conti, B.; Maccarrone, D. E.; Conte, U.; Puglisi, G., *International Journal of Pharmaceutics* **1998,** *176* (1), 85-98.
15. Borovac, T.; Pelage, J. P.; Kasselouri, A.; Prognon, P.; Guiffant, G.; Laurent, A., *Journal of Controlled Release* **2006,** *115* (3), 266-274.
16. Thompson, C. J.; Hansford, D.; Higgins, S.; Rostron, C.; Hutcheon, G. A.; Munday, D. L., *International Journal of Pharmaceutics* **2007,** *329*, 53-61.
17. Gallardo, A.; Parejo, C.; San Román, J., *Journal of Controlled Release* **2001,** *71* (1), 127-140.
18. Mizrahi, B.; Domb, A., *AAPS PharmSciTech* **2009,** *10* (2), 453-458.
19. Davaran, S.; Entezami, A. A., *Anglais* **1998,** *34* (2).
20. Liso, P. A.; Rebuelta, M.; Román, J. S.; Gallardo, A.; Villar, A. M., *Journal of Controlled Release* **1995,** *33* (3), 429-436.
21. Babazadeh, M., *International Journal of Pharmaceutics* **2006,** *316*, 68-73.
22. Schmeltzer, R.; Schmalenberg, K.; Uhrich, K., *Biomacromolecules* **2005,** *6* (1), 359-367.
23. Rosario-Meléndez, R.; Harris, C. L.; Delgado-Rivera, R.; Yu, L.; Uhrich, K. E., *Journal of Controlled Release* **2012,** *162* (3), 538-544.
24. Prudencio, A.; Carbone, A. L.; Griffin, J.; Uhrich, K. E., *Macromolecular Rapid Communications* **2009,** *30* (13), 1101-1108.
25. Schmeltzer, R. C.; Anastasiou, T. J.; Uhrich, K. E., *Polymer Bulletin* **2003,** *49* (6), 441-448.
26. Erdmann, L.; Uhrich, K. E., *Biomaterials* **2000,** *21* (19), 1941-1946.
27. DeBolt, S.; Cook, D. R.; Ford, C. M., *Proceedings of the National Academy of Sciences* **2006,** *103* (14), 5608-5613.
28. Lamidey, A.-M.; Fernon, L.; Pouységu, L.; Delattre, C.; Quideau, S.; Pardon, P., *Helvetica Chimica Acta* **2002,** *85* (8), 2328-2334.
29. Allen, T.; Cullis, P., *Science* **2004,** *303* (5665), 1818-1822.
30. Borzacchiello, A.; Ambrosio, L.; Nicolais, L.; Huang, S. J., *Journal of Bioactive and Compatible Polymers* **2000,** *15* (1), 60-71.
31. Frankenfeld, J. W.; Wright, D. L. Aliphatic diols as preservatives for cosmetics and related products. July 20, 1976, 1976.
32. Storey, R. F.; Sherman, J. W., *Macromolecules* **2002,** *35* (5), 1504-1512.
33. Schwach, G.; Coudane, J.; Engel, R.; Vert, M., *Polymer Bulletin* **1994,** *32* (5), 617-623.
34. Schwach, G.; Coudane, J.; Engel, R.; Vert, M., *Biomaterials* **2002,** *23* (4), 993-1002.
35. Dumitriu, S., *Polymeric Biomaterials*. Second ed.; Mercel Dekker Inc.: New York, NY, 2002.
36. Mizrahi, B.; Domb, A. J., *AAPS PharmSciTech* **2009,** *10* (2), 453-458.

37. Basheer, C.; Chong, H. G.; Hii, T. M.; Lee, H. K., *Analytical Chemistry* **2007,** *79* (17), 6845-6850.
38. Kim, J.; Hematti, P., *Experimental Hematology* **2009,** *37* (12), 1445-1453.

7. APPENDICES

7.1. NALBUPHINE-BASED POLY(ANHYDRIDE-ESTER): PROOF-OF-CONCEPT

Given that we had no previous experience incorporating opioids into polymer backbones, we needed to use a model compound to develop the synthetic methodology. The structural similarities between morphine and nalbuphine (Figure 7.1.1) make nalbuphine a good prototype to determine whether or not the synthesis of an opioid-based polymer was feasible. Nalbuphine is a synthetic opioid used as analgesic.[1-3]

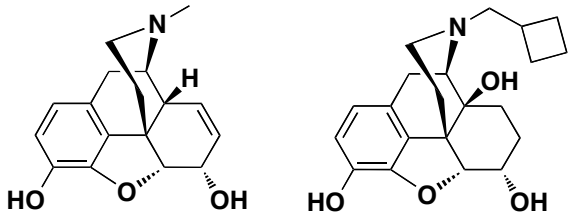


Figure 7.1.1. Chemical structures of morphine (left) and nalbuphine (right).

To synthesize the nalbuphine-based PAE, a modified two-step route was proposed. First, nalbuphine was reacted with an excess of glutaric anhydride in the presence of base (triethylamine) at room temperature to generate the diacid (Figure 7.1.2) in 98 % yield. The diacid structure was characterized using ^{1}H NMR and IR spectroscopies. The carbonyl (C=O) stretches for ester (~ 1770 cm^{-1}) and carboxylic acid

(~ 1700 cm-1) were present. Mass spectrometry (MS) was used to determine the MW of the diacid plus a proton (586.65). DSC and TGA analysis showed that the diacid has a melting point (T_m) of 97 °C and a decomposition temperature (T_d) of 215 °C.

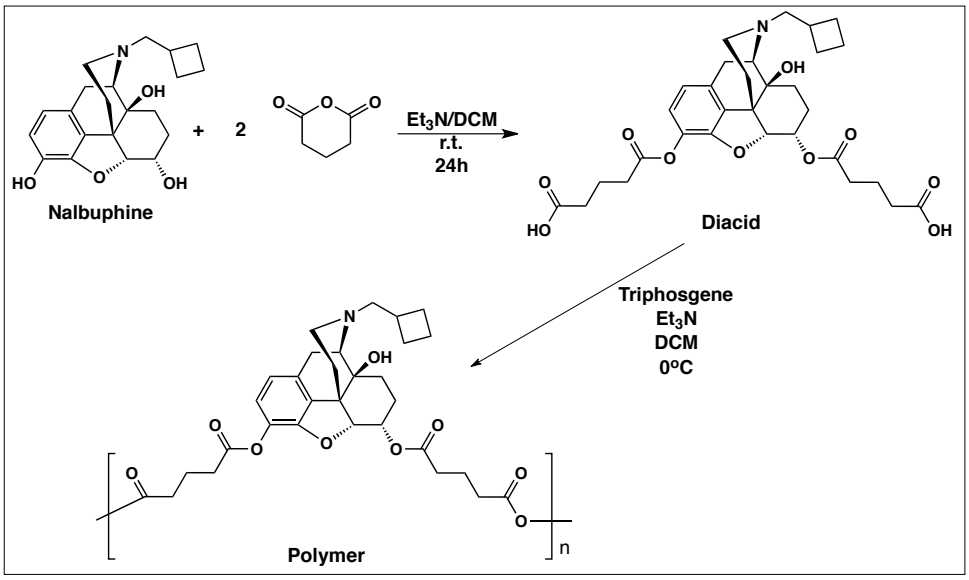


Figure 7.1.2. Synthetic scheme of nalbuphine-based diacid and polymer.

The nalbuphine-based diacid then underwent solution polymerization to form the nalbuphine-based PAE (Figure 7.1.2) obtained in ~ 40 % yield. The structure of the polymer was characterized using ^{1}H NMR and IR spectroscopies. The ^{1}H NMR spectrum showed slight peak broadening relative to the precursor diacid and the IR spectrum displays the C=O stretches for anhydrides (~ 1815 and 1760 cm^{-1}) and esters (~ 1735 cm-1). GPC indicates M_w of the polymer was ~20,000 Da. Thermal analysis using DSC and TGA denotes a glass transition temperature (T_g) of 101 °C and a T_d of 200 °C.

7.1.1. Experimental

7.1.1.6. Materials

Nalbuphine HCl was purchased from Mallinckrodt (St. Louis, MO). All other chemicals and reagents were purchased from Sigma-Aldrich (Milwaukee, WI).

7.1.1.7. Nalbuphine Neutralization

Nalbuphine HCl (0.50 g, 1.3 mmol) was added to 30 mL deionized and heated until all solid was dissolved. Concentrated ammonium hydroxide (12 drops) was added to adjust the pH to ~8. The precipitate formed was isolated via vacuum filtration and dried under vacuum at room temperature overnight. Yield: quantitative. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 8.78 (s, 1H), 6.52 (d, 1H), 6.38 (d, 1H), 4.71 (s, 1H), 4.39 (dd, 2H), 3.97 (m, 1H), 2.95 (d, 1H), 2.68 (d, 1H), 2.53 (d 1H), 2.41 (d, 4H), 2.07 (comp, 2H), 1.98 (m, 2H), 1.80 (comp, 2H), 1.60 (m, 2H), 1.43 (m, 2H), 1.31 (m, 2H), 0.92 (m, 1H). T_m = 283-297 °C.

7.1.1.8. Diacid Synthesis

Nalbuphine (0.32 g, 0.9 mmol, 1 eq) and glutaric anhydride (0.22 g, 1.9 mmol, 2.1 eq) were added to anhydrous dichloromethane (DCM) (5 mL) to give a white suspension. Triethylamine (1.3 mL, 9.0 mmol, 10 eq) was added to give a yellow

solution. The reaction was stirred overnight at room temperature for 4 days. Reaction mixture was dried under reduced pressure and the yellow viscous oil obtained dissolved in ethyl acetate (3 mL) and poured over hexanes (~ 50 mL). The solvent was decanted and the sticky white solid obtained dried under vacuum at room temperature overnight. Yield: 0.52 g (98 %) white solid. ^{1}H-NMR (500 MHz, $CDCl_3$-d_6, δ): 6.80 (d, 1H), 6.62 (d, 1H), 5.32 (m, 1H), 4.76-4.63 (dd, 1H), 3.12-1.27 (comp, 32H), 0.86 (m, 1H). IR: 1775 cm^{-1} (C=O, ester), 1692 cm^{-1} (C=O, acid). MS: M/Z = 586 [M + 1]. T_m = 97 °C. T_d = 215 °C.

7.1.1.9. Polymer Synthesis

The diacid (1.6 g, 2.7 mmol) was dissolved in anhydrous DCM (15 mL). Anhydrous triethylamine (1.7 mL, 12.0 mmol) was then added to the reaction mixture to give a clear solution. The reaction mixture was cooled to 0 °C and stirred for 15 min. Triphosgene (0.88 g, 1.1 mmol), dissolved in anhydrous DCM (9 mL), was added dropwise via syringe pump over 1 h. After stirring for 5.5 h at 0 °C, the reaction poured over excess cold diethyl ether. The precipitate formed was isolated via vacuum filtration and washed with acidic water (5 x 50 mL). The orange solid obtained was dried under vacuum at room temperature overnight. Yield: 0.66 g (42 %), orange solid. ^{1}H-NMR (500 MHz, DMSO-d_6, δ): 6.98 (d, 1H), 6.77(d, 1H), 5.32 (m, 1H), 4.76-4.63 (dd, 1H), 3.12-0.90 (comp, 30H). IR: 1816 and 1757 cm^{-1} (C=O, anhydride), 1735 cm^{-1} (C=O, ester). M_w = 10,900 Da, PDI = 1.06. T_g = 101 °C. T_d = 200 °C.

*Other methods described in Chapter 9.

7.1.1. References

1. Errick, J. K.; Heel, R. C., *Drugs* **1983,** *26* (3), 191-211.
2. Schmidt, W. K.; Tam, S. W.; Shotzberger, G. S.; Smith, D. H.; Clark, R.; Vernier, V. G., *Drug and alcohol dependence* **1985,** *14* (3-4), 339-362.
3. Sung, K. C.; Han, R.-Y.; Hu, O. Y. P.; Hsu, L.-R., *International Journal of Pharmaceutics* **1998,** *172* (1,Äì2), 17-25.

7.2. SALICYLATE-BASED POLY(ANHYDRIDE-ESTER) FORMULATION FOR DIABETIC BONE REGENERATION IN A CRITICAL SIZE DEFECT

Complications associated to inflammatory pathways (such as retinopathy, atherosclerosis, periodontitis, and impaired wound healing) are enhanced in diabetic patients.[1] Diabetes increases the intensity of inflammation and inflammatory responses last longer in diabetic individuals as compared to normoglycemic individuals.[1-3] The enhanced impaired wound healing also results in slower bone regeneration.[1, 3-5]

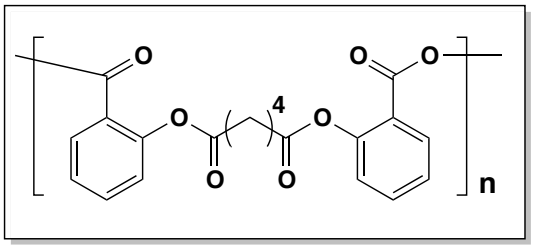


Figure 7.2.1. Chemical structure of the salicylic acid-based PAE used in the formulation for diabetic bone regeneration in a critical size defect.

A salicylate-based poly(anhydride-ester) (PAE) developed in our laboratory[6-8] (Figure 7.2.1) was used to further investigate the relationship between enhanced inflammation, diabetes, and bone regeneration. This polymer releases salicylic acid (SA), a non-steroidal anti-inflammatory drug (NSAID) capable of reducing inflammation,[9] in a controlled and sustained manner. The overall effect of NSAIDs on bone regeneration is controversial with studies supporting both positive and negative effects.[10-14] The ability of salicylate-based PAEs to promote bone regeneration was tested,[15] however, further studies are needed on the effect of NSAIDs (i.e., SA) on bone regeneration in healthy and diabetic individuals. Therefore, we collaborated with experts in the field of bone regeneration from the Department of Periodontics at the University of Pennsylvania. The formulation prepared for these studies was intended to mitigate inflammation (once the SA is released) and observe its effect on wound healing combined with a demineralized freeze-dried bone allograft (DFDBA).

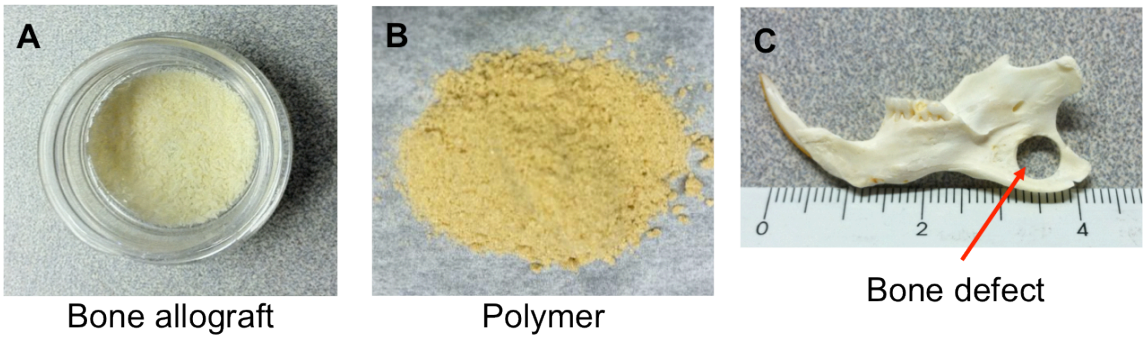


Figure 7.2.2. Images of demineralized freeze-dried bone allograft (A), salicylate-based PAE (B), and the critical bone defect (C) with 5 mm radius and 3 μm thickness in a rat mandible.

Both the salicylate-based PAE and the DFDBA (Figure 7.2.2, A and B) are very lightweight electrostatic materials and its reproducible transfer in exact amounts into the critical bone defect in a rat mandible (Figure 7.2.2 C) was challenging during surgery. The polymer and the DFDBA were formulated to obtain a non-static mixture that could be easily and reproducibly transferred into the bone defect. The two components (polymer and DFDBA) were combined in 50:50 and 75:25 weight ratios and mixed with water, ethanol, and different oils added (Table 7.2.1, **a-g**). The use of water and ethanol did not result in an "easy-to-handle" formulation (Table 7.2.1, **a** and **b**) and the addition of these solvents could result in accelerated polymer degradation, which was undesired. All the oils used (mineral oil light and heavy, vegetable, canola, and olive oil) resulted in a paste that was "easy-to-handle", did not feel greasy when touched, and the oil could protect the polymer from degrading in the first few days (Table 7.2.1, **c-g**). A preliminary *in vitro* SA release study was performed showing that oils **c** and **e-g** (Table 7.2.1) separated from the formulation when incubated in buffered media (pH 7.4) at

physiological temperature (i.e., 37 °C). The separation of the formulation was undesired and based on the results of the *in vitro* SA release study formulation **d** (containing mineral oil light) was chosen for further studies.

Table 7.2.1. Solvents used to formulate the salicylate-based PAE and DFDBA combined in 50:50 and 75:25 weight ratios, the amounts used and observations are listed.

	Solvent	Amount	Observations
a	Water	3 drops	Polymer easier to handle (sandy) Dries after ~15 min Could accelerate polymer degradation
b	EtOH	3 drops	Dries too fast Does not make the polymer easier to handle
c	Mineral oil (heavy)	2 drops	Polymer easier to handle (pasty) Does not feel greasy when touched Could create a protective coating to prevent drug release in the first few days
d	Mineral oil (light)	2 drops	Same as **c**
e	Vegetable oil	2 drops	Same as **c** but feels greasy when touched
f	Canola oil	2 drops	Same as **c**
g	Olive oil	2 drops	Same as **c**

The paste obtained from the 50:50 polymer-DFDBA and mineral oil light was transferred to the bone defect in a rat mandible to determine whether or not it stays in place. As shown in Figure 7.2.3, the formulation stayed in the defect without the necessity of an external membrane.

Figure 7.2.3. Critical size defect in a rat mandible filled with 50:50 polymer-DFDBA formulation with mineral oil light.

Preliminary results of a study indicate that PolyAspirin was able to significantly regenerate bone in critical sized defect as compared to control in healthy rats. Studies on diabetic animals are ongoing.

7.2.1. Experimental

7.2.1.1. Materials

Oils used are commercially available for consumer used. All chemicals and reagents were purchased from Sigma-Aldrich (Milwaukee, WI), unless otherwise specified.

7.2.1.2. Polymer Synthesis

Salicylic acid-based PAE was synthesized using previously reported methods.[7, 8] Properties of the polymer were as follow: M_w = 14,300 Da; T_g = 51 ^oC.

7.2.1.3. Formulation

Salicylic acid-based PAE was combined to DFDBA (LifeNet Health®, Virginia Beach, VA) in 50:50 and 75:25 weight ratios and a solvent added. Table 7.2.1 summarizes the materials used and observations (all trials used 15.0 mg of material).

7.2.1.4. *In Vitro* Studies

Samples (15.0 mg, duplicate) **c**-**g** (Table 7.2.1) were placed in scintillation vials and 2.00 mL of phosphate buffered saline (PBS) pH 7.4 added. All samples were incubated at 37 ^oC and 60 rpm. At days 2, 4, and 6, PBS was removed and replaced with fresh PBS. Samples were analyzed by UV-visible spectroscopy at λ = 303 nm.

7.2.1.5. Samples Preparation for *In Vivo* Studies

[Samples sent to the University of Pennsylvania were prepared with the help of Michelle Ouimet (Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ)]

The salicylate-based PAE (7.5 mg) and the DFDBA (7.5 mg) were combined in 50:50 weight ratio and 2 drops of mineral oil light added. The mixture was transferred to 2 mL sterile Eppendorf tubes. Samples were sterilized under UV at λ = 254 nm for 900 s. All samples were shipped in dry ice. Control samples were prepared without polymer and treated identical to the polymer containing samples.

7.2.1.6. *In Vivo* Studies

*[*In vivo *studies were performed by Dana Graves, Joseph Fiorellini, and Keisweke Wada (Department of Periodontics, University of Pennsylvania, Philadelphia, PA)]*

Formulation ***d*** *(Table 7.2.1) at 50:50 polymer-DFDBA weight ratio was transferred into the critical size bone defect (5 mm radius and 3 μm thickness).*

7.2.2. References

1. Graves, D. T.; Liu, R.; Oates, T. W., *Periodontology 2000* **2007,** *45* (1), 128-137.
2. Graves, D. T.; Liu, R.; Alikhani, M.; Al-Mashat, H.; Trackman, P. C., *Journal of Dental Research* **2006,** *85* (1), 15-21.
3. Rőszer, T., *Inflammation Research* **2011,** *60* (1), 3-10.
4. Liu, R.; Bal, H. S.; Desta, T.; Krothapalli, N.; Alyassi, M.; Luan, Q.; Graves, D. T., *Journal of Dental Research* **2006,** *85* (6), 510-514.
5. Thomas, M. V.; Puleo, D. A., *Journal of Dental Research* **2011,** *90* (9), 1052-1061.
6. Erdmann, L.; Uhrich, K. E., *Biomaterials* **2000,** *21* (19), 1941-1946.
7. Schmeltzer, R. C.; Anastasiou, T. J.; Uhrich, K. E., *Polymer Bulletin* **2003,** *49* (6), 441-448.

8. Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E., *Macromolecules* **2005,** *38* (16), 6895-6901.
9. Hersh, E. V.; Moore, P. A.; Ross, G. L., *Clinical Therapeutics* **2000,** *22* (5), 500-548.
10. Salari, P.; Abdollahi, M., *Inflammation & Allergy - Drug Targets (Formerly `Current Drug Targets - Inflammation & Allergy)* **2009,** *8* (3), 169-175.
11. Cottrell, J.; O,ÄôConnor, J. P., *Pharmaceuticals* **2010,** *3* (5), 1668-1693.
12. Vuolteenaho, K.; Moilanen, T.; Moilanen, E., *Basic & Clinical Pharmacology & Toxicology* **2008,** *102* (1), 10-14.
13. Mountziaris, P. M.; Mikos, A. G., *Tissue Eng Part B Rev* **2008,** *14* (2), 179-86.
14. Pountos, I.; Georgouli, T.; Blokhuis, T. J.; Pape, H. C.; Giannoudis, P. V., *Injury* **2008,** *39* (4), 384-94.
15. Reynolds, M. A.; Prudencio, A.; Aichelmann-Reidy, M. E.; Woodward, K.; Uhrich, K. E., *Current Drug Delivery* **2007,** *4* (3), 233-239.

7.3. AMFENAC-BASED POLY(ANHYDRIDE-ESTER)

Amfenac (Figure 7.3.1, **1**) is a non-steroidal anti-inflammatory drug (NSAID) with antipyretic and analgesic properties used to treat retinopathy and pain and inflammation associated with cataract surgery.[1] Nepafenac (Figure 7.3.1, **2**) is prodrug of amfenac with superior penetration of the cornea and scleral tissue.[1] In the eye, **2** is metabolized into **1**, which is the active ingredient.[1, 2] The major disadvantage of the topical administration of **2,** is the poor drug absorption (less than 5 % of drug) of an ophthalmic suspension into the eye.[3, 4]

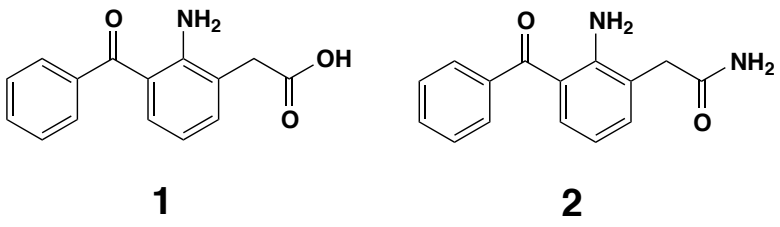


Figure 7.3.1. Structures of amfenac (**1**) and nepafenac (**2**).

Intraocular injections of **1** or **2** can provide effective concentrations in the vitreous humor of the eye.[3-5] However, the short half-life of **1** and **2** will require frequent injections that compromise patient compliance and could increase the risk of complications associated to injections.[3, 5, 6] Therefore, a new prodrug (e.g., chemical incorporation of **1** into a biodegradable polymer backbone) could be beneficial for the treatment of inflammatory diseases of the eye.

Based upon the successful chemical incorporation of various NSAIDs into poly(anhydride-ester) (PAE) backbones,[7-10] the incorporation of **1** into a PAE was proposed to yield an amfenac-based PAE (Figure 7.3.2, **7**). Using similar synthetic approaches as the one used to incorporate other NSAIDs (e.g., salicylic acid and aminosalicylates) into PAE backbones a synthetic procedure was proposed (Figure 7.3.2).[8-10]

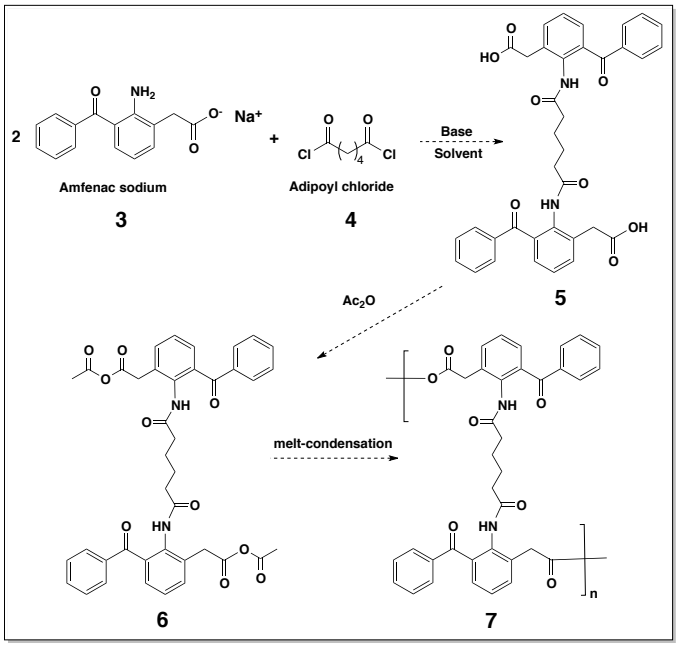


Figure 7.3.2. Proposed synthesis for the amfenac-based PAE.

Multiple attempts using different conditions (Table 7.3.1) were used for the synthesis of the amfenac-based diacid (**5**). However, the reactions were unsuccessful due to the low reactivity of the amine group and its cyclization. Characterization using proton nuclear magnetic resonance (^{1}H- NMR) and infrared (IR) spectroscopies, and differential scanning calorimetry (DSC) confirmed that the desired product was not obtained. Further work is needed in order to succeed in the synthesis of **7**.

7.3.1. Amfenac-based Diacid Synthesis (5)

Variations of previously reported methods were used to synthesize **5**. In brief, amfenac sodium **3** (2 eq) was dissolved in a solvent (see Table 7.3.1) and a base added (4

eq, Table 7.3.1). Adipoyl chloride (1 eq) was diluted in the solvent and added drop-wise to the stirring reaction mixture. The reaction mixture was allowed to stir overnight and subsequently quenched by pouring over excess water and adding concentrated HCl until pH = 2. The obtained solid was filtered, washed with water, and dried under vacuum at room temperature. Table 7.3.1 summarizes the reaction conditions used.

Table 7.3.1. Reaction conditions used to synthesize the amfenac-based diacid (**5**).

Solvent	Base	Temperature
THF	Py or TEA	r.t.
DCM	TEA or DMAP	r.t.
DCM	DMAP/TEA	0 °C to r.t.
DMF	Py or TEA	reflux
DMF	NaH	0 °C
-	Py	r.t.

7.3.2. References

1. Yanni, S. E.; Clark, M. L.; Yang, R.; Bingaman, D. P.; Penn, J. S., *Brain Research Bulletin* **2010,** *81* (2-3), 310-319.
2. Kim, S. J.; Flach, A. J.; Jampol, L. M., *Survey of Ophthalmology* **2010,** *55* (2), 108-133.
3. del Amo, E. M.; Urtti, A., *Drug Discovery Today* **2008,** *13* (3‚Äì4), 135-143.
4. Kuno, N.; Fujii, S., *Drugs & Aging* **2010,** *27* (2), 117-134 10.2165/11530970-000000000-00000.
5. Moritera, T.; Ogura, Y.; Honda, Y.; Wada, R.; Hyon, S. H.; Ikada, Y., *Investigative ophthalmology & visual science* **1991,** *32* (6), 1785-1790.
6. Herrero-Vanrell, R., Microparticles as Drug Delivery Systems for the Back of the Eye; Drug Product Development for the Back of the Eye. Kompella, U. B.; Edelhauser, H. F., Eds. Springer US: 2011; Vol. 2, pp 231-259.
7. Erdmann, L.; Uhrich, K. E., *Biomaterials* **2000,** *21* (19), 1941-1946.
8. Anastasiou, T. J.; Uhrich, K. E., *Journal of Polymer Science Part A: Polymer Chemistry* **2003,** *41* (22), 3667-3679.
9. Schmeltzer, R. C.; Anastasiou, T. J.; Uhrich, K. E., *Polymer Bulletin* **2003,** *49* (6), 441-448.
10. Schmeltzer, R. C.; Schmalenberg, K. E.; Uhrich, K. E., *Biomacromolecules* **2004,** *6* (1), 359-367.

7.4. COMBINED TREATMENT FOR ACUTE AND CHRONIC PAIN

The benefits of a combined treatment for acute and chronic pain consisting of an opioid and a non-steroidal anti-inflammatory drug (NSAID) have been reported.[1-10] A combined treatment of oral or intravenous administration of an opioid and a NSAID helps reduce the amount of opioid needed,[3, 4, 6] delays the development of tolerance,[1, 6] a well-known side-effect of morphine use,[11-13] and other side effects.[4] However, side effects associated with NSAIDs such as gastrointestinal discomfort, bleeding and ulceration increased.[3, 4, 6, 14] Therefore, an alternative approach to implement this combined treatment is needed.

Based on the need for a better system to treat acute and chronic pain, a combined treatment with opiate-based polymer (PolyMorphine)[15] and NSAID-based polymer [salicylate-based poly(anhydride-ester) (PAE)[16] or ibuprofen-based polyester[17]] may be ideal combination to treat chronic and acute pain. This system will control the release of both drugs (opioid and NSAID) ultimately delaying the development of opiate resistance and reducing the side effects associated with the NSAIDs.

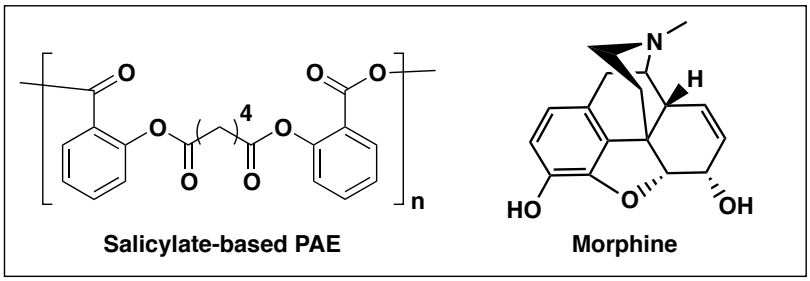


Figure 7.4.1. Chemical structures of the salicylate-based PAE (left) and morphine (right).

A salicylate-based PAE was combined with free morphine (Figure 7.4.1) and the analgesic effect assessed using the Tail-flick Latency (TFL) test. As shown in Figure 7.4.2, the free morphine provided strong analgesia for 4 h, a well-established time course for morphine,[18] by the 4 h time point the analgesic effect was completely gone. The salicylate-based PAE alone provided mild analgesia that increased with time. Interestingly, the salicylate-based PAE and morphine combination provided stronger analgesia compared to the free morphine. At the 2 h time point, the analgesia was significantly higher than the free morphine control. At the 8 h time point mild analgesia provided by the salicylate-based PAE was detected.

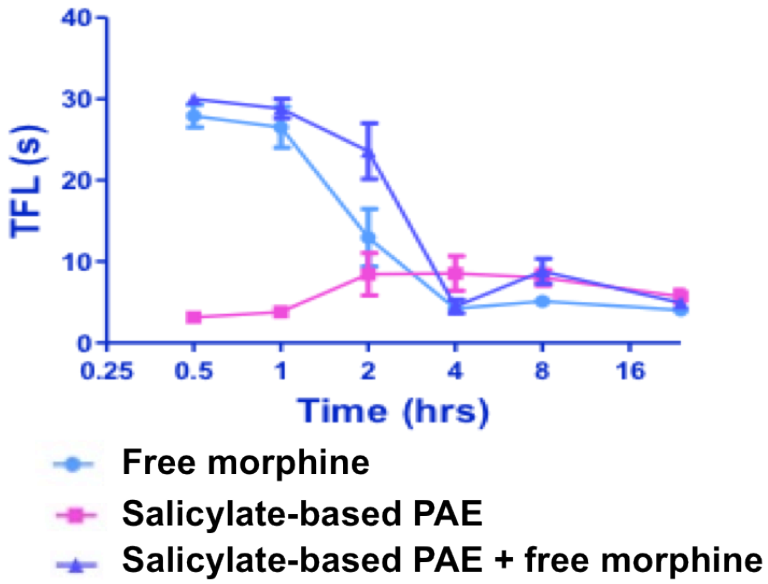


Figure 7.4.2. TFL test results at 0.5-24 h post-administration. Data are shown as mean ± standard error of mean. N = 10 for each time point.

These results show that the potential to develop a better treatment for acute and chronic pain by combining PolyMorphine and the salicylate-based PAE or PolyMorphine and Ibuprofen-based polyester. Future work involves using the aforementioned polymer combination to fully test our hypothesis.

7.4.1. Experimental

7.4.1.1. Samples Preparation

Salicylate-based PAE was synthesized using previously published procedures.[16, 19] Properties of the polymer were as follows: M_w = 17,000 Da, T_g= 55 ^oC. Polymer was grounded and particles with diameter < 75 μm using the USA Standard Testing Sieves.

Suspensions or solutions in 5 % aqueous Cremophor EL were prepared for the animal studies.

7.4.1.2. *In Vivo* Studies

*[*In vivo *studies were performed by Carolyn L. Harris and Dr. Lei Yu, Department of Genetics and Alcohol Studies, Rutgers University, Piscataway, NJ]*

Adult male C57Bl/6J mice were obtained from Charles River (Kingston, NY). Animals were approximately 10 weeks old and weighed between 19.5 – 27.7 g at the beginning of the study. Animals were housed in climate-controlled rooms with a 12:12 hour light/dark cycle, with food and water available ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Rutgers University, and consistent with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 2011). Animals were pre-handled twice a day for 3 days prior to the experiment.

All administrations were by intraperitoneal (i.p.) injection (10 animals per group). Drug dosing was as follows: free morphine (morphine HCl) at 10 mg/kg, salicylate-based PAE at 200 mg/kg, and salicylate-based PAE + free morphine at 200 mg/kg and 10 mg/kg, respectively.

Nociception in mice was measured with the TFL test. Animals were wrapped loosely in soft cloth, where each cage of animals had its own cloth to minimize cross-cage olfactory sensory stimulation. TFL was tested by immersing the distal third of the

animal's tail in a water bath at 49 °C, and the TFL time was recorded with a 30 s cutoff time to avoid tissue damage. Animals were only tested one time at each time point.

7.4.2. References

1. Koppert, W., *Acute Pain* **2007,** *9* (1), 21-34.
2. Planas, E.; Poveda, R.; Sánchez, S.; Romero, A.; Puig, M. M., *European Journal of Pharmacology* **2003,** *482* (1‚Äì3), 223-226.
3. Shapiro, A.; Zohar, E.; Hoppenstein, D.; Ifrach, N.; Jedeikin, R.; Fredman, B., *J Clin Anesth* **2003,** *15* (5), 345-50.
4. Curatolo, M.; Sveticic, G., *Best Pract Res Clin Anaesthesiol* **2002,** *16* (4), 507-19.
5. McNicol, E.; Strassels, S.; Goudas, L.; Lau, J.; Carr, D., *Journal of Clinical Oncology* **2004,** *22* (10), 1975-1992.
6. Mercadante, S.; Fulfaro, F.; Casuccio, A., *Eur J Cancer* **2002,** *38* (10), 1358-63.
7. Kolesnikov, Y.; S√µritsa, D., *European Journal of Pharmacology* **2008,** *579* (1‚Äì3), 126-133.
8. Miranda, H. F.; Prieto, J. C.; Pinardi, G., *Brain Research* **2005,** *1049* (2), 165-170.
9. Zelcer, S.; Kolesnikov, Y.; Kovalyshyn, I.; Pasternak, D. A.; Pasternak, G. W., *Brain Research* **2005,** *1040* (1‚Äì2), 151-156.
10. Aronson, J. K., *Analgesic and Anti-inflammatory Drugs*. First ed.; Elsevier: San Diego, CA, 2010.
11. Harrison, L. M.; Kastin, A. J.; Zadina, J. E., *Peptides* **1998,** *19* (9), 1603-1630.
12. Williams, J. T.; Christie, M. J.; Manzoni, O., *Physiological Reviews* **2001,** *81* (1), 299-343.
13. Ueda, H.; Ueda, M., *Front Biosci* **2009,** *14*, 5260-72.
14. Joshi, G. P., *Ambulatory Surgery* **1999,** *7* (1), 3-12.
15. Rosario-Meléndez, R.; Harris, C. L.; Delgado-Rivera, R.; Yu, L.; Uhrich, K. E., *Journal of Controlled Release* **2012,** *162* (3), 538-544.
16. Schmeltzer, R. C.; Anastasiou, T. J.; Uhrich, K. E., *Polymer Bulletin* **2003,** *49* (6), 441-448.
17. Rosario-Meléndez, R.; Uhrich, K., *In preparation* **2012**.
18. Kim, T.; Kim, J.; Kim, S., *Cancer Chemotherapy and Pharmacology* **1993,** *33* (3), 187-190.
19. Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E., *Macromolecules* **2005,** *38* (16), 6895-6901.

7.5. POLYMORPHINE MICROSPHERES FORMULATION

PolyMorphine (Figure 7.5.1) was formulated into microspheres to develop an injectable delivery system for better localization of the drug release when used *in vivo*. Polymeric microspheres are advantageous as drug delivery system because they can be injected intramuscularly or subcutaneously to the target site.[1] PolyMorphine microspheres were prepared using a previously described oil-in-water single emulsion solvent evaporation technique.[2, 3] Scanning electron microscopy images of the microspheres obtained (Figure 7.5.2) were taken to assess size and morphology. The PolyMorphine microspheres prepared have diameters of 6 ± 4 μm with porous surface.

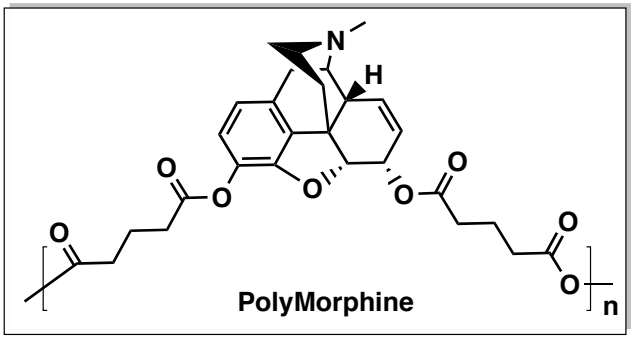


Figure 7.5.1. Chemical structure of PolyMorphine.

Future work includes the optimization of the microspheres formulation and *in vitro* and *in vivo* drug release and analgesic effect testing.

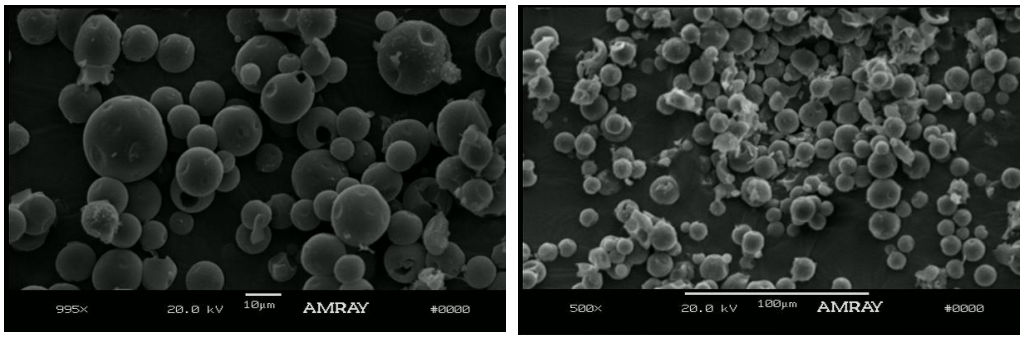


Figure 7.5.2. Scanning electron microscopy images of PolyMorphine microspheres immediately following isolation (left: 1000x, right: 500 x magnifications).

7.5.1. Experimental

7.5.1.1. Samples Preparation

PolyMorphine was synthesized using a previously published procedure.[4] Properties of the polymer were as follows: M_w = 20,000 Da, T_g= 110 ^oC.

7.5.1.2. Microspheres Preparation

Polymers were formulated into microspheres using a modified procedure of a published oil-in-water single emulsion solvent evaporation technique.[2, 3] In general, salicylate-based PAEs (0.10 g) were dissolved in dichloromethane (1.00 mL) and added drop-wise to 1% aqueous poly(vinyl alcohol) (PVA) (80 % hydrolyzed, 30-70 kDa) solution (30 mL) at room temperature. The emulsion was homogenized for 2 min using

an IKA Ultra-Turrax T8 homogenizer at approximately 10,000 rpm. The homogenized solution was left stirring for 2 h to allow microsphere formation by solvent evaporation. Microspheres were transferred to sterile 50 mL polypropylene conical tubes (30 x 115 mm style, BD Falcon, Franklin Lakes, NJ), washed with acidic water (pH 1) to remove residual PVA, and isolated by centrifugation at 3,000 rpm for 10 min. Microspheres were frozen by placing the conical tubes in a dry ice/acetone bath and lyophilized for 24 h at -40 ˚C and $133x10^{-3}$ mBar (LABCONO Freeze Dry System/Freezon 4.5).

7.5.1.3. Size and Morphology

Size and morphology of the microspheres were determined using SEM. Images for each set of microspheres were obtained using an AMRAY-1830I microscope (AMRAY Inc.) after coating the samples with Au/Pd using a sputter coater (SCD 004, Blazers Union Limited).

7.5.1. References

1. Kipper, M. J.; Shen, E.; Determan, A.; Narasimhan, B., *Biomaterials* **2002,** *23* (22), 4405-4412.
2. Yeagy, B. A.; Prudencio, A.; Schmeltzer, R. C.; Uhrich, K. E.; Cook, T. J., *Journal of Microencapsulation* **2006,** *23* (6), 643-653.
3. Rosario-Meléndez, R.; Ouimet, M. A.; Uhrich, K. E., *Polymer Bulletin* **2012,** *Manuscript accepted*.
4. Rosario-Meléndez, R.; Harris, C. L.; Delgado-Rivera, R.; Yu, L.; Uhrich, K. E., *Journal of Controlled Release* **2012,** *162* (3), 538-544.

7.6. IBUPROFEN-BASED POLY(ANHYDRIDE-ESTER)

Our group has chemically incorporated many drugs (e.g., salicylic acid, morphine)[1-6] and bioactives molecules (e.g., catechol and thymol)[7-9] into poly(anhydride-ester) (PAE) backbones. The idea of chemically incorporating a new class of drug molecules, the propionic derivative NSAIDs (e.g., ibuprofen), into PAE backbones was proposed.[10] In Chapter 6, the importance of controlling ibuprofen release and the synthesis and characterization of the ibuprofen-based diacid (i.e., polymer precursor) were described. Different polymerization methods were investigated to prepare the ibuprofen-based PAE.

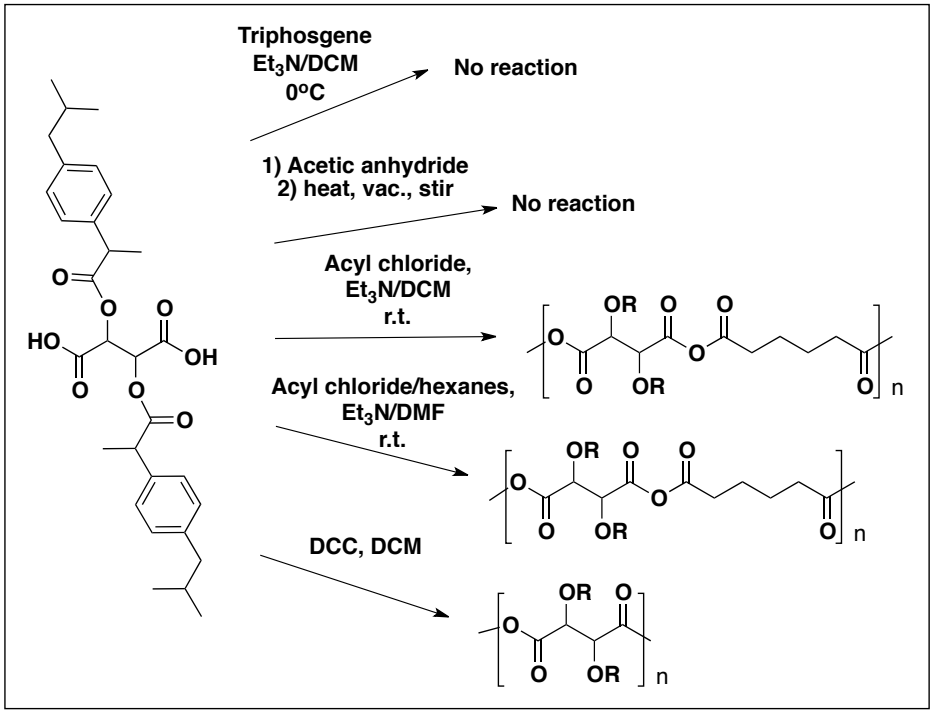


Figure 7.6.1. Polymerization reactions used for the synthesis of ibuprofen-based PAEs. These reactions resulted in starting material decomposition or impure product.

Melt-condensation polymerization was attempted; this polymerization method is known to result in high yields, high molecular weight (M_w) product, and pure polymer.[11] In addition, melt-condensation is reproducible and amenable to scale-up, from milligrams to tens of grams.[11] The first step for melt-condensation is the synthesis of a monomer via acetylation of the polymer precursor in excess acetic anhydride at room temperature. This reaction did not yield the desired product (i.e., monomer), instead the starting material decomposed. Therefore, PAE synthesis was not possible with this method.

Solution polymerization was evaluated; this method has been used extensively in our laboratory for the synthesis of PAEs.[11] This method uses triphosgene (which forms phosgene *in situ*) as the coupling agent in the presence of triethylamine (TEA).[11] Although it was successful for other PAEs synthesis,[9, 11, 12] solution polymerization resulted in starting material decomposition and no polymer formation.

Due to the unsuccessful results obtained using melt-condensation and solution polymerization, Schotten-Baumann condensation was attempted. The Schotten-Baumann condensation is a reaction extensively studied for polyamide, polyester, and polycarbonate synthesis.[13] Polyanhydrides can be synthesized at room temperature by dehydrochlorination between a acyl chloride and a diacid.[13] This reaction was attempted using one solvent or interfacial between two immiscible solvents. For both reaction conditions, characterization of the products showed impure product (containing solvent or based catalyst) with low molecular weight. Purification efforts to obtained pure polymers resulted in polymer degradation.

The last polymerization method used was dicylohexylcarbodiimide (DCC) coupling. DCC can function as a dehydrative agent for polyanhydride formation.[13] Although this reaction is known to yield oligomeric units and no high M_w polymers, we decided to try it. The results obtained support the previously published data.

Ibuprofen-based PAE synthesis was not possible after attempting the aforementioned reactions. However, our interest on the chemical incorporation of propionic acid derivative NSAIDs into a polymer backbone continued. Therefore, the synthesis of an ibuprofen-based polyester was explored and is described in Chapter 6.

7.6.1. Polymer Synthesis

7.6.1.1. Melt-condensation Polymerization

Ibuprofen-based diacid (0.11 g) was stirred in acetic anhydride (5 mL) until dissolved. Excess acetic anhydride was removed under reduced pressure at 50 °C to yield a brown paste. Characterization showed no product formation and decomposition of the starting material.

7.6.1.2. Solution Polymerization with Triphosgene

Ibuprofen-based diacid (0.38 g, 0.72 mmol) was dissolved in anhydrous DCM (5 mL) under argon and TEA (0.44 mL, 2.2 mmol) added to give a yellow solution. The mixture was cooled down to 0 °C and stirred for 15 min. Triphosgene (0.23 g, 0.79

mmol) was dissolved in DCM and added dropwise to the reaction mixture. The mixture turned dark maroon while adding the triphosgene. After 3 h, the mixture was poured over diethyl ether. The mixture was dried under reduced pressure to give a red liquid. Characterization showed no product formation and decomposition of the starting material.

7.6.1.3. Schotten-Baumann Condensation

Ibuprofen-based diacid (0.40 g, 0.75 mmol) was dissolved in anhydrous DCM (3 mL) under argon and TEA (0.21 mL, 1.5 mmol) added to give a yellow solution. Glutaryl chloride (0.10 mL, 0.75 mmol) was diluted in anhydrous DCM (5mL) and added to the reaction mixture and stirred for 2 h. The reaction mixture was added dropwise to diethyl ether. The white precipitate formed in diethyl ether was isolated via vacuum filtration, dissolved in DCM and extracted with acidic water. After drying over $MgSO_4$, DCM was removed under reduced pressure. Characterization showed impure product and the disappearance of the tartatic backbone protons. Purification efforts resulted in polymer degradation.

7.6.1.4. Interfacial Schotten-Baumann Condensation

Adipoyl chloride (0.14 mL, 0.95 mmol) was added to anhydrous hexanes (4.75 mL) under argon. Ibuprofen-based diacid (0.50 g, 0.95 mmol) was added to anhydrous DMF (4.75 mL) and added to the reaction mixture. TEA (0.26 mL, 1.90 mmol) added

dropwise over 1 h. After 6 h, the precipitate formed was isolated via vacuum filtration. The product was dissolved in DCM and extracted with acidic water. After drying over $MgSO_4$, DCM was removed under reduced pressure. Characterization showed impure product with low molecular weight. Purification efforts resulted in polymer degradation.

7.6.1.5. Dicylohexylcarbodiimide Coupling

Ibuprofen-based diacid (0.10 g, 0.21 mmol) was dissolved in anhydrous DCM (10 mL) under argon and DCC (0.36 mL 1.0 M, 0.36 mmol) added dropwise over 30 min. After stirring for 24 h, the reaction mixture was cooled to 4 °C. The precipitate formed was removed via vacuum filtration. The filtrate was concentrated under reduced pressure to yield a yellow liquid. Characterization showed impure, M_w product and the disappearance of the tartatic backbone protons. Purification efforts resulted in polymer degradation.

7.6.2. References

1. Erdmann, L.; Uhrich, K., *Biomaterials* **2000,** *21* (19), 1941-1946.
2. Anastasiou, T. J.; Uhrich, K. E., *Journal of Polymer Science Part A: Polymer Chemistry* **2003,** *41* (22), 3667-3679.
3. Schmeltzer, R. C.; Anastasiou, T. J.; Uhrich, K. E., *Polymer Bulletin* **2003,** *49* (6), 441-448.
4. Schmeltzer, R. C.; Schmalenberg, K. E.; Uhrich, K. E., *Biomacromolecules* **2004,** *6* (1), 359-367.
5. Schmeltzer, R.; Schmalenberg, K.; Uhrich, K., *Biomacromolecules* **2005,** *6* (1), 359-367.
6. Rosario-Meléndez, R.; Harris, C. L.; Delgado-Rivera, R.; Yu, L.; Uhrich, K. E., *Journal of Controlled Release* **2012,** *162* (3), 538-544.
7. Schmeltzer, R. C.; Uhrich, K. E., *Polymer Bulletin* **2006,** *57* (3), 281-291.
8. Prudencio, A.; Carbone, A. L.; Griffin, J.; Uhrich, K. E., *Macromolecular Rapid Communications* **2009,** *30* (13), 1101-1108.

9. Ouimet, M. A.; Uhrich, K. E., *Polymeric Materials: Science & Engineering* **2012,** *107*, 575-576.
10. Rosario-Meléndez, R.; Uhrich, K. E., *Polymeric Materials: Science & Engineering* **2012,** *107*, 443-444.
11. Schmeltzer, R. C.; Johnson, M.; Griffin, J.; Uhrich, K., *Journal of Biomaterials Science, Polymer Edition* **2008,** *19* (10), 1295-1306.
12. Carbone, A. L.; Uhrich, K. E., *Macromolecular Rapid Communications* **2009,** *30* (12), 1021-1026.
13. Leong, K. W.; Simonte, V.; Langer, R., *Macromolecules* **1987,** *20* (4), 705-712.
14. Mathiowitz, E.; Jacob, J.; Jong, Y.; Carino, G.; Chickering, D.; Chaturvedi, P.; Santos, C.; Vijayaraghavan, K.; Montgomery, S.; Bassett, M.; C, M., *Nature* **1997,** *386* (6623), 410-414.

8. FUTURE WORK SUGGESTIONS

In Chapter 5, it was shown that PolyMorphine provides sustained analgesia for up to 3 days, more than 20 times the analgesic time window of free morphine. To achieve longer analgesic effect (i.e., one week) from PolyMorphine, it is suggested to chemically modify the polymer structure. PolyMorphine's structure may be altered during the esterification of morphine by changing the cyclic "linker" molecule, glutaric anhydride (Figure 8.1, **1**), to a branched aliphatic linker, as shown in Figure 8.1 (**2** and **3**). The synthesis, characterization, and *in vivo* assessment of PolyMorphine containing branched aliphatic linkers can be performed as described in Chapter 5. Furthermore, the analgesic effect provided by the polymer should be tested in a chronic constriction injury animal model to determine the ability of PolyMorphine to provide sustained analgesia in chronic pain.

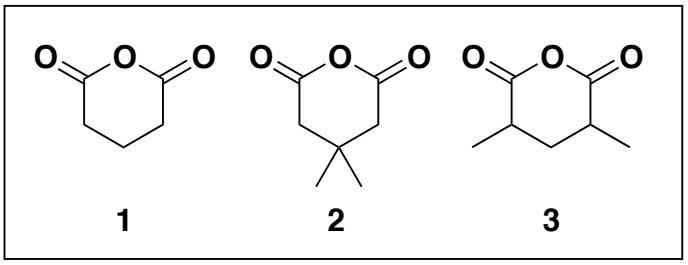


Figure 8.1. Examples of linker structures for structure variation of PolyMorphine composition (**1** linear aliphatic, **2** and **3** branched aliphatic for slow degradation rate).

Once the optimal analgesic effect from PolyMorphine is obtained, the combination of opioid-based and non-steroidal anti-inflammatory drug (NSAID)-based

polymers should be studied further. Preliminary results obtained (Appendix 7.4) suggest that having a combined treatment for acute and chronic pain consisting of an opioid and a NSAID is beneficial. Therefore, the combination of PolyMorphine with the salicylate-based poly(anhydride-ester) and PolyMorphine with the ibuprofen- or naproxen-based polymers is suggested. It is hypothesized that these polymer combinations can result in enhanced analgesia while reducing the side effects associated with both drugs (the opioid and the NSAID).

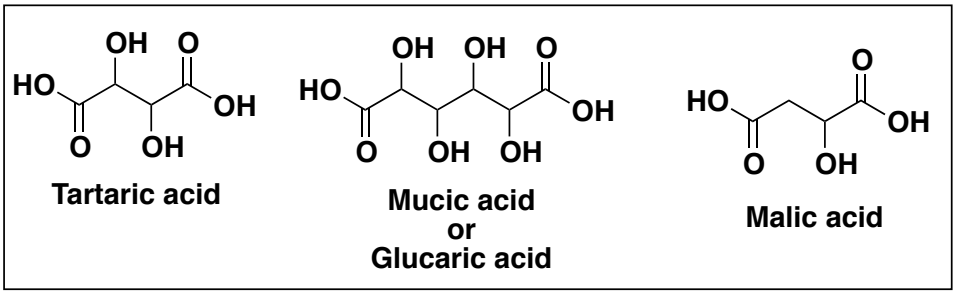


Figure 8.2. Examples of linker structures for structure variation of ibuprofen- and naproxen-based polyesters composition. Mucic acid and glucaric acid can be used to incorporate more drug molecules per repeat unit; and malic acid to incorporate fewer drug molecules.

In case the ibuprofen- and naproxen-based polymers do not provide sufficient analgesia or the degradation rate needs to be shortened or prolonged, alteration to the composition of these polymers is recommended. As described in Chapter 6, these polymers are comprised of the drug, the tartaric acid linker, and 1,8-octanediol as an extender. Both the linker and the extender can be altered to modify the physicochemical

properties and drug release rate. For example, the linker can be changed from tartaric acid to mucic acid or glucaric acid (Figure 8.2) to allow the attachment of four drug molecules to each repeat unit. The linker could be change to incorporate fewer drug molecules per repeat unit using malic acid. It is anticipated that this change will result in a modification of the physicochemical properties of the polymers and drug release profile. Figure 8.3 shows different diols that can be used for the synthesis of the ibuprofen- and naproxen-based polyesters. Preliminary results show that the properties of the ibuprofen-based polymer [e.g., glass transition temperature (T_g)] change by using different diols. As the length of the diol is increased, the T_g decreases. For example, the synthesis of the ibuprofen-based polyester with 1,3-propanediol yields a polymer with a T_g of 18 °C, 1,5-pentanediol yields a polymer with a T_g of 3 °C, and 1,8-octanediol yields a polymer with a T_g of -17 °C. The synthesis of the polymers using the proposed linkers (Figure 8.2) and extenders (Figure 8.3) will result in a library of polymers with tunable properties for different biomedical applications (e.g., the opioid-NSAID combined treatment).

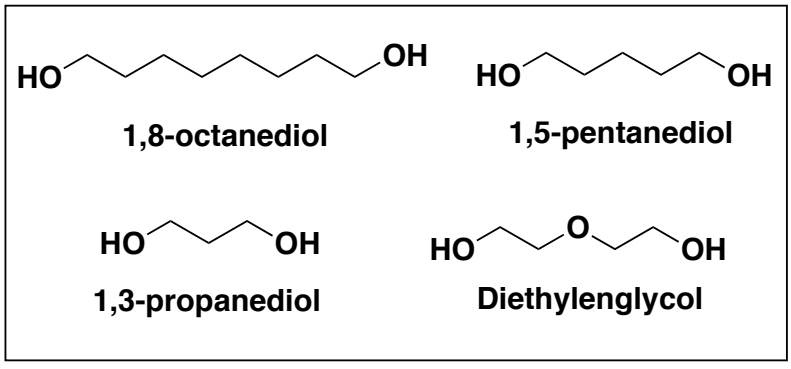


Figure 8.3. Examples of extender structures for structure variation of ibuprofen- and naproxen-based polyesters composition.

9. GENERAL EXPERIMENTAL METHODS

9.1. Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy

^{1}H-NMR spectra were obtained using a Varian 400 or 500 MHz spectrometer. Samples were dissolved (~ 5 mg/mL) in deuterated dimethyl sulfoxide (DMSO-d_6) that was used as an internal reference or in deuterated chloroform ($CDCl_3$) with trimethylsilane as an internal reference. Each spectrum was an average of 16 scans.

9.2. Carbon Nuclear Magnetic Resonance (^{13}C-NMR) Spectroscopy

^{13}C-NMR spectra were obtained using a Varian 500 MHz spectrometer. Samples were dissolved (~ 20 mg/mL) in deuterated dimethyl sulfoxide (DMSO-d_6) that was used as an internal reference or in deuterated chloroform ($CDCl_3$) with trimethylsilane as an internal reference. Each spectrum was an average of 250 scans.

9.3. Infrared Spectroscopy (IR)

Fourier transform infrared (FT-IR) spectra were obtained using a Thermo Nicolet/Avatar 360 FT-IR spectrometer. Samples (1 wt.%) were ground with KBr and compressed into a disk (13 mm diameter x 0.5 mm thick) using a hydraulic press (Carver

model M) applying pressure (10,000 psi) for 1 min or solvent-cast (1 wt.%) onto NaCl plates using dichloromethane (DCM). Each spectrum was an average of 32 scans.

9.4. Mass Spectrometry (MS)

MS was used to determine the MWs of polymer intermediates. A Finnigan LCQ-DUO equipped with Xcalibur software and an adjustable Atmospheric Pressure ionization Electrospray Ion Source (API-ESI) was used. Samples were dissolved in methanol (MeOH) and diluted to 10 μg/mL before injection using a glass syringe. Pressure during the experiments was 0.8×10^{-5} Torr and the API temperature was 150 °C.

9.5. Gel Permeation Chromatography

GPC was used to determine the M_w of the polymer. A Perkin-Elmer LC system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 advanced sample processor was used. A Dell OptiPlex GX110 computer running Perkin-Elmer TurboChrom 4 software was utilized for data collection and control. The connection between the LC system and the computer was made using a Perkin-Elmer Nelson 900 Series Interface and 600 Series Link. Samples were dissolved in DCM (10 mg/mL) and filtered through 0.45 μm polytetrafluoroethylene syringe filters (Fisher) prior to elution through a Jordi divinylbenzene mixed-bed GPC column (7.8 x 300 mm) (Alltech Associates, Deerfield, IL) at a rate of 1 mL/min for a total run time of 30 min. Weight-average molecular weights and polydispersity indexes (PDIs) were calculated relative to narrow M_w polystyrene standards (Polysciences, Dorval, Canada).

9.6. Thermogravimetric Analysis (TGA)

Thermal analysis was performed using TGA to obtain the decomposition temperatures (T_d). TGA analysis was performed using a Perkin-Elmer TGA7 analyzer with TAC7/DX controller equipped with a Dell OptiPlex Gx 110 computer running Perkin-Elmer Pyris software. Samples (~10 mg) were heated under nitrogen at a rate of 10 °C/min from 25 to 400 °C. T_d was defined as the onset of decomposition and is represented by the beginning of a sharp slope on the thermogram.

9.7. Differential Scanning Calorimetry (DSC)

Thermal analysis was performed using DSC to obtain the T_g and T_m. DSC was performed using a Thermal Advantage (TA) DSC Q200 running on an IBM ThinkCentre computer equipped with TA Instrument Explorer software for data collection and control. Samples (4-8 mg) were heated under nitrogen from -40 °C to 200 °C at a heating rate of 10 °C/min. Two heating/cooling cycles were used for each sample set. TA Universal Analysis 2000, version 4.5A was used to analyze the data.

GLOSSARY

Acute pain – pain that comes on quickly, can be severe, but lasts a relatively short time.[1]

Ad libitum – at one's pleasure.[2]

Analgesia – absence of pain in response to a stimulus that would normally be painful.[1]

Analgesic – a remedy that relieves or allays pain.[2]

Anesthesia – general or local insensibility, as to pain and other sensation, induced by certain interventions or drugs.[2]

Antioxidant – a substance that is capable of counteracting the damaging effects of oxidation in animal tissues.[2]

Biocompatible – a substance capable of coexisting with living tissues or organisms without causing harm.[2]

Biodegradable polymer – a polymer that can be broken into small segments.[3]

Biomaterial – any substance (other than a drug) or combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or part of a system, which treats, augments, or replaces any tissue, organ, or function of the body.[4]

Burst release – change in drug release rate from first-order to zero-order or no release.[5]

Calcein AM assay – is an assay designed to quantify live cell numbers based on the presence of their cytoplasmic membrane integrity. The fluorescent signal generated from the assay is proportional to the number of living cells in the sample.[6]

Chronic pain – ongoing or recurrent pain lasting beyond the usual course of acute illness or injury or, generally, more than 3 to 6 months and adversely affecting the individual's well-being.[1]

Controlled release – delivery of a drug at a predetermined rate for a definite time period.[7]

Copolymer – a polymer which contains more than one type of repeat unit.[8]

Cytotoxicity - cell destruction caused by a cytotoxic substance.[2]

Drug tolerance – a decrease in pharmacologic response following repeated or prolonged drug administration.[9]

Electron Beam radiation – ionizing radiation generated using electricity and magnetism to accelerate electrons to high energy levels.[10]

Fibroblast – a type of cell in loose connective tissue that secretes the protein ingredients of the extracellular matrix.[11]

Gamma Radiation – electromagnetic radiation emitted by man-made isotopes ^{60}Co and ^{137}Cs.[10]

Glass transition temperature – a phenomenon observed in linear amorphous polymers that occurs at a well-defined temperature when the bulk material ceases to be brittle and glassy in character and becomes less rigid and more rubbery.[12]

In vitro – made to occur in a laboratory vessel or other controlled experimental environment rather than within a living organism or natural setting.[2]

In vivo – occurring or made to occur within a living organism or natural setting.[2]

Inflammation – a localized innate immune defense triggered by physical injury or infection of tissue.[11]

Localized release – release of a drug to a particular body compartment, thereby lowering the systemic drug levels.[7]

Melt-condensation polymerization – thermal coupling of anhydride monomers, unsuitable for heat-sensitive monomers.[13]

Microspheres – solid spherical particles ranging from 1 to 999 μm.[5]

MTS Assay – is a homogeneous, colorimetric method for determining the number of viable cells in proliferation, cytotoxicity or chemosensitivity assays. The conversion of MTS into the aqueous soluble formazan product is accomplished by dehydrogenase enzymes found in metabolically active cells.[14]

Narcotic – any of a class of substances that in large quantities produce euphoria, stupor, or coma, that when used constantly can cause habituation or addiction, and that are used in medicine to relieve pain, cause sedation, and induce sleep.[2]

Nociception – the neural process of encoding and processing noxious stimuli.[1]

Nociceptor

Non-steroidal anti-inflammatory drugs – a heterogeneous group of organic acids that have analgesic, antipyretic, anti-inflammatory, and platelet inhibition actions.[15]

Opioid – compound that binds to an opioid receptor.[1]

Osteoarthritis – is a condition characterized by focal areas of loss of articular cartilage within the synovial joints, associated with hypertrophy of the bone and thickening of the joint capsule.[16]

Pain – an unpleasant sensory and emotional experience associated with actual or potential tissue damage.[1]

Polymer (homopolymer) – a large molecule made by linking monomers together.[3]

Polymer Blend – a mixture of at least two macromolecular substances.[17]

Rheumatoid arthritis – is a chronic inflammatory polyarthritis, the term rheumatism describes an ailment with inflammation, joint pain, and stiffness in the muscles.[16]

Side effect – any effect of a drug, chemical, or other medicine that is in addition to its intended effect, especially an effect that is harmful or unpleasant.[2]

Solution polymerization – one-step polymerization method at ambient or lower temperature, suitable for heat-sensitive monomers.[18]

Sterilization – the destruction of all living microorganisms, as pathogenic or saprophytic bacteria, vegetative forms, and spores.[2]

Synovial cavity – A space between the two bones of a synovial joint, filled with synovial fluid; the synovial fluid is essential for the normal joint functioning.[16]

Tail-flick Latency Test – method to measure nociception in response to thermal stimulus, the main end point is a withdrawal response.[19]

1. Institute of Medicine (US) Committee on Advancing Pain Research, C., and Education, *Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research*. The National Academies of Press: Washington, DC, 2011.
2. Dictionary.com, LLC. In *Dictionary.com*, Lexico Publishing Group, LLC: Oakland, CA, 2012.
3. Bruice, P. Y., *Organic Chemistry*. Fourth ed.; Pearsn Education: Upper Saddle River, NJ, 2004.
4. Hollinger, J. O., *An Introduction to Biomaterials*. Second ed.; Taylor & Francis Group: 2012.
5. Wright, J. C.; Burgess, D. J., *Long Acting Injections and Implants*. Springer: London, 2012.
6. Biotium Calcein AM Cell Viability Assay Kit. http://www.biotium.com/product/product_types/search/price_and_info.asp?item=30026.
7. Langer, R., *Science* **1990,** *249* (4976), 1527-1533.
8. Alger, M. S. M., *Polymer Science Dictionary*. Chapman & Hall: London, UK, 1989.
9. Dumas, E. O.; Pollack, G. M., *The AAPS journal* **2008,** *10* (4), 537-551.

10. Razem, D.; Katusin-Razem, B., *Radiation Physics and Chemistry* **2008,** *77* (3), 288-344.
11. Campbell, N. A.; Reece, J. B., *Biology*. 7 th ed.; Pearson Education Inc.: San Francisco, 2005.
12. Nichilson, J. W., *The Chemistry of Polymers*. The Royal Society of Chemistry: Cambridge, UK, 2006.
13. Leong, K. W.; Simonte, V.; Langer, R., *Macromolecules* **1987,** *20* (4), 705-712.
14. Promega CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (MTS). http://www.promega.com/products/cell-health-assays/cell-viability-assays/celltiter-96-aqueous-non_radioactive-cell-proliferation-assay-_mts_/.
15. Aronson, J. K., *Analgesic and Anti-inflammatory Drugs*. First ed.; Elsevier: San Diego, CA, 2010.
16. Bagchi, D.; Moriyama, H.; Raychaudhuri, S. P., *Arthritis: Pathophysiology, Prevention, and Therapeutics*. First ed.; CRC Press Taylor & Francis Group: Boca Raton, FL, 2011.
17. Utracki, L. A., *Polymer Blends Handbook*. Kluwer Academic Publishers: 2002; Vol. 1.
18. Domb, A. J.; Ron, E.; Langer, R., *Macromolecules* **1988,** *21* (7), 1925-1929.
19. Boyce-Rustay, J. M.; Honore, P.; Jarvis, M. F., *Methods Mol Biol* **2010,** *617*, 41-55.

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Rosario-Meléndez, R.; Lavelle, L.; Bodnar, S.; Halperin, F.; Harper, I.; Griffin, J. and Uhrich, K.E. Stability of a salicylate-based poly(anhydride-ester) to electron beam and gamma radiation. *Polym. Deg. Stab.* **2011**, 96, 1625-1630.