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# Chemically Active Rheological Modifiers for the Improved Clinical Use of Cyanoacrylates

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# CHEMICALLY ACTIVE RHEOLOGICAL MODIFIERS FOR THE IMPROVED CLINICAL USE OF CYANOACRYLATES

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ABSTRACT

Over 100 million surgical incisions, 50 million traumatic wounds, and 20 million minor lacerations from cuts and grazes are treated globally each year. One study determined that globally internal and external wounds occur for 40% and 37% of cases respectively for a total of 111 million patients that were treated for wounds. The remaining 23% of patients were treated for minor lacerations and trauma in the emergency room. These wounds require immediate medical attention including pressure application, sutures, clips, tissue cautery, and/or topical hemostatic agents to cease hemorrhage or the exit of other bodily fluids. With blood flow ceased, wound approximation or sealant is used temporarily to close the wound until fresh tissue is formed.

There are several different approaches to close a wound with each lacking in one or more properties to achieve ideal wound healing. Tissue welding and cauterization are two methods employed; however, these methods result in the formation of necrotic tissue, which is undesirable. Sutures are the wound approximation gold standard due to their flexibility and ability to resist tensile forces, but they can result in complications such as bleeding from the holes created during the suturing application required to place them. Staples and tapes are common wound approximation devices, but unlike sutures, they lack the ability to resist large tensile forces. In addition to these mechanical closure devices, fibrin and thrombin based sealants have been employed to approximate or seal tissues. These biological sealants are very biocompatibility, but they also have a low mechanical strength especially as compared to the mechanical closure devices. This low

strength has resulted in re-bleeding when this type of sealant was applied externally and also in cases when it was applied internally. In comparison, BioGlue®, a non-biological sealant has a high mechanical strength, but is limited by its poor biocompatibility. Alternatively, there have been advancements based on gecko and mussel adhesions (bioadhesion) in order to fabricate synthetic materials that mimic these naturally occurring dry and wet adhesives respectively. Studies have demonstrated that these materials have a great potential, but they still require additional research in order to render them clinically relevant for wound approximation.

Cyanoacrylate adhesives is another family of wound approximation and sealant devices. As a general overview, these materials are able to penetrate into tissue due to their liquid monomer form, rapidly polymerize due to their highly electrophilic nature, and then form bonds due to the interpenetrating networks formed. They have been fabricated in many varieties by differing the side chain for the adhesive monomer during its synthesis, blending additives into the adhesive, or mixing insoluble materials into the adhesive. A myriad of studies have demonstrated that these variations can control the properties of the adhesive in its monomer and polymer forms. Several of these properties include viscosity, mechanical strength and flexibility, polymerization rate and reaction temperature, degradation rate, and biocompatibility.

By controlling the side chain type and materials added to it, researchers are able to tailor cyanoacrylates for specific external and internal medical and industrial uses. These adhesives are well known for their typically successful external medical uses and industrial uses; however, their internal medical use has been slow to reach global use due

to the heat released during the adhesives polymerization, and the cytotoxic formaldehyde byproduct released as the polymerized adhesive degrades. In order to overcome this issue, researchers commonly synthesize cyanoacrylates for internal use by attaching long alkane side chains (e.g. 2-octyl) to them. The resulting cyanoacrylate releases a lower amount of heat during its polymerization and minimal formaldehyde as it degrades; however, several studies have demonstrated that this degradation take years, if it degrades at all, which can result in a prolonged, chronic wound healing. Nevertheless, this material has excellent clinical usefulness when the specific cyanoacrylate types are used for their specific intended uses. There is therefore great potential to modulate the adhesive to improve its clinical usefulness.

The completed research presented in this dissertation focused on using this information to formulate cyanoacrylates with potentially improved clinical usefulness. The cyanoacrylates were improved through the addition of novel chemically active polyesters (rheological modifiers). Methoxypropyl cyanoacrylate was selected for this research due to its inclusion of a short alkoxy side chain resulting in a flexible, high strength bond as well as the adhesive's proven biocompatibility. Poly(glycolide-co-caprolactone) polymers (PGCL) were synthesized as the polyesters for this research due to the fast degrading, low pH producing ability of glycolide and slow degrading, increased flexibility of ε-caprolactone. The combination of both fast and slow degrading monomers allows one specifically to control the polyester's degradation rate and thus resulting pH level for the eluent. Based on these properties, it was hypothesized that mixing PGCL as an amorphous polymer into cyanoacrylate, polymerizing the

cyanoacrylate, and then allowing the polycyanoacrylate to degrade in water or a phosphate buffered saline will allow the adhesive modifier contained within the polycyanoacrylate to also hydrolyze and self-modulate the pH of the surrounding environment to an acidic level. When a polycyanoacrylate degrades in an acidic instead of basic solution, then the formaldehyde levels released should be minimized, and the alcohol and cyanoacrylic acid released should be less toxic; thus, rendering the polycyanoacrylate potentially safer for internal use.

# DEDICATION

This work is dedicated to my wife Tiffany Killian-Garcia and my co-chair Dr. Joel Corbett for their continuous support and unwavering desire to see me reach my full potential as a scholar, and achieve the title Doctor. I could not have completed my Ph.D. journey and doctorate degree attainment without them.

I would also like to give a special dedication to my children (Sabene, Aaron, and Zoey). Doctor is a great title, but no title could ever make me happier than father, so I dedicate this work to you.

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I lastly acknowledge that as with all I do in live I strive to follow my savior Jesus Christ. I am eternally grateful for my faith and the support that only He can provide. To God be the Glory.

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# PREFACE

In 2017, it was estimated that approximately 235 million major surgical procedures are performed each year globally. One study determined that globally internal and external wounds occur for 40% and 37% of cases respectively for a total of 111 million patients that were treated for wounds. These wounds require immediate medical attention including pressure application, sutures, clips, tissue cautery, and/or topical hemostatic agents to cease hemorrhage or the exit of other bodily fluids. With blood flow ceased, wound approximation or sealant is used temporarily to close the wound until fresh tissue is formed. There are several different approaches to close a wound with each lacking in one or more properties to achieve ideal wound healing.

Cyanoacrylate adhesives are one type of wound closure material. This material is well known for its typically successful external medical uses and industrial uses; however, its internal medical use has been slow to reach global use due to the heat released during the adhesives polymerization, and the cytotoxic formaldehyde byproduct released as the polymerized adhesive degrades. ***The objective of this research was to determine a method to tailor the physical structure of cyanoacrylate adhesives to increase their biocompatibility post-polymerization thereby encouraging their internal use.*** Specifically, this research focused on first increasing the viscosity of a cyanoacrylate by blending it with novel, custom polyester modifiers. A higher viscosity adhesive would potentially have an increased biocompatibility if it was applied clinically because its probability to migrate from the application site to undesired surrounding tissues prior to polymerization would be reduced. This research also included the development of a

polymerizing adhesive thermal properties test method as well as the use of this method to measure the peak temperature change, reaction rate, and heat of polymerization for various cyanoacrylate-polyester formulations as an additional indicator of their potentially improved biocompatibility due to the addition of the polyester modifiers. The last segment of this research included determining the ability of polyester modifiers contained within a polycyanoacrylate to chemically direct the degradation of the polycyanoacrylate to lessen the formaldehyde released from it as well as improve its biocompatibility as measured by *in vitro* cytocompatibility.

The first aim of this research included the incorporation of custom synthesized polyesters into a cyanoacrylate to increase its viscosity. In Chapter 2, the polyesters were synthesized using glycolide and l-lactide monomers to form pairs of bioresorbable polymers with low or high molar percent glycolide. This synthesis also included a linear initiator and a branched initiator to form two pairs of polymers per initiator type (four polymers total). Each polyester was than blended into methoxypropyl cyanoacrylate, the base adhesive selected for this research, at 4 or 10 weight percent for each of the four polyesters to form eight adhesive formulations. The maximum t-peel load, comparative viscosity, and shelf life for each adhesive formulation was then determined as an indicator of their clinical usefulness as wound approximation or sealant materials.

The second aim as discussed in Chapter 3 was to develop a polymerizing adhesive thermal properties test method and then use this method to test the research adhesive formulations as well as medical, benchmark adhesives (n-butyl and 2-octyl). For the method development, ideal parameters were determined to not only polymerize an

adhesive, but also measure its thermal properties using a data logger as a test method with high repeatability. Subsequent to the method's development, it was used to analyze the peak temperature change, reaction rate, and heat of polymerization for the research adhesive formulations as well as n-butyl cyanoacrylate and 2-octyl cyanoacrylate.

The third aim of this research was to assess the ability of polyester modifiers contained within a polycyanoacrylate to chemically direct the degradation of the polycyanoacrylate to lessen the formaldehyde released from it as well as improve its biocompatibility as measured by *in vitro* cytocompatibility. It was hypothesized that the acidic byproducts from the degrading polyesters would decrease the local pH environment for the polymerized adhesive, and direct the polycyanoacrylate to degrade following side-chain scission instead of unzipping thereby minimizing the formaldehyde released from the degrading polycyanoacrylate. In order to test this hypothesis, an ideal, repeatable method to polymerize adhesives was first determined in Chapter 4, and executed for the adhesive blends and benchmark adhesives. The resulting adhesive films were then split into small pieces, and allowed to degrade in phosphate buffered saline. At specific time points, the polymerized adhesives were tested to determine their mass loss, eluent pH, and formaldehyde released as included in Chapter 5. The latter test was performed using an assay kit and a fluorescence plate reader. In parallel, the polymerized films were tested using an agar overlay cytocompatibility test to evaluate the *in vitro* cellular response to the expected toxic formaldehyde byproduct released, as the polymerized films were degraded.

# CHAPTER 1

# REVIEW OF LITERATURE

## 1.1. Introduction

A large number of people globally require medical attention for wounds. These injuries to tissue resulting in a cohesive failure can occur through a variety of methods. The causes of these wounds can be intentional such as a surgical incision or unintended such as diseases or glass punctures from a car accident. Furthermore, injuries can occur inside the body (internal) and outside the body (external) such as the glass in the previous example penetrating both skin and intestinal tissue. These internal and external injuries require approximately 235 million major surgical procedures each year globally based on a 2017 report.[1] Additionally, 42% of patients (22% pediatric) present with minor trauma with 4.4-11% of these minor traumas due to lacerations.[2]

Regardless of the cause and type, in most cases, wounds require immediate medical attention including pressure application, sutures, clips, tissue cautery, and/or topical hemostatic agents to cease hemorrhage or the exit of other bodily fluids. With blood flow ceased, wound approximation or sealant is used temporarily to close the wound until fresh tissue is formed. Several typical hemostatic agents and their mode of action include oxidized cellulose (forms gel mass and activates platelets when contacts blood), collagen sponges (catalyzes hemostasis), thrombin (clotting agent), gelatin (temporary plug), fibrin sealant (clotting agent), chitosan and chitin (platelet activation), amylopectin and polysaccharide hemospheres (concentrates blood components by sponge effect), and zeolite mineral based (rapid water absorption).[3]–[6] Chitosan and chitin are

positively charged hemostats that enhances the functions of wound healing items including neutrophils, macrophages, platelets, fibroblasts, glycosaminoglycans, and nucleic acids.[7]–[9] Fiber based patches of chitin have been shown to attract platelets to further improve wound healing.[10], [11] Bandages containing chitosan have been shown to cause hemostasis from platelet activation, vasoconstriction, and interactions with red blood cells through ionic forces and cell surface proteins.[12] Furthermore, after application, chitosan will depolymerize over time into n-acetyl-beta-D-glucosamine, which enhances fibroblast proliferation to improve wound healing.

### 1.2. Wounds, wound healing, and typical tissues wounded

Hemostasis (the stoppage of blood flow) and subsequent formation of new tissue (proliferation) are parts of wound healing. There are four total stages for wound healing namely hemostasis, inflammation, proliferation, and remodeling. Following the formation of a wound, primary hemostasis will occur. During this phase, vasoconstriction occurs followed by platelets activation and then migration to the wound to aggregate and adhere to form a plug. Secondary hemostasis also occurs, which is the coagulation cascade that results in the replacement of the platelet plug with a fibrin clot. This cascade follows both an intrinsic pathway and extrinsic pathway. The intrinsic pathway is activated by damage to tissue layers, while the extrinsic pathway is activated by exposure of tissue factor to blood.[13],[14] The two pathways converge to change prothrombin into thrombin, fibrinogen into fibrin, and then the fibrin polymerizes to form a clot. The entire process of hemostasis typically lasts 0-15 minutes. The next step, inflammation, typically lasts up

to three days following hemostasis. This step includes vasodilation and increased vasopermeability to cause leukocyte infiltration (mainly neutrophils) to destroy any bacteria and debris at the wound. Macrophages will also later migrate to the wound to assist with bacteria removal and subsequent neutrophil removal. Early proliferation occurs in the next four days including migration of fibroblasts, which fabricate new endothelium, epithelium, and fibronectin. Angiogenesis also occurs during this phase. The optimal pH during proliferation is 7.2-8.3. As the collagen in this newly formed granulation tissue polymerizes and crosslinks, the tissue at the wound site returns to its physical strength in a phase named late proliferation (up to 14 days after early proliferation). The final stage, remodeling, occurs over the next couple of years as the fresh tissue is renewed and replaced over time to return the tissue to nearly its initial state. Wound contraction can also occur during this phase, which results in final wound site closure. The final result is a slight scar. Optimal skin wound healing will restore blood flow to its initial pH of 7.4 and skin to its initial pH of 4-6.[3], [9], [14]–[21]

There are several types of wounds with each requiring wound healing, which can be assisted by wound approximation and sealant devices. The wound healing previously described is primary healing for acute wounds. This wound type can typically be closed immediately (closure by primary intention), so they will heal rapidly.[22] Shear wounds can be one type of acute wound.[2] Chronic wounds, in contrast, exhibit chronic wound healing, which extends wound healing time by 12 weeks at times after initial injury.[15] Some wounds such as burns exhibit secondary wound healing. These wounds include wound contraction and connective tissue growth as part of the closure for the wound.

Blunt type wounds may also require secondary wound healing because this wound type typically disrupts a larger area of tissue when the wound is generated.[2] Lastly, some wounds can become covered by bacteria, so they are left open for some time prior to closing them to reduce infection risk in delayed primary healing (tertiary intention).[18],[19]

Until late proliferation when granulation tissue is formed (including collagen deposition), the wound approximation or sealant device is important as it approximates the wounded tissue and maintains its bursting strength until the tissue recovers. The healing tissue must be able to resist pressure up to 0.02 MPa (32 N for 16 $cm^2$) for abdominal areas or 27.2 MPa for skin.[23],[24] Failure of wound approximation and sealant devices to resist pressure can cause dehiscence, which can result in wound healing complications including further hemorrhage and gastrointestinal anastomotic fluid leakage. [21],[25],[26] These morbidities can quickly lead to mortality if they are not hastily repaired. Some reports show 40000-120000 acute laparotomy wound failures (dehiscence or evisceration) each year with 20000-60000 deaths.[21] Dehiscence rate can be device or wound dependent with some studies reporting 1.8% of patients sustaining an anastomotic dehiscence.[27] A 10-30% leakage rate was reported following pancreatic and colorectal operations.[21] Other reports show that laparotomy incisions fail to heal 11% of the time. These healing failures can be due to bacterial infection, incomplete wound debridement, or hematomas produced due to anticoagulants. [21],[28],[29] Chemotherapeutic drugs can also delay wound repair by impeding the Inflammation phase and delaying or blocking angiogenesis.[21] Similarly, antiplatelet drugs to treat

atherosclerosis can increase the risk of continuous bleeding.[30] Extended presence of a wound approximation device may thus at times be needed to facilitate these examples of extended wound healing.

External wounds typically damage skin, while internal wounds can damage any of the body's tissues (epithelial, connective, nervous, or muscle). The outer surface of the human body, skin, is the body's largest organ by mass with the important function to act as a barrier against the outside world.[31], [32] It consists of three overall layers namely, from upper to lower: epidermis, dermis, and hypodermis (subcutaneous tissue) with an underlying deep fascia (dense fibrous connective tissue surrounding muscles).[2] Similar to skin, layers of epithelial tissue compose the outer layers of the small and large intestines for the human body to protect the organs and allow normal physiological events (e.g. waste transportation) to occur.

## 1.3. Methods and types of materials for wound closure

### 1.3.1. Wound approximation history

Wound approximation devices and methods have been in use throughout history including the ancient world. Early examples of wound approximation include plasters (2100 BC), adhesive tapes and sutures (1600 BC), mechanical closure (6$^{th}$ century BC), sutures, (5$^{th}$ century BC), and solid glue dissolved in water (1787). [26],[33],[34] Ancient Hindus even used insect mandibles to close skin wounds.[35] Overall, ideal wound closure devices should meet several goals including: allow for meticulous closure, rapid application, painless, minimal scarring, and low infection rate.[22]

### 1.3.2. Tissue welding and cauterization

Tissue welding and cauterization are two methods of wound approximation. These methods rely on tissue's response to thermal application. For tissue welding, tissue is briefly exposed to a laser beam causing the tissue to absorb the laser energy in turn causing an increase in the tissue's thermal energy.[36],[37] This increase in thermal energy (heat) causes bonds to form between molecules (e.g. glycosaminoglycans, GAGs) in the extracellular matrix of cells in the tissue, which creates a water-tight seal of the tissue (unlike sutures and staples).[38] Tissue welding allows for rapid wound closure (5 minutes with laser versus 15 minutes with sutures) with no tissue response due to device exposure; however, this wound approximation method can cause undesired denaturation of collagen proteins (45°C), undesired tissue coagulation (60-70°C), tissue boiling (100°C), and tissue combustion.[36] Protein solders such as fibrinogen and albumin-hyaluronic acid can be used to reduce tissue damage by providing a sacrificial layer during tissue welding, but it cannot eliminate it.[39], [40] For cauterization, an electric current (electro-cauterization) or very hot surface (thermal cautery) is applied to tissue, which causes the tissue to shrivel and become necrotic in turn sealing the tissue.[37], [41] This necrotic tissue can further slough off causing perforation of the tissue; thus, re-opening the sealed tissue.[37]

### 1.3.3. Sutures, staples, and tapes

The gold standard for wound approximation, sutures, have been in use since ancient times. Unlike cyanoacrylates, these devices can be employed in areas of high

tension due to their greater mechanical strength. They also exhibit minimal tissue reactivity.[22] Sutures can be absorbable or non-absorbable. Non-absorbable sutures are less preferred by patients, however, because they typically require a secondary medical visit for removal. Absorbable sutures will degrade over time, so they will not require a second medical visit to remove them; however, they can potentially increase scar formation if they are made of polyesters because they will hydrolyze into acidic byproducts. Nevertheless, several studies have shown equivalent cosmesis and scarring when comparing absorbable and non-absorbable sutures. [22],[34] Typical absorbable sutures lose their strength starting at day 9 as they hydrolyze, but wound strength typically returns to approximately 50% original tissue strength at this time. Sutures create point stresses on tissue, which increases the potential for tissue damage. In addition, suturing can result in complications such as bleeding from suture holes and fluid leakage during gastrointestinal anastomosis. The latter can lead to peritonitis, sepsis, and even death. Application of fibrin sealant (a wound approximation device to be later discussed) to suture holes will close them; thus, lessening the potential for these poor side effects.[42]

Staples are another wound approximation device type. They can be absorbable or non-absorbable, but like sutures, the non-absorbable type must ideally be later removed. Insorb®, one type of absorbable staple, has 50% mass loss in 10-12 weeks and 60% strength loss after two weeks.[43] Unlike sutures, staples can be applied more rapidly, up to 4-6 times faster than sutures.[35], [44], [45] They also typically have a lower rate of tissue reactivity and infection than sutures because they contact a smaller area of tissue.

Unlike suturing, there is no risk to the operator from needle sticks with stapling due to the stapler design.[35] Stapling is a less meticulous technique than suturing, so the resulting healed wound can have poor cosmesis; however, at least one study showed no significant difference in cosmesis between sutures and staples for a healed wound.[22],[44] Overall, staples are contraindicated for tissue with excessive tension.[43] In addition, when approximating lung parenchyma, staples need to be reinforced (e.g. ePTFE) to resist the 20-25 cm $H_2O$ pressure measured during uncomplicated positive pressure ventilation. Lastly, staples have been reported to shift in position after application during wound healing. These shifting staples result in difficult removal with high patient discomfort. They can also result in morbidities such as bowel obstruction, biliary stone formation, urinary calculi, and functional impairment of bone. Following hysterectomy, a shifting staple can result in dyspareunia and injury to the male partner.[46]

Tapes are another type of wound approximation device currently available. They have been historically used to approximate light load bearing tissue due to their inability to resist large loads before failing. They can also be applied without needle sticks or punctures such as with staples. For this reason, tapes avoid potential tissue necrosis and scarring from sutures and staples. After application, tapes are highly susceptible to peeling, especially with infected or moist tissue. Some patients are allergic to tapes especially latex tapes. Medical personnel must carefully apply tapes to avoid undesired tension on the wound and surrounding tissue, which can lead to swelling, constriction, and blistering. Overall, because tapes do not puncture tissue, their removal is faster and less painful than non-absorbable sutures and staples.[47] Some researchers have

developed a new tape type that may remedy the load-bearing issue. It includes molded, plastic locks and straps. Their Zip® Surgical Skin Closure device is taped on either side of a wound with a plastic component bridging the wound. After application, the straps on either side of the device are pulled to move tissue edges on either side of the wound together to approximate the wound.[48]

### 1.3.4. Biological sealants

Sealants are one type of material that can be used to both seal and approximate wounds. Some sealants have the added function of also acting as a hemostat. Sealants are either biological (natural) or non-biological (synthetic or semi-synthetic) depending on how they interact with the body. Biological sealants include fibrinogen, thrombin, Factor XIII, calcium, aprotinin, and/or tranexamic acid.[49]–[51] These components interact with the coagulation cascade during hemostasis to produce and maintain a fibrin clot at the application site at the wound. Tisseel®, one fibrin sealant, is indicated as both an adjunct to sutures for hemostasis and a tissue sealant to prevent leakage from colonic anastomoses.[52] Fibrin sealants overall can be found in liquid, powder, and foam forms. In one study, the foam form was found to be the most effective of the fibrin sealant types at reducing blood loss and mortality rate.[53] A similar study showed a 100% hemostasis effectiveness for thrombin gel as compared to an 80% effectiveness for fibrin sealant and 65% effectiveness for thrombin sealant.[54] The concentrations of thrombin and fibrinogen were also shown to affect the mechanical strength of the formed fibrin clot. Specifically, lower concentrations of thrombin and higher concentrations of fibrinogen

allowed slower polymerization and better sealant diffusion leading to higher maximum force levels at failure as compared to higher concentrations of thrombin and lower concentrations of fibrinogen.[55],[56] A 3.5 fold increase of fibrinogen specifically resulted in a 27 fold increase in the shear adhesive strength for the resulting fibrin clot.[56] The application method also affects the burst strength and re-occurrence of blood leakage after fibrin sealant treatment. One study showed a two fold increase in burst strength and increase of 1/3 depth into needle holes when comparing spray only and rub-and-spray application methods.[57] When thrombin was used to treat bleeding gastric varices, it resulted in acute hemostasis for 91% of patients treated. Recurrent bleeding still occurred, however, in 19% of patients with mortality resulting for 8% of patients.[58], [59] In another study, fibrin sealants treated bleeding gastric varices with a 75% success rate and no adverse reactions.[59] Similarly, fibrin glue treatment was 86% effective at sealing leaks during gastrointestinal anastomoses placement and 100% effective at treating bleeding peptic ulcers.[60],[61] In another study, no differences were found in the occurrence of neuronal damage, gliosis, edema, fibroplasia, axonal damage, or myelin damage between patients treated with fibrin glue and patients that did not receive fibrin glue.[62] A similar study showed that fibrin glue was more effective at nerve repair and regeneration than sutures.[63] When compared to staples during hernia mesh fixation, there was no significant difference between the two treatments for operative time, hospital stay, wound healing complications, pain post-surgery.[64] In another study, researchers performed anastomosis of abdominal aortas using several different hemostats to test their hemostasis effectiveness. For this study, the gauze only

groups had 100% mortality, the collagen group had 75% mortality, the gelatin sponge and oxidized regenerated cellulose groups had 50% mortality, the thrombin gel group had 25% mortality, and the fibrin sealant group had 0% mortality. Furthermore, even when 12 sutures were applied as a wound approximation measure to allow hemostasis, there was still blood loss measured unlike the fibrin sealant group.[65] Fibrin sealants have also been used to successfully repair Achilles tendons, anterior cruciate ligaments, bone grafts, osteochondral fractures, anastomoses in vascular grafts, hepatic ducts, and spleens.[66] Due to the xenographic or allographic origin of thrombin, there are potential safety concerns relating to antibody development to factor V, anaphylaxis, and bovine spongiform encephalopathy.[67]–[71] Based on this information, one group of researchers recommended patients receive no more than one aprotinin containing fibrin sealant treatment per year.[71] There has also been at least one case of HIV transmission attributed to the fibrinogen component.[72]

### 1.3.5. Non-biological sealant

BioGlue® is one type of non-biological sealant. This sealant contains a 45% weight/volume solution of bovine serum albumin and a 10% weight/volume solution of glutaraldehyde. It is used as a hemostat during the placement of cardiovascular anastomoses as well as during repair following acute proximal aortic dissection. It was effective in thoracic aorta repair in sheep, with no adverse effects. Anastomotic hemostasis was also achieved in 61% of patients treated with BioGlue®.[73] One other study showed that anastomotic hemostasis was achieved for 81% of patients treated with

BioGlue® and another study showed that zero of 22 patients required re-operation for bleeding after BioGlue® was applied.[74] When BioGlue® is applied to tissue, the glutaraldehyde component covalently cross-links the albumin component to the tissue proteins at the wound site.[75] The glutaraldehyde component (10% weight/volume solution) of this sealant is less than the formaldehyde component (37% weight/volume) of gelatin resorcinol formalin (GRF) glue (a similar sealant), so it is considered less histotoxic.[75], [76] Glutaraldehyde is still a highly reactive chemical, however, due to its ability to cause nerve damage, coagulation necrosis, dermatitis, asthma, mutagenic effects, and myocardial necrosis.[75], [77], [78] Due to its xenographic origin, the bovine serum albumin component of BioGlue® can contain infectious agents that can be transmitted to the patient during sealant application.[77] BioGlue® is not indicated for use on lung tissue and liver tissue; nevertheless, it was tested by some researchers to explore this potential. Severe inflammation resulted after it was applied to lung tissue. Toxic necrosis, hemorrhage, medium-grade inflammation, granulation tissue, and giant cells were found when it was tested on liver tissue.[75] In another off-label internal use, BioGlue® minimized blood loss for 100% of patients treated with this sealant during renal tumor resection.[79] When compared to fibrin and thrombin products, BioGlue® was also shown to have a greater potential for adhesion formation.[80] After application, BioGlue® becomes very rigid as it cures, which can result in increased stress for tissue. When compared to fibrin sealants Tisseel® and Crosseal™ as well as human aortic tissue, BioGlue® had a mean elastic modulus of 3122 kPa, which was much greater than aortic tissue (450 kPa), Tisseel® (103 kPa), and Crosseal™ (54 kPa) .[81] This sealant

also has a very slow degradation time, and was found at the healed wound site as granules two years after implantation; however, no chronic inflammation was observed.[82] Nevertheless, there is a high potential for an adverse reaction such as the formation of foreign body giant cells from the continuous presence of biomaterials in the body including BioGlue® granules. These non-degrading remnants can also embolize to result in a thrombosis. One study showed that when applied along a suture line, BioGlue® leaked into needle holes for 22% of fresh aorta anastomoses and 6% of prosthetic graft anastomoses tested. The cured material that leaked through was also shown to become easily dislodged, which further supports the potential for this cured sealant to embolize.[83] This inability of BioGlue® to degrade can also delay the growth of pediatric patients' tissues when the sealant is applied in a circumferential fashion.[84] Some studies have also shown BioGlue® to result in mineralization and general tissue deformation.[77]

### 1.3.6. Gecko adhesion

Several researchers have also looked to nature for an alternative approach to wound approximation. These researchers have studied adhesive methods such as gecko dry adhesion and mussel wet adhesion in order to not only better understand these types of adhesions, but also how they can be mimicked through synthetic or a blend of natural and synthetic products. These new products have been termed biomimetic adhesives or bioadhesives.[85],[86] Most bioadhesives based on gecko adhesion attempt to mimic the gecko's foot and the microscopic bristle-like structures on its feet pads.[87] Each gecko

foot contains approximately 500,000 hairs known as setae. Other data places this value as 5000-14,000 setae per square millimeter with a 100 $mm^2$ pad area. Each seta is 30-130 μm long with an approximately 5 μm diameter. On the ends of each seta are 100-1000 spatula-shaped structures (spatulae) that are 0.2-0.5 μm in size. [85],[88],[89] These micro and nano structures allow geckos to make intimate contact with surfaces at multiple points. There have several theories for the method of gecko adhesion including suction, friction, micro-interlocking, electrostatic attraction, and glue excretion; however, it is the aforementioned multi-point intimate contact and resulting intermolecular van der Waals forces that has been proven as the true gecko adhesion method. As evidence for van der Waals forces yielding the adhesion, the gecko adhesive force was measured to increase as the surface energy of the substrate increased.[89]–[91] This adhesive method has been shown to produce 10 N adhesive force per gecko foot (0.1 MPa per 100 $mm^2$ foot) and an average 20 μN force per seta. The actual van der Waals adhesive force was estimated to be 0.4 μN per spatula, which results in 40-400 μN per seta. [89],[92] The maximum gecko adhesion force was measured in a laboratory setting to be 194 μN for a seta when the seta was allowed to contact a surface and then slightly slide. If all the setae on a gecko foot achieved this maximum force value, the resulting gecko force would be 100 N.[89] There is thus great potential for gecko adhesion to be useful in both medical and industrial applications.

While gecko adhesion shows promise for medical applications, it must first attain the ability to function in the naturally occurring moist or wet environments of tissues. This water layer places a barrier between the gecko adhesive and substrate to be bonded,

which minimizes the multi-point intimate contact and resulting van der Waals adhesive forces.[93] For this reason, gecko adhesion typically only functions in dry environments. There are thus two groups of research and synthetic gecko adhesive development, namely 1) products for dry adhesion and 2) method development and resulting products for wet gecko adhesion. The dry adhesion products focus on generating surfaces with nano to micro features to mimic the setae and spatulae features found naturally on gecko foot pads. These surface features are generated using micro and nano electromechanical fabrication methods including photolithography, electron beam lithography, plasma etching, deep reactive ion etching, chemical vapor deposition, and micro-molding. Typical materials processed include polyimide, polypropylene, and polydimethylsiloxane (PDMS) because they are flexible materials that are easily fabricated. Carbon nanotubes are most recently being tested as an option to be processed for gecko adhesion. Researchers have developed carbon nanotubes synthetic setae with an adhesive strength of $1.6x10^{-2}$ $nN/nm^2$ compared to $1x10^{-4}$ $nN/nm^2$ for geckos with the ability to support 0.36 MPa.[85]

One group of researchers processed an alternative material type, poly(glycerol sebacate acrylate) (PGSA), to generate an adhesive tape with and without nano patterns. PGSA was selected because it is a tough, biodegradable, flexible elastomer. They demonstrated that the nano-patterned tape had a two times greater adhesive strength than the flat, non-patterned tape. In addition, they demonstrated the effect of varying the nano-pattern on the resulting tape adhesive strength. Specifically, their research showed that decreasing the ratio of tip diameter to pitch as well as increasing the ratio of tip diameter

to base diameter decreased adhesion strength. The PGSA tape had a maximum separation force of 0.048 MPa. It also had a minimal tissue response when implanted.[94] Another group of researchers developed nano-patterned silicone rubber, and measured an adhesion strength of 180-300 nN for the nano-pillars compared to 50-300 nN for actual gecko setae. They also demonstrated the effect of surface roughness on the formation of gecko-type adhesions. Specifically, they showed a maximum adhesion when a harder, less sticky material with fatter, shorter, compliant nano-pillars oblique to the surface were used.[95] Although currently an industrial application, a group of researchers developed a material named Geckskin$^{TM}$ in 2012 with a soft elastomer (polyurethane pad) and stiff fabric (Kevlar skin) that uses draping adhesion to produce a gecko adhesive effect. The materials are draped to result in conformal contact with a surface, while still maintaining a high elastic stiffness in the direction force is applied.[85]

In order to enable gecko adhesion in wet environments, some researchers have added coatings to their nano-patterned surfaces such as dopamine, oxidized dextran, or even cyanoacrylate. These coatings promote tissue cross-linking. Other researchers have attempted to overcome this moisture barrier using natural products that function in wet conditions such as chitosan (a material previously discussed). In one case, a nano-patterned chitosan film was fabricated and measured to have a 0.0063 MPa wet adhesion strength.[93] In another case, researchers fabricated arrays of silicon nano-rods using photolithography, and then processed some of the arrays in carbon tetrafluoride ($CF_4$) plasma to place a hydrophobic coating on the surface of the arrays. The coating was shown to increase the dry adhesive strength of the array from 50 μN per nano-rod to 462

μN.[96] Although not tested, this nano-patterned material with a hydrophobic coating will likely produce a strong gecko adhesion in wet environments because the hydrophobic coating will repel moisture from the substrate to allow multipoint intimate contact between the nano-patterned material and the substrate; thus, allowing van der Waals adhesion forces.

### 1.3.7. Mussel adhesion

Unlike gecko adhesion, mussel adhesion occurs primarily in moist or wet environments. For this reason, this type of bioadhesion is well suited for being optimized for wound approximation because it functions on moist tissue.[97] Mussel adhesion uses lipids and up to six types of phosphoproteins (including catecholic amino acid 3,4-dihydroxyphenylalanine (DOPA)) with the lipids clearing water from the surface and DOPA adhering the mussel to a surface. Mussels naturally secrete the phosphoproteins as a liquid, which then solidifies to form a byssal thread and an adhesive plaque complex. This type of adhesion is used by several marine creatures including barnacles, starfish, mussels, and limpets.[85], [86], [98]–[100] In their natural state, the California mussel has been shown to have an adhesive strength of 250-300 N.[98] Researchers have tested wet mussel adhesive strength by preparing a solution of extracted mussel adhesive proteins (MAPS) at a mussels/MAPS rate of 10,000 mussels for 1 g MAPS. The test results show a wet shear strength of 0.233 MPa (subintestinal submucosa bond) and 1.44 MPa (porcine skin bond, 24 hour cure). In comparison, n-butyl cyanoacrylate and 2-octyl cyanoacrylate were measured to have a wet shear strength of 0.057 MPa and 0.115 MPa

respectively for subintestinal submucosa bonds. Fibrin sealant and ethyl cyanoacrylate were measured to have a wet adhesive strength of 1.16 and 2.91 MPa respectively for porcine skin bonds with a 48 hour cure.[101]

When compared to the fibrin sealant and cyanoacrylate industry standards, MAPS has a similar adhesive strength thereby rendering it clinically relevant.[97], [102] Nevertheless, with its yield of 0.1 mg per mussel, MAPS is not a sustainable adhesive product. For this reason, researchers have attempted to make synthetic products using this the mussel bioadhesion technology. Some of these products have been DOPA modified PEG or HA with limited application due to the natural ability of PEG and HA to swell and put pressure on surrounding tissues.[98] Other researchers developed a polymer containing DOPA, and demonstrated its ability to not only to adhere to wet surfaces, but also seal them in 10-20 seconds after application.[85] In another example, researchers developed a copolymerized acrylate film based on mussel adhesion by incorporating aromatic (catecholic or non-catecholic), cationic, anionic, and non-polar residues. This film had a wet adhesion strength of 0.15 MPa and 0.08 MPa in seawater. Another group of researchers fabricated a bioadhesive gel based on mussel adhesions through the ultraviolet irradiation of the photocurable monomer ethylene glycol acrylate methacrylate-dopamine (EGAMA- DOPA) and ultraviolet photocrosslinkable crosslinking agent poly(vinyl alcohol) (UV-PVA) derivative. Their bioadhesive gel had a maximum adhesive strength of 0.325 MPa.[103] Other researchers have had success from developing dopamine-modified materials because dopamine is a derivative of DOPA. One group of researchers fabricated double-crosslink tissue adhesive (DCTA) comprising

a dopamine conjugated gelatin macromer, ferric ions as a rapid crosslinking agent, and a genipin as a long-term acting crosslinking agent. The DCTA mimics the double-crosslinking of MAPS in the naturally occurring byssal thread for mussels. The DCTA adhesive had a 0.025 MPa adhesive strength for a skin to fat bond with an adhesive strength of 0.015 MPa for fibrin glue in comparison.[104] Another group of researchers made a poly(dopamine-co-acrylate) (PDA) as an adhesive precursor for wound closure. They then used $FeCl_3$, $NaIO_4$, fibrinogen, and horseradish peroxide (an enzyme mediated oxidative crosslinker) as potential crosslinking agents for their PDA. They found that fibrinogen was the most efficient strong oxidative cross-linking agent of the ones tested due to its chemical structure. The PDA-fibrinogen had an adhesive strength of 0.037 MPa after 15 minutes curing compared to a fibrin sealant with an adhesive strength of 0.01 MPa after 1 hour curing. Furthermore, the developed adhesive was measured to have a 59% mass loss after 1 month in PBS at 37°C.[105] This adhesive would thus have great potential for wound closure due to its adherence and degradation abilities.

### 1.3.8. Gecko-mussel adhesion

Another group of researchers combined knowledge from both geckos and mussels to develop a bioadhesives material that functions in both dry and wet environments. They fabricated nano-patterned PDMS coated with a mussel-mimetic polymer film, namely poly(dopamine methacrylamide-co-methoxyethyl acrylate) (p(DMA-co-MEA). The uncoated material was tested in air and water with adhesion strength results of 40 nN and 5.9 nN respectively. This result demonstrates the typical shortcoming of gecko adhesion,

moisture, or rather, more specifically, a failure of the synthetic bioadhesive material to make intimate contact with a substrate due to a moisture layer. When the mussel-mimetic polymer film was placed on the material, and then the material was tested, the material had an adhesion strength of 120 nN in air and 86 nN in water. Although the adhesion strength from air to water was still reduced, it was only reduced by 28% compared to an 85% reduction without the film applied. Furthermore, this material with the film applied was shown to retain 85% and 98% of its initial adhesion strength after 1100 contact/release cycles in water and air respectively as a measure of adhesion fatigue.[106]

## 1.4.Cyanoacrylate adhesive as a wound closure method

### 1.4.1. Cyanoacrylate description, types, and general properties

Cyanoacrylate adhesive is another type of material that is capable for some wound approximation requirements, but they are not yet indicated for all types of wounds. Due to their current lower mechanical strength including burst strength, they are contraindicated in areas of high tension or repetitive movement such as joints and hands.[22],[34] They are also not indicated for nerve anastomosis because in at least one study their application caused a foreign body inflammatory reaction and retractile fibrosis, reducing the nerve diameter by up to two-thirds its initial size.[107] Prior to cyanoacrylate application, hemostasis must have occurred or vessels otherwise clamped shut, so that the wound edges are dry. Adhesive in the wound otherwise will impair wound healing by diverting resources from the wound to the adhesive material to remove it from the body. Also, although cyanoacrylates have been tested for treating bleeding

lesions, for several of these cases, they were shown to cause episodes of abdominal, pulmonary, and intracerebral embolization as well as infarction.[108]–[110] In other off-label cases, cyanoacrylates successfully bonded cartilage and bone.[111]–[114] Researchers have found some success utilizing cyanoacrylates for internal use, but for fast setting cyanoacrylates such as n-butyl cyanoacrylate, they had to mix the cyanoacrylate with lipiodol to reduce the setting time. This mixture allowed endoscopic administration of the cyanoacrylate without the adhesive adhering to the surgical instruments prior to application. The lipiodol chemical imparted the added benefit of allowing the cyanoacrylate to be visible using fluroscopy.[115] When properly used for wound approximation, one study shows cyanoacrylates are at least as safe as nylon sutures.[116] The currently approved human use cyanoacrylates for both internal and external applications are n-butyl and 2-octyl cyanoacrylate.

When a cyano group (C≡N) and acrylate group ($CH2=CHCOO^-$) are combined, they form a cyanoacrylate. All cyanoacrylates contain several functional groups including a carbon alkene bond, cyano/nitrile bond, ester bond, and variable side chain group (R) as illustrated in Figure 1-1.

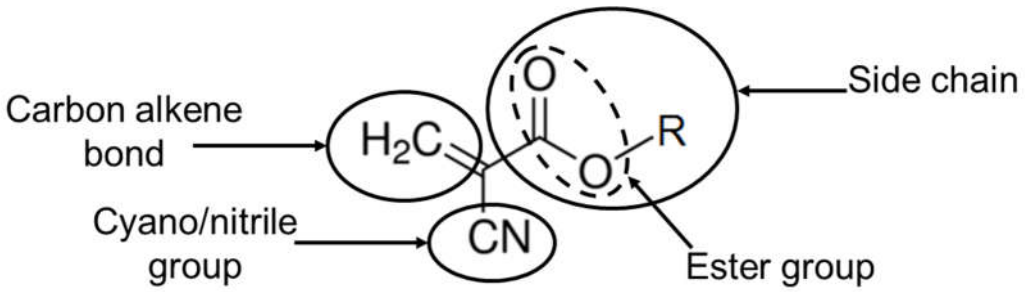



Figure 1-1: General Cyanoacrylate Structure

Cyanoacrylate monomers can anionically polymerize into poly-cyanoacrylates. This polymerization starts with initiation. During this phase, a nucleophile donates an electron pair to the cyanoacrylate monomer. Due to the high electron withdrawing nature of the cyano and ester groups, even a weak base such as water can serve as a nucleophile. This highly reactive monomer renders the alkene double bond present in the monomer very polarized and thus highly susceptible to nucleophilic attack. Once one cyanoacrylate monomeric unit has received an electron pair, the unit's double bond is broken, and the unit can initiate other cyanoacrylate units. This further initiation by multiple monomeric units results in propagating chains. This chain propagation phase is terminated when all the cyanoacrylate monomer units have been exhausted.[117] Figure 1-2 illustrates a typical cyanoacrylate anionic polymerization as discussed.

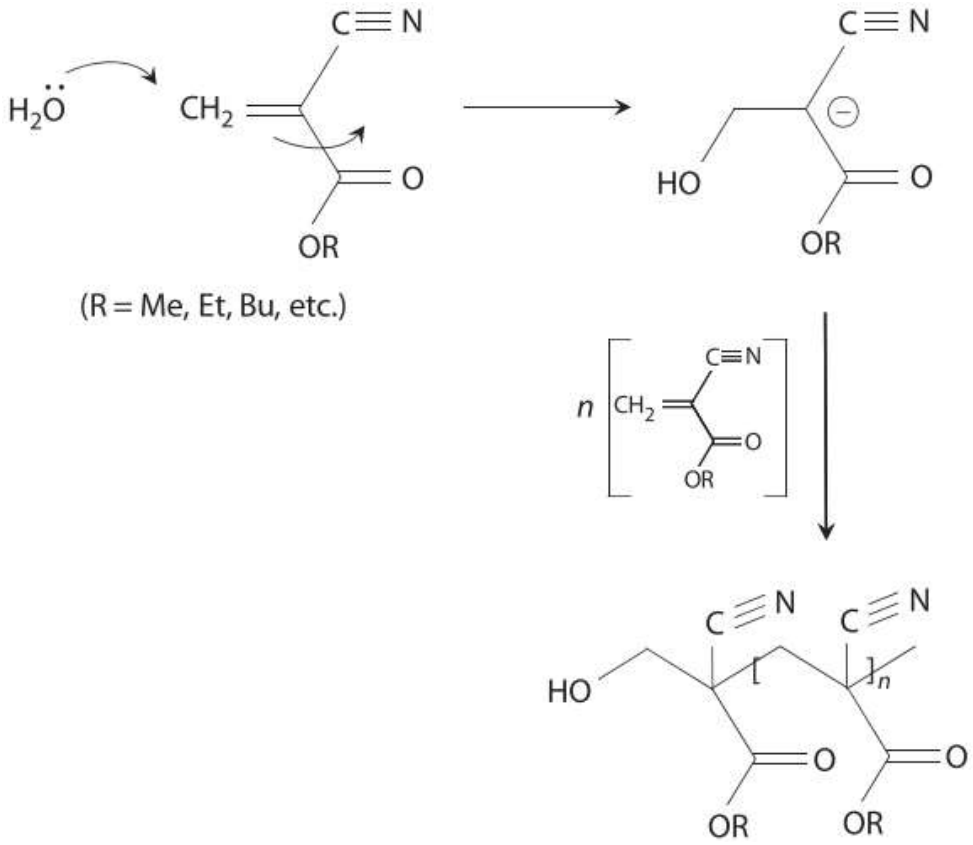



Figure 1-2: Cyanoacrylate Anionic Polymerization[117]

The variable side chain group for cyanoacrylates can vary in both length (typically number of carbon alkane bonds) as illustrated in Table 1-1 and complexity (inclusion of a functional group such as ester, ether, and phenyl) as depicted in Table 1-2. Scientists have determined that increasing the side chain size will increase the viscosity of the resulting cyanoacrylate monomer, with complexity having a greater impact than chain length.[118] The reason for this latter observation is that the greater complexity causes a greater flow resistance and thus viscosity as compared to the linear compounds.[119] In addition, following polymerization, as the polycyanoacrylate chain length and thus molecular weight of the polycyanoacrylate is increased, the hydrolysis

rate for the polymer decreases.[118] The larger polycyanoacrylate compound experiences a greater amount of steric hindrance thereby increasing the effort required to degrade it. Polymerized cyanoacrylate flexibility and degradation are also affected by the size of the cyanoacrylate side chain. Longer side chain lengths such as for 2-octyl cyanoacrylate produce more flexible, slower degrading material. In comparison, shorter chain lengths such as n-butyl cyanoacrylate produce stiffer, faster degrading material.[120] In one study, after applying n-butyl cyanoacrylate for internal liver fixation, the polymerized adhesive was found to be degraded and completely absorbed in 9 months.[121] The reason for the difference in flexibility is the decreased intermolecular forces for longer side chains producing a greater movement for the molecule than short chains. This flexibility is further increased with the addition of ether groups because the oxygen atom has an increased ease of rotation. Cyanoacrylates polymerize through an exothermic reaction, so the tissue the liquid monomer is applied to will experience a variable amount of heat as the adhesive cures.[122], [123] This heat of polymerization (HOP) varies depending on the type of cyanoacrylate. For HOP and polymerization rate, there is an inverse relationship between side chain length and complexity versus HOP and polymerization rate due to steric hindrance.[117], [124], [125] Cyanoacrylates with longer side chains such as hexyl, 2-octyl, and decyl or more complex side chains such as ethoxyethyl and methoxypropyl will generally have slower polymerization rates and lower HOP. In comparison, cyanoacrylates with short side chains such as methyl and ethyl or less complex side chains such as propyl will generally have faster polymerization rates and increased HOP. Other adhesive modifiers such as plasticizers can also be mixed

into the cyanoacrylate to modulate its polymerization rate and HOP. There is an inverse relationship between the amounts of adhesive modifier mixed into adhesives and the polymerization rate and HOP of the resulting adhesives because 1) the adhesive modifiers act as heat sinks and 2) there is less overall adhesive present to polymerize.[125]–[127]

Table 1-1: Cyanoacrylates with Variable Side Chain Lengths

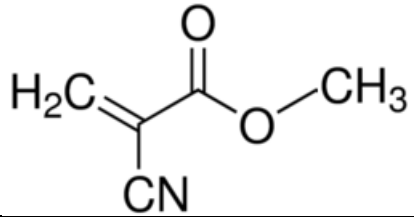
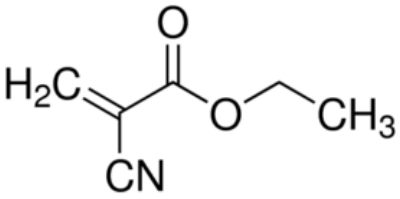
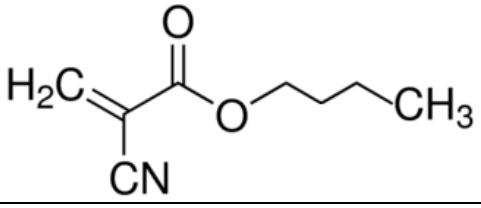
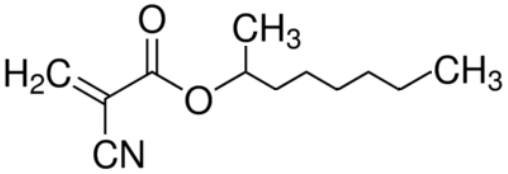
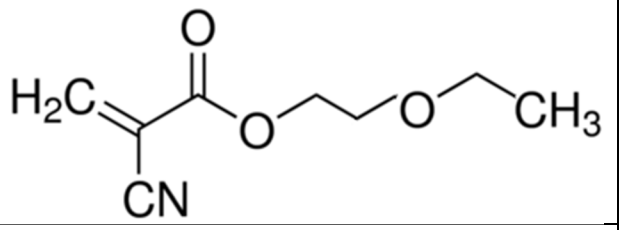
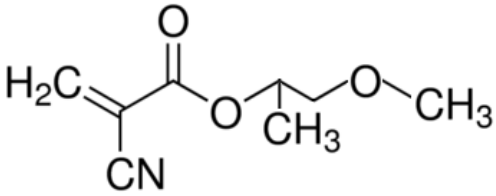
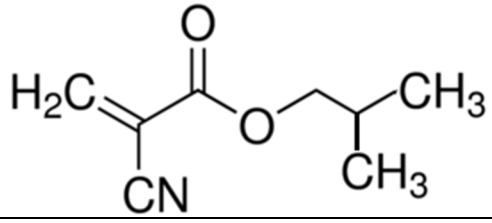
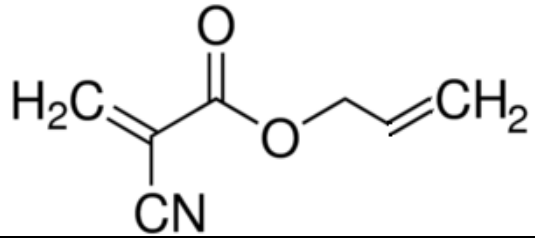
| Name | Structure |
|---|---|
| Methyl Cyanoacrylate |  |
| Ethyl Cyanoacrylate |  |
| Butyl Cyanoacrylate |  |
| 2-Octyl Cyanoacrylate |  |

Table 1-2: Cyanoacrylates with Variable Side Chain Complexity

| Name | Structure |
|---|---|
| Ethoxyethyl Cyanoacrylate |  |
| Methoxypropyl Cyanoacrylate |  |
| Isobutyl Cyanoacrylate |  |
| Allyl Cyanoacrylate |  |

Over time, polycyanoacrylates will degrade following two hydrolysis mechanisms. The first, a reverse Knoevenagel reaction causing depolymerization (unzipping), is started because the high electron withdrawing nitrile and ester groups in the polymer repeat unit (see Figure 1-2) cause the carbon atom between them to be highly activated. In the presence of water, this activated carbon will attract an electron pair from a water molecule, breaking up the polymer chain. This charged compound in turn will act as a nucleophile to attack other polycyanoacrylate chains. This depolymerization reaction

as summarized in Figure 1-3 results in the formation of 2-cyanoacetate and formaldehyde. A basic, high pH environment shifts the equilibrium towards increased depolymerization and an overall increased degradation rate for polycyanoacrylates.[117],[118] Acidic, low pH environments polycyanoacrylates strongly degrade by a second, alternative hydrolysis mechanism. Through this mechanism, the polycyanoacrylates experience side chain scission at the ester groups as summarized in Figure 1-4 resulting in the formation of 2-cyanoacrylic acid and alcohol.[117]

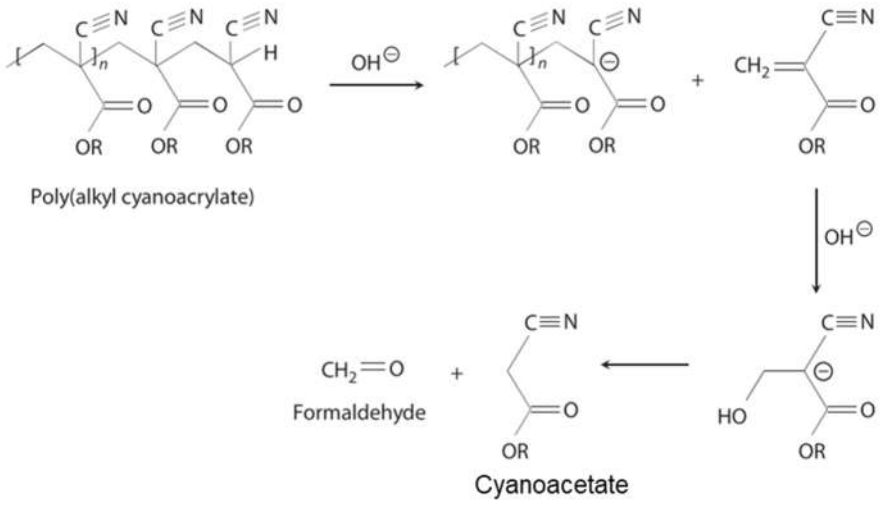



Figure 1-3: Polycyanoacrylate Degradation by Depolymerization[117]

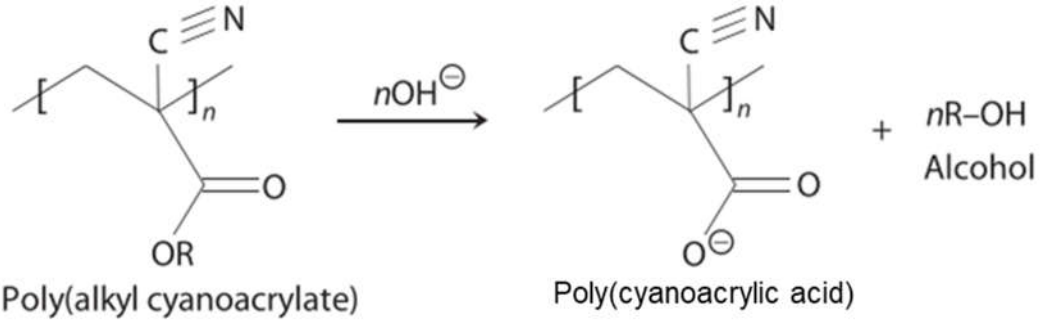



Figure 1-4: Polycyanoacrylate Degradation by Side Chain Scission[117]

One of the potential complications from cyanoacrylate usage is the release of formaldehyde, a known toxic chemical, from degrading polycyanoacrylates. Post-application, polycyanoacrylates may degrade into cyanoacetate and formaldehyde, with the latter generating a foreign body response.[118] For this reason, before an adhesive is applied to approximate a wound, hemostasis at the wound site must have first occurred.[34] Formaldehyde has been shown to bind extracellular protein components including amino and sulfhydryl groups commonly found in growth medium.[128] It can also cause acute and chronic inflammation. Amounts of 0.005 μg formaldehyde per 100 μg polycyanoacrylate as shown from poly(isobutyl cyanoacrylate) have been shown to cause no effect on cell growth. In comparison, amounts of 3.621 μg formaldehyde per 100 μg polycyanoacrylate as shown from poly(methyl cyanoacrylate) have been shown to inhibit cell growth. There was overall a relationship observed between inhibition of cell growth and formaldehyde concentration when testing several different polycyanoacrylates.[118] For this reason, formaldehyde is labeled as a human carcinogen by the International Agency for Research on Cancer and a World Health Organization panel. It is also listed as a probable human carcinogen by the United States Environmental Protection Agency.[117] As previously discussed, polycyanoacrylates with longer side chains will degrade slower; thus, they will release formaldehyde at a slower rate. Similarly, scientists have shown that a 5.3 fold increase in molecular weight for a cyanoacrylate can result in an 18 fold decrease in formaldehyde release.[124]

### 1.4.2. Methyl and ethyl cyanoacrylate external and internal use

Methyl and ethyl cyanoacrylates are two of the original cyanoacrylates and the simplest in chemical structure as depicted in Table 1-1. They were first invented by Alan Ardis in 1949 although their function as an adhesive was not invented until 1956 by Dr. Harry Coover.[129], [130], [131] Methyl cyanoacrylate (originally sold as Eastman 910®) contains one methyl group as the variable side chain while ethyl cyanoacrylate (commonly sold as Krazy Glue®) contains one ethyl group as the variable side chain for the cyanoacrylate.[132] These two glues were initially fabricated for industrial usage with some usage as hemostats during the Vietnam War. Due to their fast application and setting time as well as the strong bond they produce upon curing (0.066 MPa and 0.082 MPa for approximating small and large tissue wounds respectively), some medical personnel and researchers began testing these materials as wound approximation devices with mixed outcomes.[133] In one case, ethyl cyanoacrylate was applied to brain tissue, which caused severe superficial cortical necrosis without any actual tissue bonding.[134] In another case, methyl cyanoacrylate was used to treat an intracranial aneurysm; however, this attempted treatment resulted in a complication of late arterial thrombosis and an aneurysm.[135] Ethyl cyanoacrylate has also been applied to the left cruciate cortex and left neurovascular bundle in cat femurs to determine their effect on nerves and surrounding tissue. The result was meningeal necrosis, neuronal and axonal degeneration, vascular wall degeneration, thrombosis, and an inflammatory reaction.[136] When ethyl cyanoacrylate was also applied to rabbit corneal lesions, it resulted in a moderate inflammatory reaction in the initial phases of wound healing.[137] Lastly, there have

been some cases of skin irritation from repeated exposure to the methyl and ethyl cyanoacrylate adhesives, especially during the sloughing off of the polymerized adhesive.[138] There have also been some successes reported from the usage of ethyl cyanoacrylate to treat wounds. In one study, a suture-less pericardial patch was glued to the myocardium using ethyl cyanoacrylate with no reported mediastinal infections, and instead fully healed lacerations.[139] In fact, in a separate study, when comparing ethyl cyanoacrylate and Vicryl® absorbable sutures as wound approximation devices, researchers showed no major structural macroscopic differences in the healed wound site for small and large wounds, and no toxic reactions, infections, or inhibited wound healing due to the ethyl cyanoacrylate usage.[140]

### 1.4.3. Butyl and isobutyl cyanoacrylate external use

Butyl and isobutyl cyanoacrylate are the other two original cyanoacrylates invented by Alan Ardis in 1949.[129], [130] These cyanoacrylates contain four carbons as a butane group in their side chain as illustrated in Table 1-1 and Table 1-2. Butyl (typically n-butyl) cyanoacrylate has a linear side chain, while isobutyl has a complex side chain. Typical product names include Indermil®, Histoacryl®, LiquiBand®, and Glubran® 2. There are also products such as LiquiBand® Surgical S that contain a mixture of both butyl cyanoacrylate and 2-octyl cyanoacrylate in order to yield a product with the benefits of each cyanoacrylate type such as fast cure and flexibility. This product specifically is made from a mixture of 90% butyl cyanoacrylate and 10% 2-octyl cyanoacrylate. Butyl cyanoacrylate has been used for both external and internal

applications with variable results for each. In one external application, LiquiBand® was compared to non-absorbable monofilament sutures with no significant difference observed in terms of wound complications and cosmesis after 3-4 weeks and 3 months post-application. In this same study, there were also fewer patients required additional wound dressings post-application of LiquiBand® (21%) as compared to the sutures (97%) indicating the adhesive acted as a better hemostat than the sutures.[141] In a similar study, LiquiBand® Surgical S and sutures were applied externally with no serious adverse complications reported, a similar rate of patients with minor complications (22-23%), and a similar cosmesis upon wound healing completion. In addition, fewer patients required additional wound dressings post-application of LiquiBand® Surgical S (5%) as compared to the sutures (92%) indicating the adhesive acted as a better hemostat than the sutures.[142] A different study looked at the effect of Histoacryl® external application for pediatric patients. In this study, 88% of the patients had full wound healing.[143] In another study, Indermil® was applied for treating hand wounds. For this study, three of the wounds had minor dehiscence.[144] Due to cases such as these, as previously discussed, cyanoacrylates are contraindicated for areas of repeated movement. Other studies also showed poor wound dehiscence when using butyl cyanoacrylate. In one study, Histoacryl® was used to treat 1033 patients with 1.1% (11 patients) experiencing wound dehiscence.[145] In another study, Indermil® was used to treat 18 patients, with four of them experiencing wound dehiscence.[146] This wound complication is especially problematic because it delays the wound healing cycle, re-introduces the wound to the environment and thus the potential for infection, and requires additional

treatment to re-close the wound. Butyl cyanoacrylate has a sufficient tensile strength, so this wound complication, as shown above, has a low rate of occurrence. When the mechanical properties Glubran® 2 was compared to Tissucol™ (a fibrin sealant product) as tested using fresh pig skin, the adhesive was found to have a shear strength of 0.033 MPa and t-peel strength of 0.269 N/cm as compared to the fibrin sealant with a shear strength of 0.002 MPa and t-peel strength of 0.045 N/cm.[126]

### 1.4.4. Butyl and isobutyl cyanoacrylate internal use

Butyl cyanoacrylate has also been used for internal applications with variable results. In one study, Histoacryl® was implanted into 44 animals, with 11 of them developing sarcomas at the implantation site.[147] In another study, extensive biocompatibility testing was performed for internal use of Histoacryl®. Extracts of this adhesive were shown to be non-mutagenic, non-irritating, resulting in no systemic toxicity, passing all prescribed ISO 10993 tests. When the adhesive was implanted, it showed good local tolerance with no cell or tissue necrosis. There was also a 100% survival rate one year after implantation into animals. When it was used to tack mesh in place internally, researchers noted that a spot-wise application was necessary to limit areas of stiffness and limited tissue in-growth into the mesh from the polymerized adhesive.[148] Glubran® 2 was also used in a separate study to affix mesh for hernia repair for 20 patients with only one patient having a hernia re-occurrence one year later.[127] Similarly, when Histoacryl® was applied for mesh fixation in pelvic floor surgery for sheep, there was a low inflammatory reaction observed with good integration

of tissue with the mesh.[149] When butyl cyanoacrylate or Vicryl® sutures were used to affix mesh for hernia treatment in a separate study, in a seven day follow-up, patients receiving the adhesive reported significantly less pain than the patients receiving sutures.[150] In a large study for hernia repair, 552 patients had a mesh tacked in place using butyl cyanoacrylate, while 89 patients had the mesh tacked in place using titanium spiral tacks. The adhesive treated group had a 10% seroma rate, while the tacks treated group had a 23% seroma rate.[151] In a different study, Glubran® 2 was used to tack mesh to tissue during hernia repair. The polymerized adhesive caused the tissue to become very stiff with a 0.5 mm elastic deformation after 3 months as compared to a normal elastic deformation of 4000 mm. Overall, the adhesive treatment resulted in severe inflammation, polymerized glue appearing as sharp edged irregular structures, inhibited tissue in-growth, and significantly reduced elastic deformation and elasticity of mesh and abdominal wall.[152]

Butyl cyanoacrylate has also been tested as a sealant. In one study, researchers tested Histoacryl® as a treatment method for gastric variceal bleeding and esophageal varices. They compared the Histoacryl® results to a typical treatment technique, band ligation. Histoacryl® was shown to rapidly polymerize and plug the lumen when injected into varices. It produced initial hemostasis for 87% of patients and obliteration of gastric varices for 51% of patients. Adverse results for patients included 31% rebleeding rate and 29% mortality rate. In comparison, band ligation resulted in the strangulation and necrosis of varices. It produced initial hemostasis for 45% of patients and obliteration of gastric varices for 45% of patients. Adverse results for patients included 54% rebleeding

rate and 48% mortality rate.[153] Other than band ligation, other bleeding gastric varices treatments currently employed include shunts, banding, sclerosis with sodium tetradecyl sulfate, sclerosis with endoclip, Sengstaken-Blakemore tube, and β-blockers only. In one study, these treatment methods were compared to treatments with Histoacryl®. Of the 11 non-cyanoacrylate treated patients, 45% had a 3 month survival rate and 40% had a one year survival rate. In comparison, of the 17 Histoacryl® treated patients, 88% had a 3 month survival rate and 82% had a one year survival rate.[154] Histoacryl® thus was shown as a potential improvement over non-cyanoacryate methods for treating bleeding gastric varices. A separate study demonstrated that butyl cyanoacrylate treatment as a hemostat for chronic gastric ulcers with active bleeding can result in successful hemostasis for one patient and intra-abdominal arterial embolization and resulting death for a second patient.[155] In a similar study, 60 patients with major peptic ulcer hemorrhage were treated with Histoacryl® injection. Although there was initial hemostasis for 95% of the patients and rebleeding for only 12% of the patients, two of the patients had an arterial embolization with infarction with one patient dying.[156] In another study, 29 patients with bleeding gastric varices were treated with butyl cyanoacrylate. Once again, the initial hemostasis and re-bleeding rates were promising (93% and 25% respectively); however, the mortality rate was 38% and complications rate was 46%.[157] In another case study, isobutyl cyanoacrylate was used as an endoscopic obturation method for esophageal varices, which caused hemiplegia, polymerized adhesive in cerebral arteries, and death.[158] Similarly, when a different patient had Histoacryl® injections for bleeding gastric varices, the results were a tumorous gastric

varix with ulceration and spleen infarctions.[159] In a different case, a patient presented with a right ganglionic parenchymal hemorrhage due to a ruptured lenticulostriate artery aneurysm associated with ipsilateral middle cerebral artery occlusion. For this case, the aneurysm and feeding artery were occluded with endovascular injection of n-butyl cyanoacrylate thereby obliterating the aneurysm with no adverse reactions reported.[160] In another case, Trufill® was tested for treating cerebral arteriovenous malformations (AVM). Trufill® is an n-butyl cyanoacrylate liquid embolic system indicated for the embolization of cerebral arteriovenous malformations (AVMs) when pre-surgical devascularization is desired.[161] Polyvinyl alcohol (PVA) sponges, a current treatment method, was also tested in comparison. The Trufill® and PVA treatment methods had a similar success rate indicated by a full AVM embolization (80% for Trufill® and 87% for PVA). Adverse events from the treatment methods included parenchymal hemorrhage (6% Trufill® and 12% PVA), pulmonary embolism (0% for Trufill® and 2% for PVA), hemorrhagic complications (13% for Trufill® and 29% for PVA), and death (2% for Trufill® and 6% for PVA).[162]

In the orthopedic field, butyl cyanoacrylate has also been explored as a treatment option for fixation as an adjunct or primary method of bone, cartilage, tendons, and deep tissue repair. In one study, Histoacryl® was applied to connect a Lactosorb® biodegradable plate to bony fragments. Upon follow-up, a bony union was observed between the bone fragments.[163] Similarly, butyl cyanoacrylate treatment was shown to result in complete healing within 6 months of an osteochondral fracture of the patella and the medial femoral condyle.[164] In addition, when isobutyl cyanoacrylate was applied

around the fracture surface of the knees for 16 dogs, 81% of the fractures re-united. Furthermore, the bone healed around the sites of polymerized adhesive, and the monomer appeared non-toxic to the adjacent bone.[165] In a different study, however, when Glubran® 2 was applied for mandibular repair using onlay grafting procedures, there were no bony bridges observed between the fractured bones and grafts at four and 12 weeks. Instead, there was total graft necrosis observed for two patients after four weeks and three patients after 12 weeks.[166] When butyl cyanoacrylate was used in a separate study to secure rabbit auricular cartilage autografts, it was shown to result in viable tissue after two weeks and one year.[167] Similarly, when Histoacryl® was applied to the stapes region of the middle ears of nine baboons there was no damage or injury to the stapes, footplate, labyrinth, or middle ear with only minor inflammatory reactions observed.[168] Indermil® was also tested for ear repair. Specifically, it was used to repair the external surface of the tympanic membrane for 33 patients with all skin incisions healing by primary intention.[169] In a separate study, however, when Histoacryl® was applied adjacent to well-vascularized soft tissue with no graft in one rabbit ear it resulted in increased acute inflammation and a prolonged foreign-body giant-cell response.[170] In a different study, isobutyl cyanoacrylate was compared to silk sutures for tendon repair. Although the cyanoacrylate had a weaker tensile strength (9 N) than the silk sutures (23 N), when the cyanoacrylate was applied over the silk sutures, the combination resulted in a greater tensile strength (40 N) than the two singly.[171] In a different study, Indermil® was used to close perineal skin following episiotomy or second degree tears at vaginal delivery in 20 patients, with three of the patients reporting

a burning sensation as the only adverse event.[172] In a separate study, Indermil® was used to close full thickness, deep tissue back defects after first allowing hemostasis to occur. At a two week check-up, the wounds approximated with adhesive were more rigid than similar wounds closed with sutures; however, the two treatment types appeared to result in equivalent wound repair at a 4 week check-up. Also at the two week check-up, one of the adhesive treated wounds had partial dehiscence unlike the sutures treatment group with no dehiscence.[173]

Other than bone and ears, butyl cyanoacrylate has been used for other internal applications such as oral surgery and liver repair. When it was applied orally for one study, it resulted in complete hemostasis.[174] In a separate study, Indermil® was applied during oral surgery for 10 rats. The researchers noted no significant differences between controls (blood collected from the rats prior to surgery) and the Indermil® treated rats 2, 14, 21, 65 days post-surgery for liver and kidney functions based on blood screening. There were also no pathological changes.[175] In a different case with 50 patients, n-butyl cyanoacrylate was used to fix the left lateral lobe of the liver to the diaphragm during upper abdominal procedures with no complications at a one month check-up.[121]

### 1.4.5. Octyl and 2-octyl cyanoacrylate external use

2-Octyl cyanoacrylate (2-OCA) marketed as Dermabond® is the gold standard for cyanoacrylate wound care due to its larger side chain resulting in a more flexible material with a slower degradation rate and thus formaldehyde release rate. This protracted

degradation time is preferred due to the decreased formaldehyde release; however, during wound healing, undegraded polymer fragments can impede proliferation and thus overall wound healing. The fragments decrease the surface area available for collagen to bridge wound edges to heal and strengthen wounded tissue.[118] There have also been some medical uses of n-octyl cyanoacrylate (n-OCA). When correctly applied, 2-OCA and n-OCA effectively approximate most wounds. Correct application includes keeping the wound approximated for the entire setting time duration of the adhesive, which would otherwise result in immediate wound dehiscence.[176] Other than Dermabond®, 2-OCA is also marketed for use in the USA as SecureSeal™, Surgiseal®, and Exofin®. In 1998, Dermabond®, a product of Ethicon®, Inc., became the first cyanoacrylate tissue adhesive approved by the FDA for USA marketing.[117] Dermabond®, like most cyanoacrylates, is contraindicated as the sole treatment for deep dermal wound approximation, and contraindicated for tissue that experiences high tension such as joints. It is also contraindicated for wet wounds (water, blood, or other fluid) because the adhesive will typically cure to the moisture present and not the tissue itself. For this reason, medical personnel must temporarily approximate the wound, typically by pressure or clamps, and allow hemostasis to occur before Dermabond® is applied. Currently, Dermabond® is indicated for topical use only because the body will not absorb the polymerized adhesive, and the polymerized adhesive can elicit a foreign body reaction. Lastly, Dermabond® is a one time use product unit because the liquid adhesive mixes with an accelerator in the applicator tip for the unit as the adhesive is applied, so any remaining adhesive in the unit will cure thereby preventing future uses of the unit.[122], [123],[176] Once applied,

Dermabond® will cure to hold the wound approximated, and then slough off in 5-10 days.[177] It is important that the polymerized adhesive slough off within this time period because in at least one case, failure of the polymerized adhesive to slough off in 14 days resulted in an infection.[176]

In one study comparing the effectiveness of 2-OCA to fibrin glue when applied for urinary tract wound closure, 2-OCA showed no leakage and fibrin glue showed 50% leakage 28 days post-application. 2-OCA did, however, demonstrate significantly greater inflammation than the fibrin sealant.[178] In a study where 2-OCA was used for head and neck surgery, of 52 patients treated, there were no wound dehiscence events reported, and there was only a 4% rate of minor wound complication.[179] In one study where 2-OCA was used for pediatric neurosurgery wound closure, 3% of the patients treated had cerebrospinal fluid leakage indicating at least partial wound dehiscence.[180] In another neurosurgery study, 2-OCA as Dermabond® or SecureSeal™ was used to close wounds for 365 patients after posterior spinal surgery. This wound closure method resulted in a 0.8% cerebrospinal fluid leakage rate and 1.4% wound dehiscence rate.[181] When a group of researchers pooled the results from several journal articles regarding the use of Dermabond®, they calculated that Dermabond® has a 0.9% wound dehiscence rate.[182] In another study, Dermabond® was used to close wounds after circumcision, with a 1% rate of re-bleeding.[183] In comparison, Surgiseal® was used in a study to close 154 incisions with a 3% rate of wound dehiscence and a 3% rate of re-bleeding.[177] 2-OCA has also been shown to provide an antimicrobial effect. Specifically, in one study, 2-OCA was used for skin closure of sternal incisions in cardiac surgery. The wounds closed with

2-OCA had an infection rate of 2%, while the wounds closed without 2-OCA had an infection rate of 5%.[184] When Dermabond® or sutures were used to close intentionally infected wounds, there was a 20% infection rate remaining after 5 days for the Dermabond® treated tissues, while there was a 65% infection rate remaining after 5 days for the sutures treatment group.[185] In another study, deep and topical sutures only or deep sutures and topical 2-OCA were used as treatment methods for cardiac device implantation wound closure. A bacterial skin infection developed for 0.9% of the sutures only group, while there was a 0% infection rate for the sutures/2-OCA group. In addition, one patient from each group (0.3% sutures and 0.8% sutures/2-OCA) required device removal due to internal infection.[186]

In general, 2-OCA has a higher wound burst strength than most other cyanoacrylates because of its increased flexibility due to its longer side chain as previously discussed. One study compared the wound bursting strength of Dermabond® (2-OCA), Histoacryl® (n-butyl), and surgical tape. For this study, each product was first applied to approximate incised tissue. Next, vacuum pressure was applied to the approximated tissue at increasingly greater levels. The maximum pressure before the approximated tissue re-opened was measured. This study demonstrated that Dermabond® has a greater wound bursting strength than Histoacryl® (298±58 mm Hg vs. 199±87 mm Hg). Both of the cyanoacrylates tested also had a greater wound burst strength than the tape (129±67 mm Hg). The failure modes of each of these treatment types also varied. Specifically, the tapes and Histoacryl® experienced adhesive failure (peel/tear off tissue), while 56% of the Dermabond® samples experienced cohesive failure (tearing apart of

polymerized adhesive film).[187] In two separate studies measuring the maximum intra-abdominal pressures generated during normal daily activities, coughing and jumping were determined to produce the second (150 mm Hg) and first (252 mm Hg) greatest pressures respectively. It is thus important to note that of the three treatment types tested, Histoacryl® and Dermabond® would likely withstand coughing, but only Dermabond® would likely withstand jumping.[188], [189]

In a study comparing 2-OCA and sutures for laparoscopic port-site wound closure, the sutures had an 8% rate of dehiscence, while 2-OCA only had a 2% rate of dehiscence.[190] 2-OCA and sutures were also compared in a separate study comparing their ability to close wounds from laparoscopic surgery. In the study, 2-OCA had a 4% wound dehiscence rate, while sutures had a 0% wound dehiscence rate based on the reported results. In addition, both closure methods had a similar rate of seroma formation (2.5% for 2-OCA and 1.8% for sutures). This wound complication is nearly as problematic as wound dehiscence because the treatment for seromas includes re-opening the wound, draining it, and then allowing it to heal by secondary intention.[191] In a different study where PDO sutures or Dermabond® was used to close 99 laparoscopic cholecystectomy wounds (approximately 50 wound per treatment type), there were no complications reported for both treatment types.[192] A different study also produced similar results when Dermabond® and sutures or staples were used to close approximately 40 incisions each, and there was no wound dehiscence reported for either group.[193] In another study, Dermabond® or sutures were used to close wounds from breast surgery. Of the 69 Dermabond® patients and 64 sutures patients, there was no

wound dehiscence, hematoma, or infection reported. The researchers did however report that sutures produced an initial increased inflammatory reaction at the wound site (less tissue reaction for Dermabond®), but this inflammation was not observed a later follow up.[194] In a different study where Dermabond® (19 wounds) or suture (26 wounds) were used as treatment methods for facial wounds, there was no wound dehiscence or infection reported during the first two weeks after surgery.[195] In a study where skin was closed after coronary artery bypass grafting (saphenous vein harvesting), 11% suture and 9% Dermabond® treated patients showed signs of inflammation, hematoma, or exudation.[196] When Dermabond® or sutures were used to close simple lacerations, 1% of Dermabond® treated patients and 6% of sutures treated patients had wound erythema or swelling. In addition, 1% of each treatment type had minor wound dehiscence.[197] In a large study comprising 455 Dermabond® treated wounds and 469 sutures treated wounds, there was a 1.6% wound dehiscence rate for Dermabond® and 0.9% for sutures, and there was an 18.5% Dermabond® wound erythema rate and 36.4% for sutures.[198] In a smaller study comprising 106 2-OCA treated wounds and 103 sutures treated wounds, there was a 10% rate of erythema for 2-OCA and 13% for sutures.[199] In a study where Dermabond® or Monocryl® sutures were used to close inguinal hernia repair incisions, Dermabond® closed wounds had a 17% rate of wound dehiscence, while Monocryl® treated wounds had a 0% wound dehiscence rate.[200] In a separate study, Dermabond® was used to close wounds during mammoplasties with a wound dehiscence rate of 2% and a total minor wound complication rate of 14%. In a similar study using sutures, the wounds closed with sutures had a 20% rate of total minor wound

complications.[201] In regards to cosmetic appearance as the wound heals, one study showed that wounds treated with Monocryl® had a 67% scarring rate after 8 weeks, but only a 20% scarring rate after 1.8-2.7 years. In comparison, Dermabond® had a 40% scarring rate after both 8 weeks and 1.8-2.7 years.[202] In another study comparing 2-OCA and sutures, the researchers demonstrated that wounds treated with 2-OCA and wounds treated with sutures have a similar cosmetic appearances three months later.[203] A separate study comparing Dermabond® and sutures, demonstrated that 5-7 days after wound treatment, sutures treated wounds had increased inflammation and erythema, while Dermabond® treated wounds did not. Nevertheless, at a 90 day check up, both treatment types had no evidence of healing abnormalities. In addition, at both check up time points, neither treatment type resulted in wound dehiscence.[204] When Dermabond® or conventional head dressings was used to treat 17-20 patients each after ear correction surgery in a different study, the conventional head dressing resulted in a 30% wound complication rate, while Dermabond® resulted in a 9% wound complication rate.[205]

### 1.4.6. Octyl and 2-octyl cyanoacrylate internal use

As previously discussed, cyanoacrylates have been used both externally and internally. Other than the previously discussed external uses, 2-OCA has also been used internally with at least one product, Omnex®, already having been approved for marketing in the USA by the FDA.[117] Omnex® is a blend of 2-OCA and butyl lactoyl cyanoacrylate. Once polymerized, Omnex® is reported to degrade by hydrolytic chain

scission over approximately 2.5 years. This product is indicated for use in as an adjunctive for vascular anastomosis for achieving hemostasis by sealing areas of leakage around the vessel, but not inside the vessel. As an adjunctive, the product is not made to be used alone, but rather with other devices such as sutures or staples. Similar to other cyanoacrylates including external uses, the application site must be dry to avoid curing the adhesive on non-tissue areas such as fluid. Although the adhesive is reported to be degradable, in one 2 year study in rats, there was no significant degradation observed. There was also no adverse local reactions, systemic toxicity, or evidence of carcinogenicity. In a large clinical study of 151 patients containing 101 Omnex® treated patients and 50 oxidized regenerated cellulose (control), the two treatment types had similar complication rates for various morbidities. In this study, there was a 5% rate of dehiscence for Omnex® and 0% for the control. In addition, there was a 5% thrombosis rate for Omnex® and 6% for the control. Overall, thrombosis and thromboembolism are two potential adverse reactions for Omnex®. It is also important for Omnex® not to cure within the vessel because it can delay wound healing and even result in a local embolic vascular obstruction.[206]

Additionally, there have been some off-label internal uses of n-OCA & 2-OCA. In one study, auricular cartilage grafts were glued together using n-OCA or sutured together. The study resulted in no histological or graft migration difference between the two treatment types.[207] One of the more frequently off-label uses of 2-OCA is for cornea repair. In one study, 2-OCA was used to approximate a perforated cornea. The cornea healed without scarring, vascularization, or thinning. In addition, the polymerized

adhesive fell out after 6 weeks.[208] In another study, octyl cyanoacrylate or ethyl cyanoacrylate was used to repair corneal lesions. The octyl cyanoacrylate resulted in slow reepithelization and collagen organization with a discrete inflammatory reaction in the initial phases. When ethyl cyanoacrylate was applied, the result was a moderate inflammatory reaction in the initial phases.[137] When 2-OCA was used to close nephrostomy tubes removal sites in one study, the urinary leakage ceased immediately, and there were no urinary tract or wound infections. One of the 25 patients, however, developed renal pain due to swelling. In comparison, the control group that received only wound dressing had a 10% rate of urinary infections and urinary leakage lasting over 24 hours for 20% of patients.[209] In another study, the auricular vein of eight rabbits was injected with Dermabond® or Histoacryl® (eight ears per treatment type). After four hours, minimal inflammation was seen for Dermabond®, but none for Histoacryl®. After 24 hours, tissue necrosis was seen for only Dermabond®. After one week, tissue necrosis was seen for both Dermabond® and Histoacryl®. Furthermore, as an initial test, after injecting each treatment type, the auricular veins were flushed with saline, which resulted in Dermabond® becoming free (loss of adherence). During the study, one animal died due to a thromboembolic event with thrombi observed in the pulmonary vessels, which was attributed to Dermabond® based on its ability to be readily flushed. The researchers also recommended that the study be repeated with increased amounts of Dermabond® to determine whether the adhesive could be easily flushed because there was an insufficient amount injected to fully adhere in place.[210] In another study that included 2-OCA injections, 25 patients were treated with 2-OCA to fill large gastric fundal varices. There

was a 100% success rate for immediate control of the variceal bleeding post-injection of 2-OCA. Re-bleeding occurred for 4% of the patients. Some of the patients (12%) also died prior to the check up during the study.[211] When 2-OCA was applied to a fractured tooth in another case study, there was an initial warmth and a mild burning sensation reported during the 15-20 seconds post-injection of the 2-OCA. At a check up two days later, the tooth was found intact with no recurrent pain; however, the oral surgeon still had to remove and replace the tooth due to the damage around the tooth after the tooth's fracture.[212]

### 1.4.7. Uncommon cyanoacrylate types by chemical synthesis and/or blending

Other than these common types of cyanoacrylates, researchers have explored alternative cyanoacrylates. Some researchers have synthesized new cyanoacrylate types by attaching additional functional groups to the side chain of common cyanoacrylates. In one study, researchers attached an ethoxy group to the ethyl side chain of ethyl cyanoacrylate to form ethoxyethyl cyanoacrylate.[124] In a similar study, researchers attached methoxy, ethoxy, propoxy, butoxy, and hexoxy to ethyl cyanoacrylate to form novel cyanoacrylates with a variable alkoxy-ethyl side chain length.[213] These two sets of researchers determined several property relationships for varying alkane and alkoxy side chain length for cyanoacrylates. They showed an inverse relationship between alkane and alkoxy side chain length versus polycyanoacrylate tensile bonding strength, polymerization rate and peak temperature, and glass transition temperature. They also showed a direct relationship between alkane and alkoxy side chain length versus

polycyanoacrylate molecular weight and cytotoxicity cell viability. Formaldehyde release was also shown to increase as the alkoxy side chain length was increased, and decrease as the alkane side chain length was decreased. Overall, these studies show how varying the alkane and alkoxy side chain length and thus the steric hindrance and ease of rotation for the overall polycyanoacrylate affects many of the properties for the polymerized adhesive.

Other researchers have functionalized the cyanoacrylate itself such as in one study that included the addition of hydrophilic polymers to the adhesive as cross-linking materials.[214] Specifically, crosslinking agent poly(ethylene glycol)-dicyanoacrylate (PEG-DCA) was added to butyl cyanoacrylate (BCA). Researchers showed that the degradation of the resulting polymerized adhesive could be modulated by increasing the amount of BCA (and thus steric hindrance) to decrease the degradation, and increasing either the PEG amount or molecular weight (and thus hydrophilicity) to increase the degradation. Degradation was found to occur at the ester group in the BCA side chain (polycyanoacrylate side chain scission degradation) and at the known hydrophilic PEG group. When the adhesive was formulated with PEG 20000 Da molecular weight, the polymerized adhesive fully hydrolyzed at 28 days *in vitro* and three months *in vivo*. In comparison, 100% BCA had only a 26% mass loss after 28 days in vitro and was still present after 15 months in vivo. For the *in vivo* degradation, 70.1% of the hydrolyzed PEG adhesive was shown to be excreted in urine and feces as compared to only 20.2% for the degraded 100% BCA. Lastly, the PEG adhesive was shown to have similar

cytotoxicity cell inhibition results and mechanical properties including burst strength as compared to the 100% BCA.

Other than changing the properties of cyanoacrylates through their synthesis, several studies have shown that blending additives into the cyanoacrylate can also affect the resulting adhesive formulation's properties. In one study, poly(lactide-co-caprolactone) (PLCL) was mixed into ethyl cyanoacrylate and allyl cyanoacrylate.[215] This study showed the existence of a direct relationship between PLCL weight percent in cyanoacrylate and porcine skin bond strength for the adhesive formulation. This relationship was potentially due to the increased viscosity of the formulation resulting in more of the adhesive staying at the application site when it was applied to the porcine skin for the bond strength testing. In addition, this study showed a direct relationship between caprolactone amount in the PLCL dissolved into the cyanoacrylate and the flexibility of the polymerized adhesive. In another study, when acrylic compounds were mixed into ethyl cyanoacrylate, the acrylic compounds were shown to partially inhibit the polymerization of the adhesive.[216], [217] When compared to silk sutures, there was no significant difference reported in wound healing at seven and ten days after wound approximation following hernia repair. The sutures were shown, however, to result in less wound tension. This result is likely due to the known typical increased flexibility of thin filament sutures as compared to a polymerized adhesive film layer. Lastly, in a different study, PLLA dissolved in chloroform was mixed into partially pre-polymerized allyl 2-cyanoacrylate (PACA).[218] There was an inverse relationship identified between PLLA mass mixed with the PACA and bond strength. This result was likely due to the decrease

in adhesive in the mixture as more PLLA was added. The prepared mixtures were fully polymerized, and then analyzed by FT-IR, which showed no shifts of peaks comparing PACA to the polymerized PACA/PLLA mixture; thus, the PLLA blended into the PACA, and did not combine with the PACA polymer chain.

As similar work, Dr. Shalaby W. Shalaby and Dr. Charles L. Linden, Jr. modified a cyanoacrylate by adding a methoxypropyl side chain to the cyanoacrylate during its synthesis, and blending absorbable polymeric oxalates into the adhesive.[219], [220] The resulting adhesive was shown to have a higher adhesive strength than isobutyl cyanoacrylate when approximating soft tissue. When the adhesive was implanted subcutaneously, it was shown to completely degrade in less than 568 days. Based on analysis, it was primarily excreted in urine. This testing also demonstrated a similar level of cytotoxicity as compared to isobutyl cyanoacrylate for the first 90 days post-implantation.

### 1.4.8. Additives for cyanoacrylates and their effects

The materials added to cyanoacrylates can thus affect the properties of the typical (e.g. ethyl cyanoacrylate) and atypical (e.g. methoxypropyl cyanoacrylate) synthesized cyanoacrylates. The additives themselves as insoluble or soluble materials can be varied with differing results once they are mixed with the cyanoacrylate. Absorbable polyesters lend themselves well to this application due to their ability to mix with cyanoacrylates without polymerizing them as shown in the methoxypropyl work above. Absorbable polyesters are also well known biocompatible materials due their ability to hydrolyze into

natural byproducts such as lactic acid and glycolic acid depending on the polyester type due to the ester functional group they contain. Similar to how the properties of cyanoacrylates can be varied by differing their side chain, the properties of polyesters can be varied by differing their monomers and synthesis methods. For example, when D,L-lactide is synthesized, it produces a fast degrading amorphous polymer as compared to the slow degrading, crystalline polymer produced when L-lactide is synthesized.[221] These outcomes occur because the increased complexity of D,L-lactide as compared to L-lactide results in the decreased ability for D,L-lactide to form crystalline structures. Similarly, using a branched (complex) or linear (simple) initiator during polymer synthesis has been shown to result in a polymer with decreased or increased crystallinity respectively.[222] In addition, varying the ratio of ε-caprolactone and glycolide in a poly(glycolide-co-caprolactone) copolymer (PGCL) has been shown to result in a varied degradation for the copolymer. Specifically, increasing the amount of glycolide increased the degradation rate, while increasing the amount of ε-caprolactone decreased the degradation rate.[223] These outcomes occur because ε-caprolactone has an increased steric hindrance as compared to glycolide. For the PGCL, the pH of the eluent resulting from the degradation would be expected to be more acidic for high glycolide amounts (fast degrading) and less acidic for high ε-caprolactone amounts (slow degrading). The PGCL hydrolysis mechanism is summarized in Figure 1-5. The increased steric hindrance for polycaprolactone also results in a more amorphous, elastic material. In comparison, the decreased steric hindrance for polyglycolide results in a more crystalline and stiff material.[224]–[226]

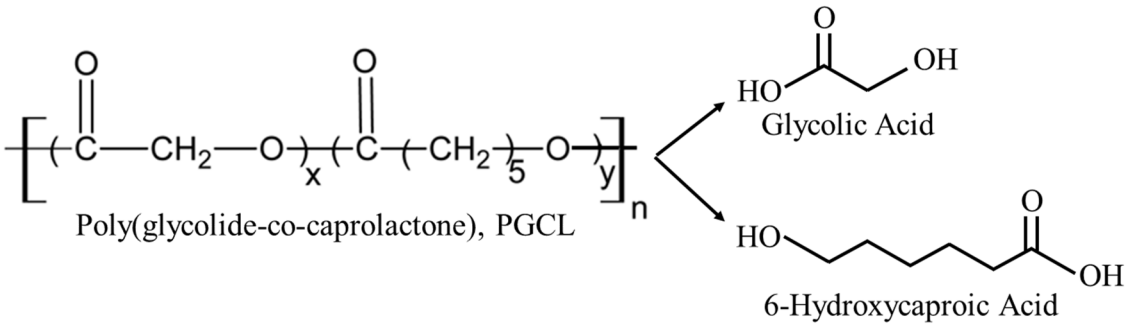



Figure 1-5: PGCL Hydrolysis

## 1.5. Conclusion, introduction to research, and hypotheses

Based on this presented review, there is a current need for wound approximation both externally and internally. The several different approaches to accomplish this goal that were discussed are lacking in one or more properties to achieve ideal wound healing. Tissue welding and cauterization are two methods employed; however, these methods result in the formation of necrotic tissue, which is undesirable. Sutures are the wound approximation gold standard due to their flexibility and ability to resist tensile forces, but they can result in complications such as bleeding from the holes created during the suturing application required to place them. Staples and tapes are common wound approximation devices, but unlike sutures, they lack the ability to resist large tensile forces. In addition to these mechanical closure devices, fibrin and thrombin based sealants have been employed to approximate or seal tissues. These biological sealants are very biocompatibility, but they also have a low mechanical strength especially as compared to the mechanical closure devices. This low strength has resulted in re-bleeding when this type of sealant was applied externally and also in cases when it was applied internally. In comparison, BioGlue®, a non-biological sealant has a high mechanical

strength, but is limited by its poor biocompatibility. Alternatively, there have been recent advancements based on gecko and mussel adhesions (bioadhesion) in order to fabricate synthetic materials that mimic these naturally occurring dry and wet adhesives respectively. Studies have demonstrated that these materials have a great potential, but they still require additional research in order to render them clinically relevant for wound approximation.

Cyanoacrylate adhesives is another family of wound approximation and sealant devices. As a general overview, these materials are able to penetrate into tissue due to their liquid monomer form, rapidly polymerize due to their highly electrophilic nature, and then form bonds due to the interpenetrating networks formed. They have been fabricated in many varieties by differing the side chain for the adhesive monomer during its synthesis, blending additives into the adhesive, or mixing insoluble materials into the adhesive. These variations have been demonstrated in a myriad of studies to control the properties of the adhesive in its monomer and polymer forms. Several of these properties include viscosity, mechanical strength and flexibility, polymerization rate and reaction temperature, degradation rate, and biocompatibility. By controlling the side chain type and materials added to it, researchers are able to tailor cyanoacrylates for specific external and internal medical and industrial uses. These adhesives are well known for their typically successful external medical uses and industrial uses; however, their internal medical use has been slow to reach global use due to the heat released during the adhesives polymerization, and the cytotoxic formaldehyde byproduct released as the polymerized adhesive degrades. In order to overcome this issue, researchers commonly

synthesize cyanoacrylates for internal use by attaching long alkane side chains (e.g. 2-octyl) to them. The resulting cyanoacrylate releases a lower amount of heat during its polymerization and minimal formaldehyde as it degrades; however, this degradation has been demonstrated in several studies to take years, if it degrades at all, which can result in a prolonged, chronic wound healing. Other complications resulting from the external or internal use of cyanoacrylates including off-label uses includes wound dehiscence resulting in re-bleeding, infarction due to embolization, excessive heat during polymerization causing burns, inhibition of tissue growth, reduced tissue elasticity, and seromas. Nevertheless, this material has excellent clinical usefulness when the specific cyanoacrylate types are used for their specific intended uses. There is therefore great potential to modulate the adhesive to improve its clinical usefulness.

Based on this literature information, this dissertation research focused on using this information to formulate cyanoacrylates with improved clinical usefulness. The cyanoacrylates were improved through the addition of novel chemically active polyesters (rheological modifiers). Methoxypropyl cyanoacrylate was selected for this research due to its inclusion of a short alkoxy side chain resulting in a flexible, high strength bond as well as the adhesive's proven biocompatibility. Poly(glycolide-co-caprolactone) polymers were synthesized as the polyesters for this research due to the fast degrading, low pH producing ability of glycolide and slow degrading, increased flexibility of ε-caprolactone. The combination of both fast and slow degrading monomers allows one specifically to control the polyester's degradation rate and thus resulting pH level for the

eluent. The dissertation research attempts to prove the following main hypotheses in Table 1-3 for the research formulations containing the novel polyesters.

Table 1-3: Dissertation Hypotheses by Chapter

| Chapter Number | Hypothesis |
|---|---|
| Chapter 2 | 1. There is a direct relationship between adhesive modifier weight percent versus adhesive viscosity. |
| Chapter 3 | 2. There is an inverse relationship between adhesive modifier weight percent versus adhesive peak temperature change, reaction rate, and estimated heat of polymerization. |
| Chapter 4 | 3. There is a direct relationship between adhesive modifier weight percent versus polymerized adhesive flexibility. |
| | 4. There is a direct relationship between adhesive modifier ε-caprolactone molar percent versus polymerized adhesive flexibility. |
| Chapter 5 | 5. There is a direct relationship between adhesive modifier weight percent versus polymerized adhesive degradation rate. |
| | 6. There is a direct relationship between adhesive modifier glycolide molar percent versus polymerized adhesive degradation rate. |
| | 7. There is an inverse relationship between adhesive modifier weight percent versus polymerized adhesive eluent pH, formaldehyde release amount, and cytotoxicity. |
| | 8. There is an inverse relationship between adhesive modifier glycolide molar percent versus polymerized adhesive eluent pH, formaldehyde release amount, and cytotoxicity. |

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# CHAPTER 2

# POLYMERIC SYNTHESIS AND ADHESIVE FORMULATION

## 2.1. Introduction

The properties of cyanoacrylate adhesives can be modified through the addition of other components including soluble and insoluble inorganic and organic materials (e.g. polymeric adhesive rheological modifiers). For example, the addition of insoluble silica or crystalline polymer microparticles such as polyglycolide can increase the viscosity of the adhesive effectively turning the liquid adhesive into an adhesive paste.[1], [2] In addition, dissolving polymers with a glass transition temperature ($T_{g)}$ below room temperature (18-23°C) and body temperature (37°C) such as polycaprolactone ($T_g$= -60°C) into cyanoacrylates can increase the flexibility of the polymerized adhesive film once it is formed because the dissolved polymer itself is in a rubbery state.[3]–[11] When mixed into cyanoacrylate, adhesive modifiers can also increase the bond strength for the adhesive formulation.[11] This relationship was potentially due to the adhesive modifier acting as a rheological modifier by increasing the viscosity of the cyanoacrylate resulting in more of the adhesive staying at the application site when it was applied during bond strength testing.

Adhesive modifiers can either be obtained commercially or custom synthesized for usage. The synthesis method varies depending on the material to be generated. Many bioresorbable polymers are made from the chain growth ring opening polymerization of ring-structure monomers such as l-lactide, glycolide, ε-caprolactone, and trimethylene carbonate. These monomers are initiated by a nucleophile such as a hydroxyl group from

an alcohol donating an electron pair to a monomer, and thereby breaking the monomer's carbonyl double bond. The resulting initiated, anionic monomer will further initiate other monomer groups (propagation) to form a polymer chain. The polymerization is terminated when the monomer has been exhausted, or another material reacts with the propagating chains to bond with the chain ends thereby terminating their growth.[12], [13],[14] This polymerization can occur for one monomer or a batch of several monomers. Random co-polymerization occurs when several monomers are mixed together and allowed to polymerize. Alternatively, polymerizing one monomer, and then adding a second monomer and allowing it to polymerize will result in a block or graft bioresorbable co-polymer.

There are several key attributes of bioresorbable polymers including appearance, molecular weight, moisture content, melting point, crystallinity, and composition with monomer content. The polymer can be colored through oxidation or the addition of a dye during the polymerization. Color can be an aesthetic design feature or an indicator of an undesired status for the polymer. For example, a dark colored polymer with a light colored core can indicate an incomplete polymerization. The polymer can also be a liquid, gel, or a solid. Each polymer also has a specific size and shape ranging from nanoparticles to a solid block. These physical states and sizes affect the ability for the polymer to be utilized for specific applications. A polymer's molecular weight affects the ability for the polymer to hydrolyze and to be further processed. For example, a high molecular weight polymer can be more readily processed, but it will typically have a longer hydrolysis time. Moisture content not only affects a polymer's processing ability,

but also affects its stability. A polymer with a high moisture content will typically degrade when exposed to high temperatures during specific tasks such as melt extrusion. High moisture content can also cause the polymer to degrade pre-maturely. A polymer's melting point and crystallinity affect the polymer's processing capability. For example, polymers with a low melting point and low crystallinity (amorphous state) are sufficient for blending into liquids, but insufficient for high temperature melt extrusions. Lastly, the composition for a polymer is a critical attribute. Although a polymer is synthesized with known charges of monomers, initiators, catalysts, and other desired materials, the theoretical composition does not always match the actual composition. Sources of variation include non-homogenous mixing during synthesis, incomplete polymer synthesis or purification, or degradation for the charge compounds prior to their usage or during the synthesis.

Select key attributes for bioresorbable polymers can be modified by the polymerization initiator used for their synthesis. This compound can control the polymer's molecular weight, chain structure, and crystallinity. Specifically, increasing the amount of initiator added during polymer synthesis will typically decrease the molecular weight of the resulting polymer. Polymers with decreased molecular weight can be useful because they require less chain disentanglement to dissolve into solvents.[15]

Other than molecular weight, the chemical structure of initiators can also affect the polymer's chain structure. When used to synthesize a polymer, linear initiators (e.g. 1,3 propane diol) will result in a polymer with one axis, while branched initiators (e.g.

trimethylolpropane) will result in a polymer with multiple axes. Figure 2-1 illustrates example polymers with single and multiple axes.

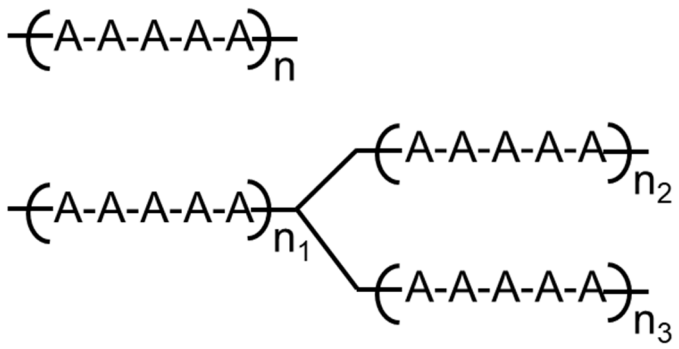


Figure 2-1: Polymer of Monomer "A" Example with Single Linear Axis (Top) and Three Branched Axes (Bottom)

A polymer synthesized to have a single axis using a linear initiator will typically result in a single long polymer chain. If a polymer is synthesized with multiple axes (branching), the resulting polymer will typically have multiple polymer chains.

In addition to molecular weight and chain structure, initiators can also affect the crystallinity for polymers. Linear initiators are more likely to result in a crystalline polymer, while branched initiators are more likely to result in an amorphous polymer.[16] Branched initiators typically form amorphous polymers during polymerization due to the increased polymer chain complexity resulting in a decreased packing efficiency for the propagating polymer chains.[14]

Solid state polymerization of bioresorbable polymers is typically performed under low moisture, dry conditions, at elevated temperatures, with a catalyst also added. Moisture can initiate the polymerization, so a dry environment will decrease undesired side reactions; thus, typically increasing the molecular weight of the resulting

polymer.[14] There are several methods to maintain a dry environment during polymer synthesis. Namely, 1) using a desiccant chamber or other dehumidifier to continuously remove moisture from the area, 2) using a vacuum chamber, and 3) using an inert, dry gas (nitrogen or argon preferred) rich environment instead of air that contains moisture. Elevated temperatures and a catalyst are useful to not only hasten a reaction, but also drive it closer to full polymerization; all monomer consumed. Elevated temperatures are also necessary in order to melt the monomers, so that the monomer molecules can move and combine to form the polymer.

Following polymerization of a bioresorbable polymer, it can be useful to perform extractions or purifications to reduce any monomer amount remaining in the polymer. Bioresorbable polymers are formed from acidic monomers or cyclic ring monomers that degrade into acidic compounds. A large concentration of monomer can thus shift the pH below the homeostatic condition of 7.4 pH, which can reduce cellular activity when not properly controlled. One method to purify a polymer includes dissolving the polymer in a solvent such as dichloromethane, precipitating out the polymer in cold isopropanol, and then vacuum drying the precipitate to remove all solvent from it. Distillation can also be performed for polymers; however, the high temperatures that can be required for distillation can degrade the polymer. Monomer can alternatively be extracted by placing a polymer into a non-solvent such as acetone or ethyl acetate. Following extraction, the polymer is typically vacuum dried to remove all remaining non-solvent from it.

Once the polymers are formed and processed as desired, they can be mixed into the cyanoacrylate adhesive to fabricate adhesive formulations. Certain precautions must

be taken during the mixing to ensure the adhesive does not polymerize during the mixing process. Similar to the polymerization, the mixing is performed under low moisture conditions. Dry conditions are necessary because of the highly reactive nature of cyanoacrylates and their ability to polymerize by the addition of water. Unlike the polymerization, however, there is no additional initiator or catalyst needed. The mixing temperature can also be slightly elevated to allow better mobility of polymer modifiers and adhesive by decreasing the viscosity of them, but not boiling either component.[17] A higher temperature, however, can also increase the potential for the cyanoacrylate to polymerize, which more readily occurs at elevated temperatures.

Once formulated, an adhesive can be tested *in vitro* to determine its general clinical relevance as a wound approximation device. In their guidance document for 510(k) premarket submission, the FDA recommends specific *in vitro* testing for topical use adhesives. The *in vitro* testing includes analysis to determine an adhesive's bond strength, degradation rate, heat of polymerization, and shelf life. Bond strength is an important measure of the functionality for the adhesive to adhere and thus approximate wounds. Degradation rate and heat of polymerization affect the biocompatibility for the adhesive. Specifically, the heat released during the polymerization of the adhesive during its application as well as the byproducts released as the adhesive subsequently degrades can cause tissue irritation and potential necrosis. Lastly, the adhesive can be tested to determine its shelf life (expiration date) as a further clinical relevance test. In order for an adhesive to be clinically useful, it must be able to maintain its properties in storage, so that it performs as desired when it is needed. The FDA recommends testing the viscosity

of the adhesive monomer in addition to other tests during the shelf life study. Viscosity can also be tested as a measure of an adhesive's handling properties and its ability to maintain its position once applied to a wound surface.

Accordingly, it was of interest to understand the underlying factors in the formulation of absorbable tissue adhesives towards the modulation of their physico-mechanical and degradation properties. These properties include: viscosity, shelf life (stability), heat of polymerization, hydrolysis rate, and biocompatibility resulting from degradation products.

## 2.2. Materials and Methods

### 2.2.1. Summary

In order to generate custom made adhesive formulations, one of the first selected tasks was to synthesize poly(glycolide-co-caprolactone) polymers as adhesive rheological modifiers. Chain growth ring opening random co-polymerization of the ring-structure monomers (glycolide and ε-caprolactone) was the planned synthesis method for these polymers. Glycolide was selected for this research due to its relative hydrophilicity and rapid degradation to produce glycolic acid. ε-Caprolactone was selected for this research due to its low glass transition temperature and thus high flexibility as a polymer. In order to understand the effect of these two monomers as polymers on the properties of a cyanoacrylate adhesive, it was planned to generate custom polymers with controlled ratios of the two monomers. The properties of the resulting polymers both before and after blending into cyanoacrylate would then be measured. Both linear and branched

initiators were used for polymer synthesis in order to determine their effect on the resulting polymer both prior and after blending with a cyanoacrylate. Furthermore, polymer synthesis was planned to include high monomer:initiator (M/I) ratios to result in polymers with reduced molecular weights. Low molecular weight polymers would facilitate the blending of them into cyanoacrylate. The plan for this blending was to prepare both low (four weight percent) and high (ten weight percent) adhesive modifier amounts to be blended into cyanoacrylate. These two weight percent values were selected in order to test the hypothesis that there is a direct relationship between adhesive modifier weight percent versus adhesive viscosity. This property would provide an indication for the clinical ability of the adhesive to remain in position once applied.

Methoxypropyl cyanoacrylate, MPC (obtained from Permabond, LLC. as Permabond® 930) was selected as the cyanoacrylate for this research due to its inclusion of a short alkoxy side chain resulting in a flexible, high strength bond as well as the adhesive's proven biocompatibility.[18] This adhesive was the base adhesive for the research adhesive formulations discussed later in Chapter 2, and the main control for the dissertation work. In addition to this adhesive, Histoacryl®, Dermabond®, n-butyl cyanoacrylate (BCA), and 2-octyl cyanoacrylate (2-OCA) were also tested in order to compare the results of the research adhesives to benchmark, medical grade adhesives. Histoacryl® (obtained from schoolhealth.com) was selected as a BCA product. Dermabond® (obtained from esutures.com) was selected as a 2-octyl cyanoacrylate product. BCA and 2-OCA were selected as medical grade monomers because they are used to formulate Histoacryl® and Dermabond® products respectively. In addition to

these adhesives, ethoxyethyl cyanoacrylate (EEC) was also tested for this research in order to test a cyanoacrylate with a longer alkoxy side chain than MPC. The EEC test data would potentially allow comparisons to be made between increased adhesive modifier in MPC and increased alkoxy length for a cyanoacrylate's side chain. BCA, 2-OCA, and EEC were obtained from Afinitica Technologies S.L.

Polymeric rheological modifiers (plasticizers) were first synthesized using random co-polymerization as opposed to segmented (block, graft) polymerization. These polymers were crafted from glycolide (Gly.) monomer obtained from Purac (Corbion) and ε-caprolactone (Cap.) monomer obtained from Acros Organics. Each polymerization was catalyzed with 0.2M tin (II) 2-ethylhexanoate (SnOct, obtained from Alfa Aesar) dissolved in toluene (obtained from Fisher Scientific). Each polymerization also contained either 1,3 propanediol (1,3 P.) as a linear initiator or trimethylolpropane (TMP) as a branched initiator, obtained from Sigma Aldrich. In total there were four polymers produced with variable theoretical molar percentages (mol. %) of the monomers and either 1,3 propanediol as a linear initiator or trimethylolpropane as a branched initiator as outlined in Table 2-1.

Table 2-1: Theoretical Monomer and Initiator Charges for Polymeric Synthesis

| **Polymer** | **Gly. (mol. %)** | **Cap. (mol. %)** | **Initiator** |
|---|---|---|---|
| G10C90L | 10 | 90 | 1,3 P. |
| G40C60L | 40 | 60 | 1,3 P. |
| G10C90T | 10 | 90 | TMP |
| G40C60T | 40 | 60 | TMP |

### 2.2.2. Polymeric Synthesis

Polymerizations were performed in a 2 L glass kettle submerged in an oil bath with a stir rod containing a wide PTFE blade connected for mixing. Before performing each polymerization, the glassware and plastic-ware was first thoroughly cleaned and dried. The glassware was then heat dried for at least 15 hours. For each of the G10C90L and G40C60L polymers, the 1,3 P. was pre-dried under room temperature vacuum prior to use.

During synthesis, the monomers were first vacuum dried at 40°C in the kettle for approximately 0.5-1.0 hour. The temperature for the kettle's oil bath was then slowly ramped up to 100°C. During this ramp, the monomers slowly melted. Next, while the molten monomers were being mixed at a stir rate between 80-100 RPM, the initiator and catalyst were added. The mixture in the kettle was then allowed to polymerize at a temperature between 140-160°C and a rate between 80-100 RPM until nearly full monomer conversion to polymer. This conversion was estimated by relative peak ratios of polymer and monomer from gel permeation chromatography (GPC) chromatograms.

### 2.2.3. Analysis of Custom Synthesized Polymers

GPC analysis for the in-process polymer as well as the final polymer was conducted using a Waters GPC. The GPC was equipped with four columns, Styragel® HR sizes 0.5, 2, 4, and 6. Dichloromethane (DCM) was used as the eluent and the system was calibrated using polystyrene standards. Samples were dissolved in DCM to a concentration of 4 mg/ml, shaken for an hour until dissolved, and filtered with a 0.45 um

filter. An injection volume of 25 µl and a run time of 50 minutes were used for all samples.

In addition to the GPC analysis, proton nuclear magnetic resonance (NMR) was conducted for the final polymers using a JEOL 300 ECX spectrometer. Samples were dissolved in deuterated chloroform at concentrations of 20 mg/mL, and data was collected with a 16-scan profile. Peak ratios were evaluated to determine sample composition.

### 2.2.4. Adhesive Stabilization and Formulations with Custom Synthesized Polymers

With the polymers synthesized, the MPC adhesive was then obtained and processed prior to generating the adhesive formulations. A sample of the MPC was first set aside in storage for later testing. The stock MPC was then anionically stabilized by Aspire Biotech, Inc. by adding sulfur dioxide gas ($SO_2$) until there was 304 ppm present in the MPC based on mass as measured by Aspire Biotech, Inc.

The anionically stabilized MPC (MPC-S) adhesive was then mixed with the 10 and 40 molar percent (low and high) glycolide polymers previously synthesized to obtain adhesive formulations of 4 or 10 weight percent (low and high) polymer for this research as outlined in Table 2-2.

Table 2-2: Theoretical Adhesive Formulations Composition

| Adhesive | Polymer | Polymer Weight Percent |
|---|---|---|
| Cy96-G10C90L4 | G10C90L | 4 |
| Cy90-G10C90L10 | | 10 |
| Cy96-G40C60L4 | G40C60L | 4 |
| Cy90-G40C60L10 | | 10 |

| Adhesive | Polymer | Polymer Weight Percent |
|---|---|---|
| Cy96-G10C90T4 | G10C90T | 4 |
| Cy90-G10C90T10 | | 10 |
| Cy96-G40C60T4 | G40C60T | 4 |
| Cy90-G40C60T10 | | 10 |

The mixing was performed in a two-neck 100 ml glass round bottom flask submerged in an oil bath with a glass stir rod containing a small PTFE blade connected for stirring. Before performing each mixing (adhesive formulation), the glassware and plastic-ware was first thoroughly cleaned and dried. As an additional step, the glassware cleaning included a rinse with a 5 weight percent solution of hydrochloric acid to attempt to eliminate all hydroxide groups from the surface of the glassware to lessen any premature polymerizing of the cyanoacrylate. The glassware was then heat dried for at least 15 hours.

During mixing, the polymeric modifier was first melted and vacuum dried at 50°C in the flask over approximately 2-3 hours. Next, while the molten polymer was being stirred at 100 RPM, MPC-S was added to the flask. The mixture was then stirred at 100 RPM for approximately 1.0-1.5 hours until the research polymer was visibly mixed into the MPC-S in the flask to form one homogenous mixture.

### 2.2.5. Analysis of Adhesive Formulations

The formulated adhesives, benchmark adhesives, MPC, and MPC-S were then tested for their comparative viscosity, peak t-peel load, and/or shelf life to determine their clinical relevance.

For the comparative viscosity test, a new 1 ml BD syringe was connected to a new BD 18 G 1.5 inch beveled or blunt tip needle, and then suspended vertically with the syringe opening facing up.[19] A 1 ml aliquot of adhesive was then deposited into the syringe. The time required for the adhesive to travel from the 1.0 ml graduation on the syringe to the 0.7 ml graduation on the syringe was measured using a stopwatch. The adhesive's comparative viscosity was defined as the measured flow time.

The tensile test used for this research segment was a fabric t-peel test.[20] This test uses fabric instead of animal skin because of the large amount of skin needed and the variability between skin samples.[6] For the test, the fabric is first soaked in a phosphate buffer, and then blotted to remove any excess buffer. The adhesive is then spread between parts of two soaked fabric strips to form a "T" (see Figure 2-2), and allowed to polymerize with 15 minutes under a 1 kg load. After polymerizing, the remaining parts of the strips are placed in a tensile testing apparatus (see Figure 2-2). The apparatus pulls the two strips apart with the peak load required to perform this action reported.

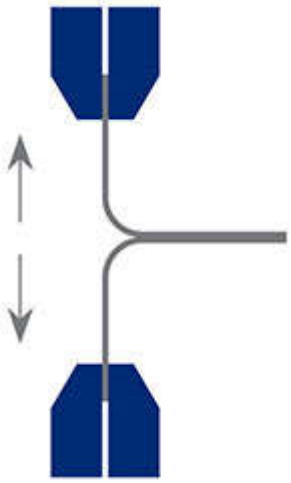

Figure 2-2: T-peel Testing Setup[21]

Shelf life was determined by aging adhesive in 2 ml polypropylene centrifuge tubes at 50°C to create accelerated aging condition. Samples were removed at pre-determined time points of 5.6 days and 15.6 days, and then their comparative viscosity was measured. The viscosity change over time was then determined.

## 2.3. Results and Discussion

### 2.3.1. Molecular Weight and Composition for Custom Synthesized Polymers

The molecular weight and composition of each of the final synthesized research polymers (n=1 per polymer per test) can be found in Table 2-3 and Table 2-4.

Table 2-3: Molecular Weights for Research Polymers

| Polymer | Molecular Weight (Mn, Da) | Molecular Weight (Mw, Da) |
|---|---|---|
| G10C90L | 5905 | 9802 |
| G40C60L | 5323 | 8943 |
| G10C90T | 4923 | 6843 |
| G40C60T | 4157 | 6152 |

Table 2-4: Compositions for Research Polymers

| | Charging Amounts (Theoretical) | | NMR Results (Actual) | |
|---|---|---|---|---|
| **Polymer** | **Cap. (mole %)** | **Gly. (mole %)** | **Cap. (mole %)** | **Gly. (mole %)** |
| G10C90L | 90 | 10 | 92.2 | 7.8 |
| G40C60L | 60 | 40 | 62.1 | 37.9 |
| G10C90T | 90 | 10 | 91.7 | 8.3 |
| G40C60T | 60 | 40 | 63.3 | 36.7 |

In this research segment, glycolide and ε-caprolactone were synthesized using random co-polymerization in order to increase the chain structure randomization to allow an equal opportunity for each monomer type to be found throughout the polymer. The polymers were also synthesized with a moderate amount of initiator in order decrease the molecular weight of the resulting polymer. A more amorphous, lower molecular weight polymer will typically degrade faster than a crystalline, higher molecular weight polymer because less energy is required to hydrolyze it.[22] Polymers that were more amorphous, less crystalline and lower molecular weight were selected for this research to facilitate their hydrolysis within the polymerized adhesive. Glycolide monomer was selected because polyglycolide will rapidly hydrolyze due to reduced steric hindrance into glycolic acid, a low pH compound. Glycolic acid can then potentially decrease the pH of the local environment. ε-caprolactone was selected because polycaprolactone has a high flexibility due to the ether bond and long alkane chain in the polymer repeat unit. Additionally, the polymer has a slower degradation rate due to the increased steric hindrance on the ester bond in the polymer repeat unit. Controlling the molar ratio of glycolide and ε-caprolactone for a polymer's synthesis would thus allow one to control

the degradation rate and flexibility of the resulting polymer. For this research, a set of polymers with a linear chain initiator and a set of polymers with a branched chain initiator were each synthesized. These two different sets were fabricated in order to determine the effect from varying the theoretical crystallinity of the polymer as previously discussed.

Polymers were synthesized to have low molecular weights by using low monomer:initiator (M/I) ratios to synthesize each polymer. The data in Table 2-3 indicates that the goal of low molecular weight polymers was met. This data also demonstrates the effect of testing triaxial polymers (G10C90T and G40C60T) using a GPC. Prior to performing GPC for a sample, the GPC is calibrated using linear polystyrene calibration standards of various known molecular weights to determine the retention time for each molecular weight using the GPC. During sample analysis, the GPC separates polymers by hydrodynamic volume, and compares the retention time results to the results from the initial calibration in order to estimate the molecular weight for the sample. When a single chain polymer is dissolved in solvent for a GPC, it will have a larger hydrodynamic volume as compared to the branched polymer. Due to their smaller hydrodynamic volume as compared to the linear research polymers, the GPC reports the triaxial research polymers' molecular weight as lower than the linear research polymers' molecular weight (G10C90L and G40C60L) although they were synthesized from nearly identical monomer charges and M/I ratios. Nevertheless, the triaxial and linear polymers' molecular weights were similar. The data in Table 2-4 demonstrates that the goal of generating polymers with controlled ratios of glycolide:caprolactone was met.

### 2.3.2. Stabilized Adhesive Testing

The comparative viscosity and maximum adhesive t-peel load values for MPC and MPC-S can be found in Table 2-5. These properties were tested initially and after anionically stabilizing the adhesive to confirm the stabilization did not adversely affect the adhesive.

Table 2-5: MPC and MPC-S Initial Test Results

| **Adhesive** | **$SO_2$ Amount (ppm)** | **Comparative Viscosity (mean ± standard deviation, s)** | **Maximum T-Peel Load (mean ± standard deviation, N)** |
|---|---|---|---|
| MPC | 0 | 9.70±0.29 | 29.558±3.769 |
| MPC-S | 304 | 10.05±0.37 | 39.892±9.519 |

Based on the results in Table 2-5, there appeared to be no difference between the MPC and MPC-S for the comparative viscosity test. In addition, the MPC-S appeared to have a maximum t-peel load that was equal or greater than the MPC maximum t-peel load. Each pair of data sets was further analyzed using a statistical t-test to compare the means for MPC versus MPC-S with resulting p-values of 0.27 (viscosity) and 0.18 (t-peel). These p-values are greater than a significance level ($\alpha$) of 0.05, which indicates there is no statistically significant difference between the MPC and MPC-S for these two tests. The anionic stabilization thus did not adversely affect the MPC adhesives handling and adhesion properties.

### 2.3.3. Adhesives Formulation Additional Information and Testing Overview

Prior to fabricating each adhesive formulation, the glassware was cleaned, which included a rinse with a 5 weight percent solution of hydrochloric acid. This rinse attempted to eliminate all hydroxide groups from the surface of the glassware to lessen any premature polymerizing of the cyanoacrylate.

For this research, 4 and 10 weight percent adhesive modifier formulations were fabricated and tested for comparative viscosity, t-peel, and shelf life. The results from testing the adhesives can be found as Figure 2-3, Figure 2-4, and Figure 2-5.

### 2.3.4. Adhesives Formulations Comparative Viscosity

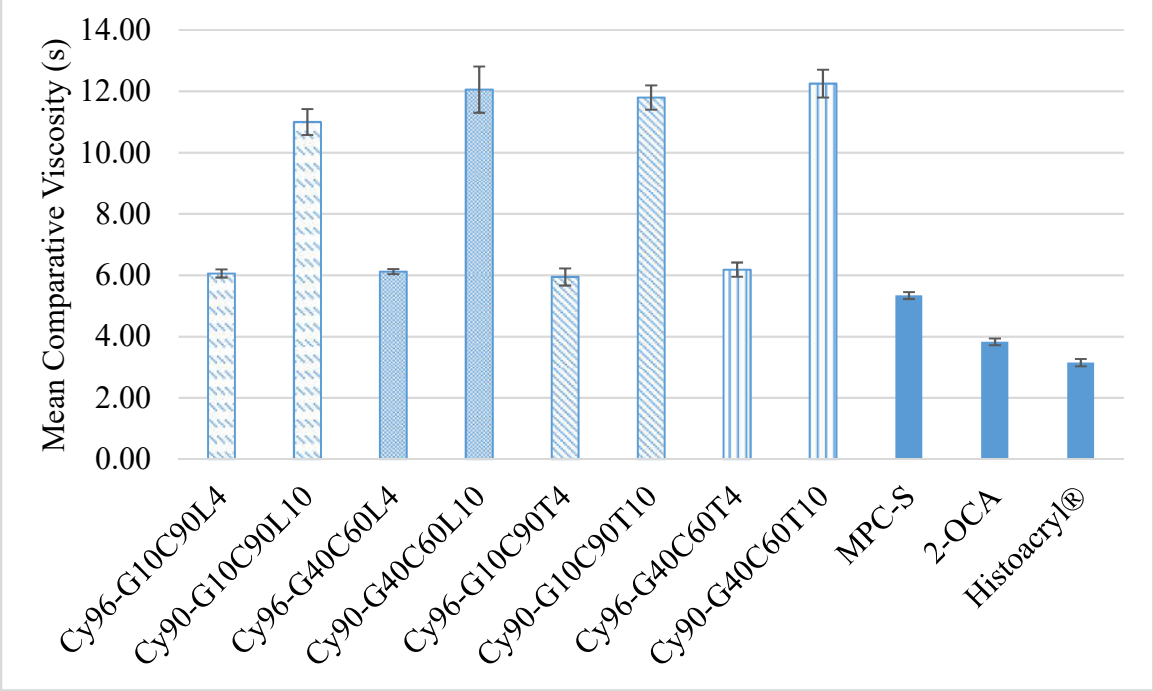


Figure 2-3: Initial mean comparative viscosity for adhesives (n=3 per adhesive, error bars = 1 standard deviation)

For the initial viscosity testing, a slight modification was made to the comparative viscosity testing. Specifically, the BD 18 G 1.5 inch beveled needle was changed to an 18 G 1.5-inch blunt tip needle supplied by McMaster-Carr due to a safety concern regarding the use of beveled needles. This change in test method was performed after the MPC and MPC-S were initially tested (see Table 2-5). The difference in tip type should not have caused a discrepancy between the test results; however, this change may have resulted in the difference between the MPC-S results in Table 2-5 and the results in Figure 2-3 because it was the only difference between the test results.

The initial viscosity testing results included in Figure 2-3 for the research adhesives demonstrate a direct relationship between adhesive modifier weight percent and adhesive viscosity. Specifically, as the amount of polymeric modifier was increased, the viscosity increased. There was additionally a similar viscosity among the set of 4 weight percent adhesives and among the set of 10 weight percent adhesives. The adhesive modifier thus appeared to affect the viscosity for the MPC-S similarly regardless of the modifier type. Additionally, the MPC-S and research adhesives had a greater viscosity than the 2-OCA and Histoacryl®. Their increased viscosity is expected to result in their increased resistance to gravitational and physical forces subsequent to their application at a wound site that would typically act to shift the adhesive out of its applied location. It is important for the adhesive to remain at the application site because less adhesive present will decrease the bond strength and thus functionality of the adhesive. In addition, if the adhesive travels away from the application site, it can flow into the wound or any other

nearby wounds, which can result in internal polymerized adhesive. This unplanned result can cause biocompatibility issues such as decreased cellular activity depending on the adhesive.

### 2.3.5. Adhesive Formulations T-Peel

As depicted in Figure 2-4, the MPC-S had a large t-peel load, so it was expected that the research adhesives would also have t-peel loads close to the MPC-S t-peel load. The MPC-S t-peel load additionally was a much greater t-peel load than Histoacryl® and Dermabond®, which indicates MPC-S would have an acceptable level of adhesive ability for clinical use. The adhesive t-peel loads measured for the research adhesives were, however, lower than expected. This decrease in mechanical strength as compared to MPC-S may be due to the minor increase in heat during the mixing process resulting in the adhesive becoming partially aged and polymerized. It can also be due to the addition of polymeric modifier to the MPC-S resulting in less total adhesive to polymerize. If the mixing process was the cause of the lower mechanical strength, it could potentially be optimized to achieve better t-peel loads in future adhesive formulations.

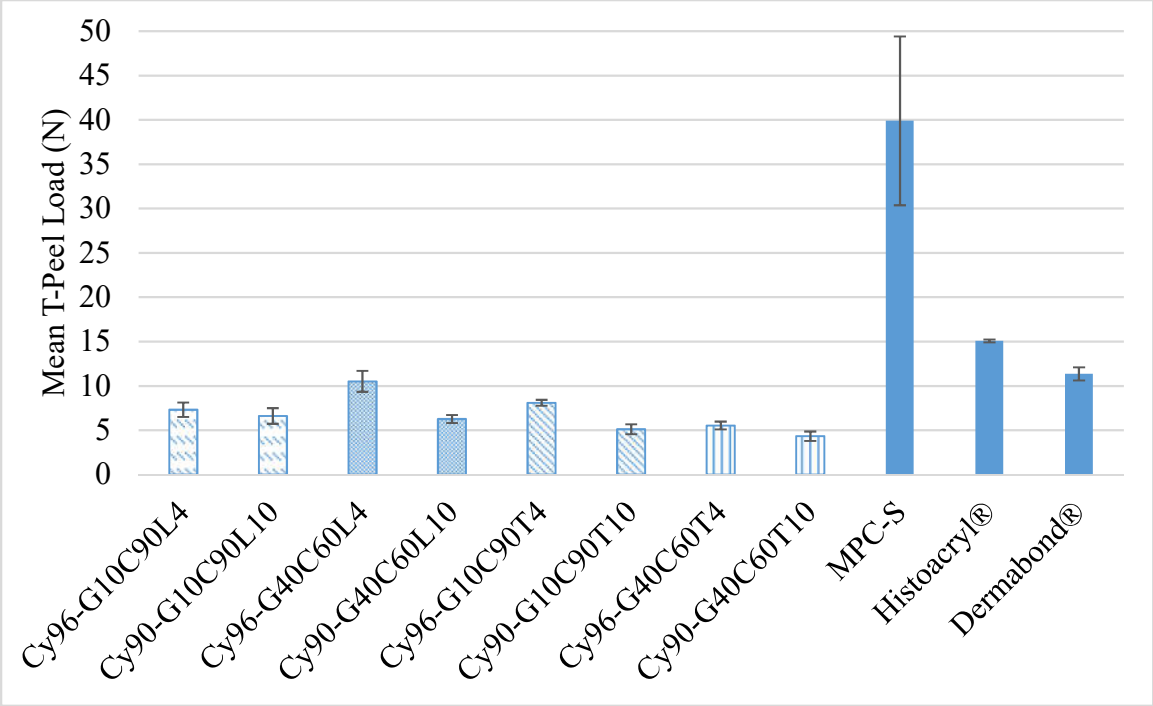


Figure 2-4: Mean adhesive t-peel load values (n=3 per adhesive, error bars = 1 standard deviation)

### 2.3.6. Adhesive Formulations Shelf Life

The shelf life stability for the adhesives was measured using the comparative viscosity test. This test was used to measure shelf life because the adhesive will thicken as it polymerizes, so a viscosity increase for the adhesive is an indicator of the adhesive's instability. The shelf life study time points were calculated based on the Arrhenius reaction rate equation with the assumption that $Q_{10}$=2 for the equation.[14],[23] The actual time points used were 5.6 days and 15.6 days at 50°C, which approximates to 1 month and 3 months at 25°C respectively by the Arrhenius equation. Based on Figure 2-5, the research adhesives, MPC-S, and 2-OCA were deemed stable. There was an initial

increase in viscosity at the first time point; however, this value appeared to stabilize at the second time point. Several of the research adhesives appear to have decreased in viscosity from the first time point to the second time point. These decreases are within the one standard deviation range for comparing the first and second time points. The adhesives are thus predicted to have sufficient stability for clinical usefulness.

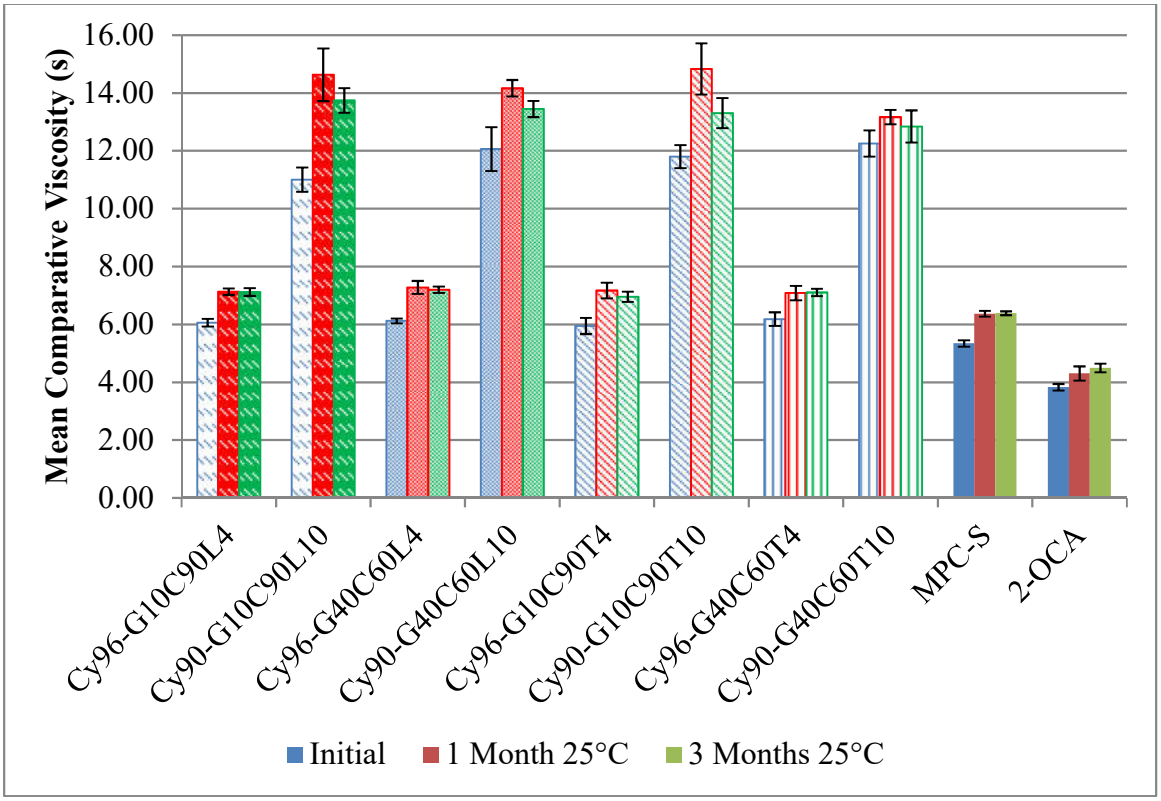


Figure 2-5: Shelf life by mean comparative viscosity for adhesives (n=3 per adhesive per time point, error bars = 1 standard deviation)

## 2.4. Conclusions

The polymer synthesis and adhesive formulation was successfully completed for this research based on the fabrication of a desirable amount of each material for evaluation. The goals of fabricating low molecular weight polymers with two of them,

G10C90T and G40C60T, initiated using a branched initiator to generate triaxial polymers were met. Polymers with controlled ratios of glycolide:caprolactone were also successfully fabricated, which also met a goal of this research. In regards to MPC, the anionic stabilization of MPC to MPC-S did not increase the viscosity or decrease the maximum t-peel load for the stabilized adhesive. This result was important because MPC-S was the base cyanoacrylate for the entire research. For the research, when the adhesive modifiers were mixed into the MPC-S, and the comparative viscosity of the resulting adhesive formulations was measured, a direct relationship between adhesive modifier weight percent and adhesive viscosity was observed. The data thus provides evidence that the previously introduced hypothesis may be true. The formulated adhesives also each had a viscosity greater than or equal to the benchmark adhesives of 2-OCA and Histoacryl®, so they should be clinically useful by staying in position when applied. Once applied, the goal of the adhesives would be to approximate wounds and resist tensile forces that would result in wound dehiscence. Based on the t-peel testing results, the research adhesives would be able to resist a smaller level of tensile forces as compared to the benchmark adhesives tested. Lastly, the test results demonstrate that the research adhesives, MPC-S, and 2-OCA were all stable with predicted stability for clinical usefulness. Suitable properties for the adhesive modifiers and formulations were achieved, so it was thus recommended to proceed with further analysis of the research adhesives' properties to understand the underlying factors in the formulation of absorbable tissue adhesives towards the modulation of their physico-mechanical and degradation properties.

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CHAPTER 3

ADHESIVES THERMAL ANALYSIS METHOD DEVELOPMENT AND TESTING

## 3.1. Introduction

The polymerization of a cyanoacrylate adhesive is typically a rapid, highly exothermic reaction. The heat from this reaction (heat of polymerization, HOP) can elevate human tissue beyond the homeostatic temperature of 37°C. As tissue temperature is elevated beyond this point, nerve receptors will report the heat change as an increase in warmth at a specific magnitude. The body will then respond to the homeostatic deviation. Depending on the magnitude of the temperature change and time of exposure, the body's response will range from no change in tissue to full tissue necrosis and with no pain response to excruciating pain. In one study, the exact effect of time and temperature on human skin's response was determined, with a summary of the study's results in Figure 3-1.

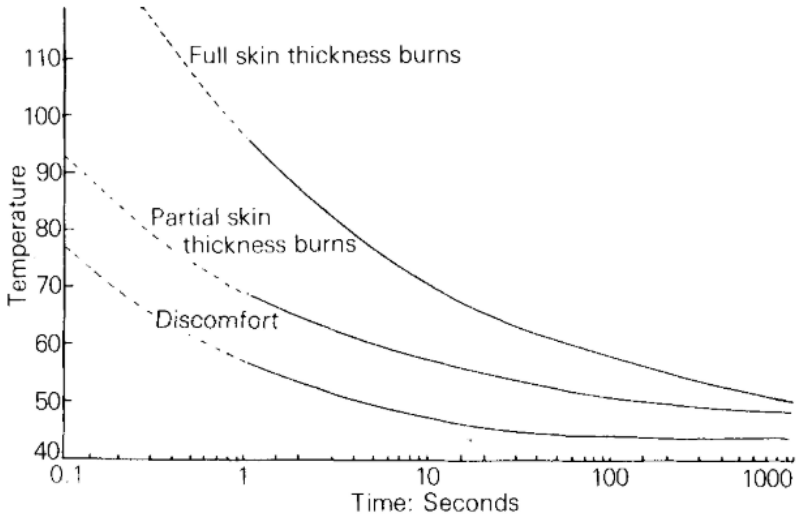


Figure 3-1: Human Skin's Response to Temperature Elevation (Magnitude and Time)[1]

When a cyanoacrylate adhesive is applied during wound approximation or sealant applications, patients typically report a sensation of warmth as the adhesive polymerizes, which would indicate an estimated 1-40°C increase in temperature for likely 0.1-100 seconds (see Figure 3-1).[2]–[8] In several cases, full thickness burns or tissue necrosis has resulted from cyanoacrylate adhesive polymerization, which would indicate an estimated 20-100°C increase in temperature for likely 0.1-100 seconds (see Figure 3-1).[9]–[11] There is thus a need to modulate cyanoacrylate adhesives in order to remove this disadvantage, while maintaining other functional properties, and thus increase their biocompatibility.

In order to decrease their HOP, cyanoacrylate adhesives can be modulated through side chain chemistry or by mixing in other components to the adhesive. HOP is also dependent on the polymerization reaction rate for the adhesive, which can also be modulated. As previously discussed, the side chain for cyanoacrylates controls many of their properties. For HOP and polymerization rate, there is an inverse relationship between side chain length and complexity versus HOP and polymerization rate due to steric hindrance.[7], [12], [13] Cyanoacrylates with short side chains such as methyl and ethyl or less complex side chains such as isopropyl will generally have faster polymerization rates and increased HOP. In comparison, cyanoacrylates with longer side chains such as hexyl, 2-octyl, and decyl or more complex side chains such as ethoxyethyl and methoxypropyl will generally have slower polymerization rates and lower HOP. Other adhesive modifiers such as plasticizers can also be mixed into the cyanoacrylate to modulate its polymerization rate and HOP. There is an inverse relationship between the

amounts of adhesive modifier mixed into adhesives and the polymerization rate and HOP of the resulting adhesives because 1) the adhesive modifiers act as heat sinks and 2) there is less overall adhesive present to polymerize.[7], [14], [15]

Although cyanoacrylate adhesives can be tailored to have reduced polymerization rates and HOP, there must be a balance between biocompatibility and clinical relevance. Adhesives with side chains that are very long (e.g. decyl) or very bulky (e.g. hexoxy) have much lower tensile strength than the short chain, less bulky side chain cyanoacrylates.[11], [16] In addition, these long or bulky side chain cyanoacrylates (including 2-octyl) typically require an accelerant such as benzalkonium chloride in order for them to polymerize, which provides an additional opportunity for the body to have an adverse reaction to the exposure of a synthetic material. Lastly, adding excess adhesive modifiers to cyanoacrylates can greatly increase their viscosity rendering them difficult to apply. A highly viscous, thick adhesive with poor tensile strength and excess synthetic products would have a reduced clinical relevance.

In order to select ideal synthesis and formulation modifications for cyanoacrylates to balance polymerization rate and HOP with clinical relevance, a test method is needed to measure polymerization rate and HOP for adhesives. Thermogravimetric analysis (TGA) has historically been used to measure the extent of reactions; however, this thermal analysis method does not measure heat flow ($\Delta H$), so the HOP could not be measured. In addition, cyanoacrylate polymerization is too rapid to measure reaction rate by TGA. Differential scanning calorimetry (DSC) has historically been used to measure heat flow for materials, as it can be used to measure reaction rates and HOP under

isothermal conditions; however, there is a potential for cyanoacrylate vapor to travel through the DSC chamber and other components, polymerize at these undesired locations, and then damage the DSC equipment.[17] In order to prevent this damage, it is not advised to perform cyanoacrylate polymerization with a DSC.

Although not recommended, researchers have used a DSC to measure the heat of polymerization for cyanoacrylates. Other researchers have used alternative methods such as a thermocouple to measure the peak temperature change (from the initial temperature) and/or overall peak temperature during cyanoacrylate polymerization.[10], [16] Several of the measured values from the DSC tests and alternative methods tests can be found in Table 3-1.

Table 3-1: Cyanoacrylate Polymerization Peak Temperature Change, Peak Temperature, and Heat of Polymerization[11],[16],[18]

| **Cyanoacrylate Type by Side Chain Type** | **Peak Temp. Change (°C)** | **Peak Temp. (°C)** | **Heat of Polymerization (J/g)** |
|---|---|---|---|
| Methyl | 4 | 41 | 264.78 |
| Butyl | Not tested | Not tested | 372.15 |
| Hexyl | 1.8 | 38.8 | Not tested |
| Methoxy | 18 | 55 | Not tested |
| Ethoxyl | 14 | 51 | Not tested |
| Butoxy | 11 | 48 | Not tested |
| 2-Octyl (Dermabond®) | 11 | 48 | 229.08 |
| Methoxypropyl | Not tested | Not tested | 278.83 |

Although these values vary depending on the research study, both demonstrate the presence of an inverse relationship between cyanoacrylate side chain length versus peak temperature change during adhesive polymerization. This relationship was expected

because of the increased steric hindrance for the cyanoacrylates with longer side chains. In addition, the literature values demonstrate that cyanoacrylates with shorter alkoxy side chains (e.g. butoxy) can have a similar peak temperature change during adhesive polymerization as cyanoacrylates with long alkane side chains (e.g. 2-octyl). This observation may be due to the bulky side chain increasing the time required for the propagating cyanoacrylate to bond with other cyanoacrylate monomer. Although these literature methods can measure peak temperature change for polymerizing cyanoacrylates, they do not report the HOP or polymerization rate. These latter properties are also important for predicting the effect of the polymerizing adhesive on tissue because increased reaction rates and heat can burn tissue.[10]

This research proposes an alternative novel method for measuring a polymerizing adhesive's thermal properties (PATP) inspired by these different techniques. The goal for this method was to use a data logger to measure the temperature over time while in contact with a polymerizing adhesive. Ideally, the data from this testing would at a minimum capture the peak temperature change, peak temperature, and reaction time from the known exothermic cyanoacrylate polymerization reaction. The measured change in temperature over time data would ideally allow one to calculate the reaction rate for the polymerization. Lastly, measuring the total reaction time and temperature during the reaction would ideally allow one to calculate the heat of polymerization using the area under the time versus temperature curve from the measured data. The development of this new method was expected to allow one to test the hypothesis introduced in Chapter 1,

that there is an inverse relationship between adhesive modifier weight percent versus adhesive reaction rate, peak reaction temperature, and heat of polymerization.

## 3.2. Materials and Methods

### 3.2.1. Polymerizing Adhesives Thermal Properties Test Method (PATP) Apparatus

A thermometer with data logger capability, namely the EL-EnviroPad-TC manufactured by Lascar Electronics was selected as the data logger for the PATP test method. A 4 ml glass vial obtained from VWR with a 15 mm outer diameter and 45 mm height was selected as the container for the test method's apparatus. This container type also included a separate PTFE lined lid. Potassium phosphate dibasic salt was added to the vial to aid in polymerizing adhesives for most trials. Methoxypropyl cyanoacrylate anionically stabilized with 304 ppm sulfur dioxide (MPC-S) was used as the adhesive for all test method development trials. Figure 3-2 illustrates the PATP test method apparatus (calorimeter).

Figure 3-2: PATP Test Apparatus

### 3.2.2. PATP Method Development

The method development first included evaluating several different adhesive polymerization methods using the 4 ml glass vials. Deionized water and ACS reagent grade potassium phosphate dibasic salt were the first two materials attempted for polymerizing the adhesive. An anhydrous grade potassium phosphate dibasic salt was then tested. A test was then completed using a known, small amount of deionized water added to 120°C dried anhydrous salt. Several combinations of adhesive volume, salt mass, salt drying time, and water volume were then tested to determine the combination that produced the sharpest temperature versus time peak.

Additional test method parameters to better control variability were then determined as part of test method development. The effect on test method variability from packing the salt down after mixing the salt and water amounts was first measured. Trials using a minimum time of 15 minutes between the water addition and adhesive addition, equilibration of the probe at 30°C prior to testing, and a temperature fluctuation rate for steady state were then completed. These trials included different settings for these additional parameters to measure the resulting temperature versus time peak sharpness.

### 3.2.3. Final PATP Method

Based on the test method development work, a final polymerizing adhesive thermal properties method was selected. Anhydrous potassium phosphate dibasic salt as obtained from Fisher Scientific was first added as 0.7000±0.0100 g to a 4 ml glass vial, with the actual salt mass recorded. The vial's lid was set aside in a sealed bag at room temperature. The salt filled vial was then placed at 115-140°C in a drying oven for at least 3 hours to dry the salt in the vial. After this elapsed time, the dried salt filled vial was then removed, and immediately capped with its lid. Subsequent to this step, the total drying time was recorded. The dried salt filled vial was then allowed to cool to the touch at room temperature over approximately 5 minutes. A 20-200 µl pipette was then used to measure and apply 25 µl of deionized water to the dried salt in the vial. The salt and water in the vial was then stirred using a small, clean stainless steel spatula. The filled vial was then left closed at room temperature for at least 15 minutes. A plunger from a 1

ml syringe was then used to flatten the moist salt in the vial to form a layer at the bottom of the vial. The filled vial was then left closed at room temperature until it was desired to test an adhesive.

Prior to testing an adhesive, a vial of salt was prepared as previously described, and then the data logger and probe were initiated as follows. The metal probe was first connected to the data logger. Acetone and a lint-free wipe were then used to clean the metal probe in order to remove any particulate including adhered salt and adhesive from other tests. After the probe air-dried approximately one minute at room temperature, the data logger was powered on, and the probe was pre-heated. For the pre-heating method, the data logger was first set to Monitor mode. A nitrile glove covered hand was then used to grasp the bottom of the probe until the data logger reported a temperature of 30.0°C. The probe was then released. The data logger was then set to log temperature at a rate of one data point per second. With the data logger logging in progress, the probe was placed into the pre-prepared vial. As needed, the vial was gently tapped to shift the moist salt in the vial to fill any space in the salt layer that may have been created from where the probe was placed into the salt layer. The salt in the vial was then inspected to ensure it fully surrounded the probe. The probe was then allowed to equilibrate with the moist salt for 5-7 minutes. After this time, the data logger was reviewed to ensure there was no fluctuation in temperature as defined as <0.2°C change in temperature over a 60 second period. If there was no temperature fluctuation, the temperature displayed on the data logger was recorded as the initial temperature. A 100-1000 µl pipette was then used to measure and apply 400 µl of adhesive to the moist salt in the vial. This aliquot was

carefully dispensed to ensure none of the adhesive was deposited on the probe itself. The temperature data being logged was then monitored. During testing, the temperature increased and then returned to approximately the initial temperature. The data logger was stopped when the displayed temperature was at least 1°C away from the initial temperature. The measured temperature over time data set appeared as a parabolic curve with a sharp peak (see Figure 3-3). As final steps, the logged data was then saved to the data logger, the probe was then removed from the vial and cleaned with acetone, and the vial now containing polymerized adhesive and moist salt was then disposed of accordingly.

### 3.2.4. PATP Method Data Collection and Thermal Properties Calculations

The measured temperature data from this test method was used to determine the reaction rate, peak temperature, peak temperature change, and heat of polymerization. Reaction rate was calculated as the linear slope of the temperature versus time plot using a high point and low point for the plot (see Figure 3-3 and Equation 3-1). Peak reaction temperature was determined by reviewing the data to locate the maximum measured temperature (see Figure 3-3). Peak temperature change was calculated by subtracting the peak reaction temperature from the initial reaction temperature (see Figure 3-3). Lastly, heat of polymerization was calculated by first calculating the area under the peak for the plotted curve, and then applying this value along with a calculated mean calorimetric constant of 0.025 J/(°C*s) and the mass of adhesive tested to the heat equation (see Equation 3-2) to calculate heat of polymerization. The area under the peak for the plotted

curve was determined by calculating a curve of best fit for a generated normalized temperature versus normalized time plot using Microsoft Excel, and then integrating the curve between the initial temperature and final temperature. For this plot, normalization was defined as subtracting the initial value (temperature or time) from all other values (temperature or time respectively). The mean calorimetric constant was calculated by obtaining the mean result from using Equation 3-3 with MPC, BCA, and 2-OCA.

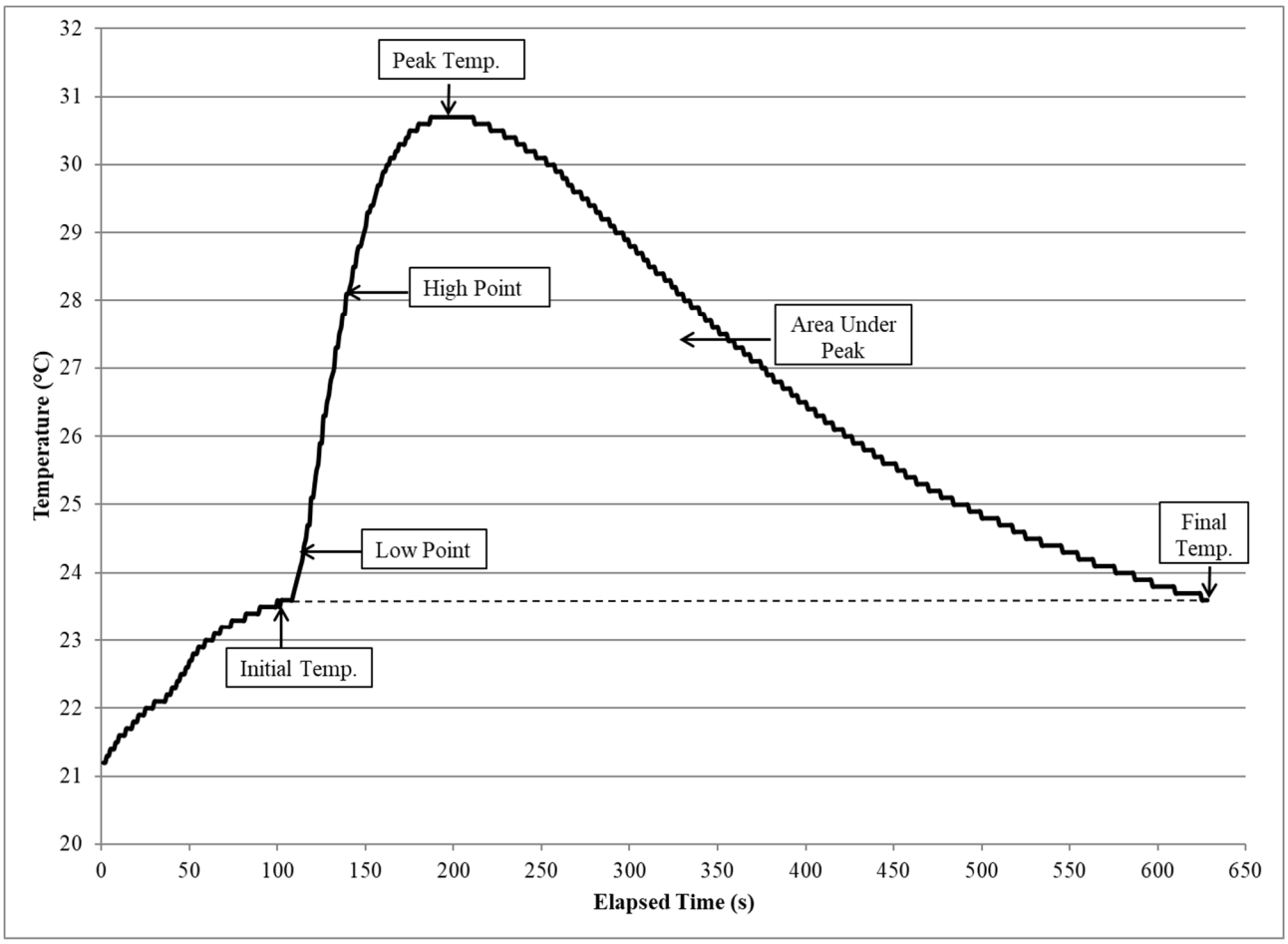


Figure 3-3: Example Temperature vs. Time Plot

$$Reaction\ Rate\ (°C/s) = \frac{y_2 - y_1}{x_2 - x_1}$$

Equation 3-1: Reaction rate formula with ($X_1$,$Y_1$) and ($X_2$,$Y_2$) obtained as specific low and high points randomly selected for the linear portion of the temperature versus time plot (see Figure 3-3)

$$HOP\ \left(\frac{J}{g}\right) = \frac{K * A}{m}$$

Equation 3-2: HOP formula with K estimated as 0.025 J/(°C*s), A as the calculated area under the peak for the temperature versus time plot (°C*s), and m as the mass of adhesive[19]

$$K\ (\frac{J}{°C * s}) = \frac{Lit.HOP}{A/m}$$

Equation 3-3: Calorimetric constant (K) calculated using A and m discussed in Equation 3-2 as well as an HOP value obtained from literature HOP (Lit. HOP) and included in Table 3-1

### 3.2.5. Adhesives Analysis Using PATP Method

This developed polymerizing adhesive thermal properties method was used to test the research adhesives and benchmark adhesives in triplicate. In order to calculate the HOP for an adhesive, the adhesive's density was first determined. The density of each research adhesive formulation was determined based on their measured mass by a balance and measured volume by a graduated container (±2% accuracy). The benchmark adhesives' density values were determined based on their data sheets from their respective manufacturer. The density values can be found in Table 3-5.

## 3.3. Results and Discussion

### 3.3.1. PATP Apparatus

The EL-EnviroPad-TC was primarily selected as the instrument for the new cyanoacrylate polymerizing adhesive thermal properties test method due to the ability of the data logger's metal probe to measure temperature over time repeatedly in the presence of a polymerizing cyanoacrylate. Specifically, the probe was able to withstand the high temperatures produced during cyanoacrylate polymerization. Cyanoacrylates were also able to make intimate contact with the probe to better transfer the input from the polymerizing adhesive to the probe. Although this intimate contact resulted in the adhesive adhering to the probe, at the conclusion of each test, the adhesive could be removed from the probe by wiping it with acetone. The ability to fully remove the adhesive between tests was important to decrease contamination and thus potential variability between tests. This data logger was also helpful due to its ability to log data rapidly as compared to most other temperature data loggers, which accommodated the known rapid polymerization rate for cyanoacrylates. The instrument's high accuracy and calibration were also desired to be able to make acceptable comparisons between data sets. Lastly, it was helpful that the instrument could perform multiple data logging runs (adhesive polymerization tests) with many points recorded for each run without needing to off-load data between each run. This instrument's overall parameters are in Table 3-2.

Table 3-2: Data Logger Equipment Parameters

| Parameter | Desired Setting | Actual Setting |
|---|---|---|
| Measurement Capability | Thermometer with data logging | Thermometer with data logging |
| Probe Temperature Capability | 15-100°C or wider | -100-700°C |
| Probe Surface | Non-removable surface (e.g. PTFE or metal) | Metal surface |
| Accuracy | High (±2°C or less) | ±1.5°C |
| Calibration | Calibrated device | Calibrated device |
| Measurement Rate | 1 point/second or faster | 1 point/second |
| Storage Space | 5 or more runs | 100 runs |
| Readings per Run | 1800 readings or more | 65,500 readings |

Similar to the data logger's probe, the 4 ml glass vial size was selected to maximize the contact area between material in the vial and the data logger probe. This vial type also minimized the amount of material in the vial including the adhesive, so that there would be a sufficient amount of adhesive for other testing. Lastly, the glass material for the vial acted as an insulator, so that the data logger was able to measure the heat generated from the polymerization reaction with minimal expected effect from the surrounding environment.

### 3.3.2. PATP Method Development

Initial testing performed with this vial type and data logger utilized a variety of materials as attempted initiators for adhesive polymerization. Water and potassium phosphate dibasic salt were the first two materials tested due to their utilization in the t-peel testing previously performed. From the t-peel testing, it was known that adhesives would polymerize on cotton fabric soaked with a solution of potassium phosphate in

deionized water (phosphate buffer). The specific phosphate buffer was a mixture of a potassium phosphate monobasic and potassium phosphate dibasic at a ratio of approximately 1:3 monobasic:dibasic to produce a typical 0.1 M 7.4 pH buffer solution.[20], [21] The buffer containing a solution of potassium phosphate in deionized water was able to help polymerize the adhesive, so the two main components from the buffer (deionized water and potassium phosphate dibasic salt) were the first two materials attempted for polymerizing the adhesive for the PATP test method.

When initially tested, water appeared to act as a heat sink with only a minimal increase in temperature observed with and without a small amount of salt added. Based on this result an anhydrous grade was selected for the potassium phosphate dibasic salt. A small amount of water was then added to the anhydrous salt to dissociate the salt's ions without creating a heat sink as observed with large amounts of water.

In addition to measuring temperature and time values, initial results from the data logger were plotted to observe the temperature versus time curve for polymerizing adhesives. Representative plots for this initial testing can be found as Figure 3-4. Each generated plot was reviewed to determine which testing parameters resulted in the least heat-sink effect (broad peak) as indicated by a sharper peak. Peak sharpness was also representative of a fast reaction rate and polymerization reaction time.

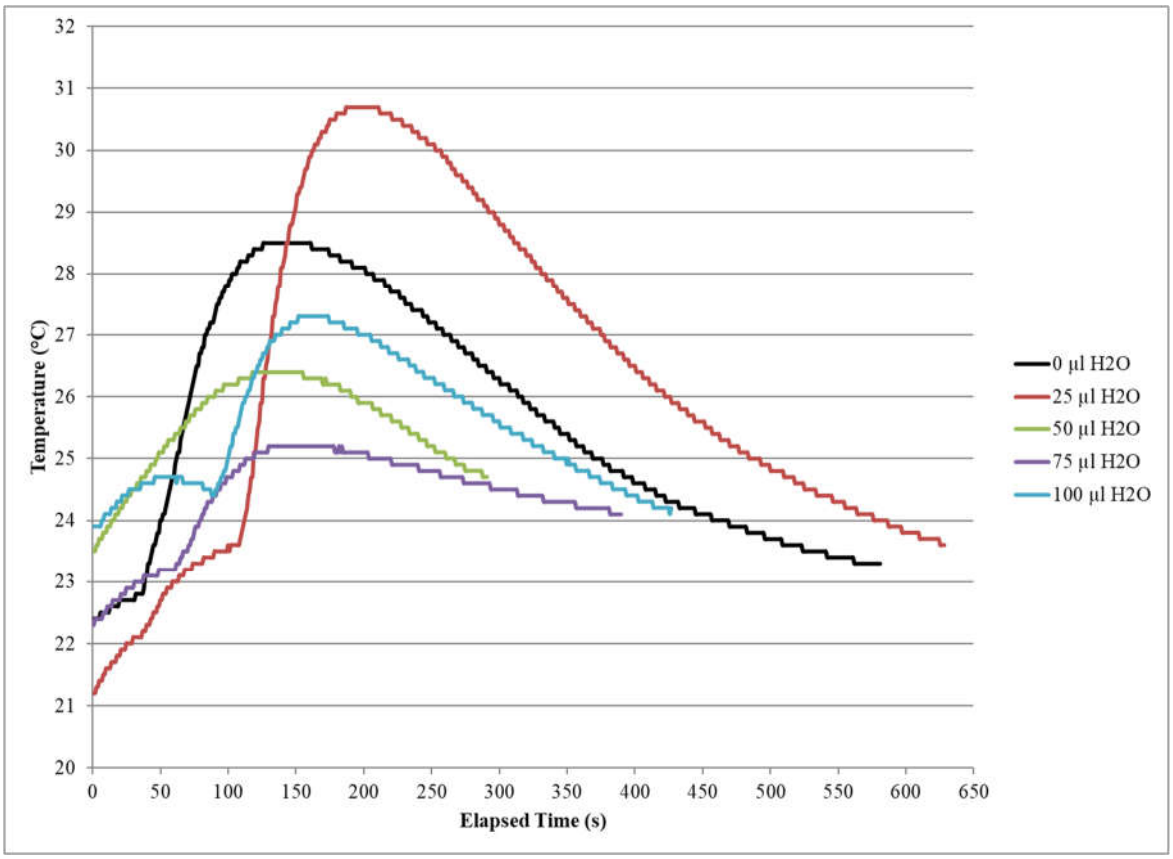


Figure 3-4: Example Initial Polymerizing Adhesive Thermal Properties Test Method Development Plots

Test method parameters to better control variability were also determined during initial testing. One parameter, wet salt packing, was explored due to its ability to provide a more consistent surface area exposure of the wet salt to the instrument's probe, thereby increasing the test method's repeatability. Adding water to the salt dissociated the potassium and phosphate ions, which was an exothermic reaction. For this reason, the temperature of the moist salt mixture would briefly be elevated before cooling back to room temperature. In order to increase the test method's repeatability, a minimum time of 15 minutes between the water addition and adhesive addition was implemented for each test. Lastly, the initial temperature of the instrument's probe would vary depending on the

actual room temperature. The metal probe was more susceptible to cool temperatures as well. Additional heat would thus be required to raise the temperature of a cold metal probe versus a warm probe. For this reason, after the 15 minute wait previously discussed, the probe was then pre-heated to 30°C before it was placed in the moistened salt. The temperature was then monitored with the adhesive added after a minimum allowable temperature fluctuation (steady state) of 0.2°C/minute was observed.

Initial testing results included in Table 3-3 indicate that the peak type varied regardless of the MPC-S volume added, salt dry time, and water amount added to the salt. For one set of tests, the water amount was varied from 0 to 100 µl, with 25 µl producing a sharp peak, while the other water amounts produced broad peaks (see Figure 3-4). Overall, the initial test method had excessive variability as demonstrated by the results in Table 3-3. As an example, when the same parameters were used, there were two different results as demonstrated by the first and second tests in Table 3-3.

Table 3-3

Table 3-3: Polymerizing Adhesive Thermal Properties Test Method Development Results (Initial Tests with 0.7 g Salt)

| MPC-S (µl) | Salt Dry Time (hours) | Water (µl) | Peak Type |
|---|---|---|---|
| 550 | 0 | 100 | Sharp |
| 550 | 0 | 100 | Broad |
| 500 | 0 | 150 | Broad |
| 550 | 3 | 3.25 | Broad |
| 550 | 3 | 7.75 | Broad |
| 550 | 3 | 22.35 | Broad |
| 550 | 3 | 12 | Sharp |
| 550 | 3 | 18.5 | Sharp |

| MPC-S (µl) | Salt Dry Time (hours) | Water (µl) | Peak Type |
|---|---|---|---|
| 550 | 3 | 64.9 | Sharp |
| 550 | 3 | 89.6 | Sharp |
| 550 | 3 | 143.7 | Broad |
| 362 | 5 | 0 | Broad |
| 362 | 5 | 25 | Sharp |
| 362 | 5 | 50 | Broad |
| 362 | 5 | 75 | Broad |
| 362 | 5 | 100 | Broad |

Due to the excessive variation, additional parameters were investigated to attempt to reduce the test method's variation. Using the refined test method, the variation previously observed was better controlled as demonstrated in Table 3-4.

Table 3-4: Polymerizing Adhesive Thermal Properties Test Method Development Results (Refined Tests)

| Initial Parameters | | | | Additional Parameters | | | Result |
|---|---|---|---|---|---|---|---|
| MPC-S (µl) | Salt (g) | Salt Dry Time (hours) | Water (µl) | Salt Packed? | Wait Time After Water Addition (mins.) | Probe Pre-Heat? | Peak Type |
| 362 | 0.7 | 5 | 25 | Yes | Not Reported | No | Broad |
| 400 | 0.7 | 5 | 25 | Yes | 15 | Yes | Sharp |
| 400 | 0.7 | 5 | 25 | Yes | 15 | No | Broad |
| 400 | 0.7 | 5 | 25 | Yes | 15 | No | Broad |
| 400 | 0.7 | 5 | 25 | Yes | 15 | Yes | Sharp |
| 400 | 0.7 | 5 | 25 | Yes | 15 | Yes | Sharp |
| 400 | 0.7 | 5 | 25 | Yes | 15 | Yes | Sharp |

The revised test method with new parameters added improved the ability of the test method to produce a sharp peak repeatedly test to test. Specifically, the last three

tests in Table 3-4 used the same parameters, and produced the same test result. Based on the improved repeatability for the test method, the method development (data generation) was complete.

### 3.3.3. PATP Method Heat of Polymerization Calculation

As a final parameter for the method development, an equation was determined to calculate a heat of polymerization value for each adhesive using the data from each adhesive polymerization test run. This equation included a calorimetric constant, which accounts for heat changes due to the calorimeter type.[19] A calorimetric constant represents the amount of heat required to raise the temperature of the calorimeter 1°C, so it is also known as the heat capacity of the calorimeter.[22] The calorimeter constant was calculated for MPC, BCA, and 2-OCA using Equation 3-3. These adhesives were used with this calculation because data from their heat of polymerization testing was obtainable in this research as well as literature. For this equation, the calculated area (A) under the peak for the temperature versus time plot (A=°C*s) and the mass of adhesive (m) were obtained from the PATP method test results. Each literature HOP value for Equation 3-3 is included in Table 3-1. Using Equation 3-3, the PATP data, and the literature data, the calorimetric constants were determined to be 0.025, 0.023, and 0.026 J/(°C*s) for MPC, BCA, and 2-OCA respectively.[23] The mean calorimetric constant was calculated to be 0.025 with a standard deviation of 0.001 J/(°C*s). This very low standard deviation provides evidence that this calorimetric constant is appropriate for the PATP method's calorimeter for variable types of cyanoacrylate adhesives.

In comparison, the calorimetric constant for a 150 ml lidded, double polystyrene foam cup calorimeter and a 3 L dewar flask were calculated to be 24 J/°C and 447 J/°C respectively.[22],[24] These two values have units that differ from the PATP method due to the difference between the calculated units for the area under the peak for the foam and flask values (A=Wx°C, heat flow versus temperature plot) and the PATP method (A=°C*s, temperature versus time plot). Although the units differ slightly, the comparison between the three calorimetric constants demonstrates that the PATP method constant is much lower than the other two constants. This difference is attributed to the very small, 4 ml volume container used for the PATP method as compared to the 150 ml and 3 L containers used for the foam cup and flask.

In addition to the calorimetric constant, the heat of polymerization equation also included the mass of the adhesive. Each adhesive was applied as a volume, so the mass added during testing had to be calculated based on the density for each adhesive. The density values are included in Table 3-5.

Table 3-5: Density Values for Adhesives

| **Adhesive** | **Formulation Mass (g)** | **Formulation Volume (ml)** | **Density (g/ml)** |
|---|---|---|---|
| Cy96-G10C90L4 | 53.2 | 50 | 1.064 |
| Cy90-G10C90L10 | 57.8 | 50 | 1.156 |
| Cy96-G40C60L4 | 53.3 | 50 | 1.066 |
| Cy90-G40C60L10 | 55.3 | 50 | 1.106 |
| Cy96-G10C90T4 | 53.2 | 50 | 1.064 |
| Cy90-G10C90T10 | 56.1 | 50 | 1.122 |
| Cy96-G40C60T4 | 53.4 | 50 | 1.068 |
| Cy90-G40C60T10 | 56.5 | 50 | 1.13 |

| Adhesive | Formulation Mass (g) | Formulation Volume (ml) | Density (g/ml) |
|---|---|---|---|
| MPC-S | N/A | N/A | 1.05 |
| 2-OCA | N/A | N/A | 1.05 |
| BCA | N/A | N/A | 0.983 |
| EEC | N/A | N/A | 1.06 |

### 3.3.4. Adhesives Testing Using PATP Method Overview

The newly developed method was then used to test the research and benchmark adhesives, with the peak temperature change, reaction rate, and heat of polymerization results included in Figure 3-5, Figure 3-6, and Figure 3-7 respectively

### 3.3.5. Adhesives Peak Temperature Change

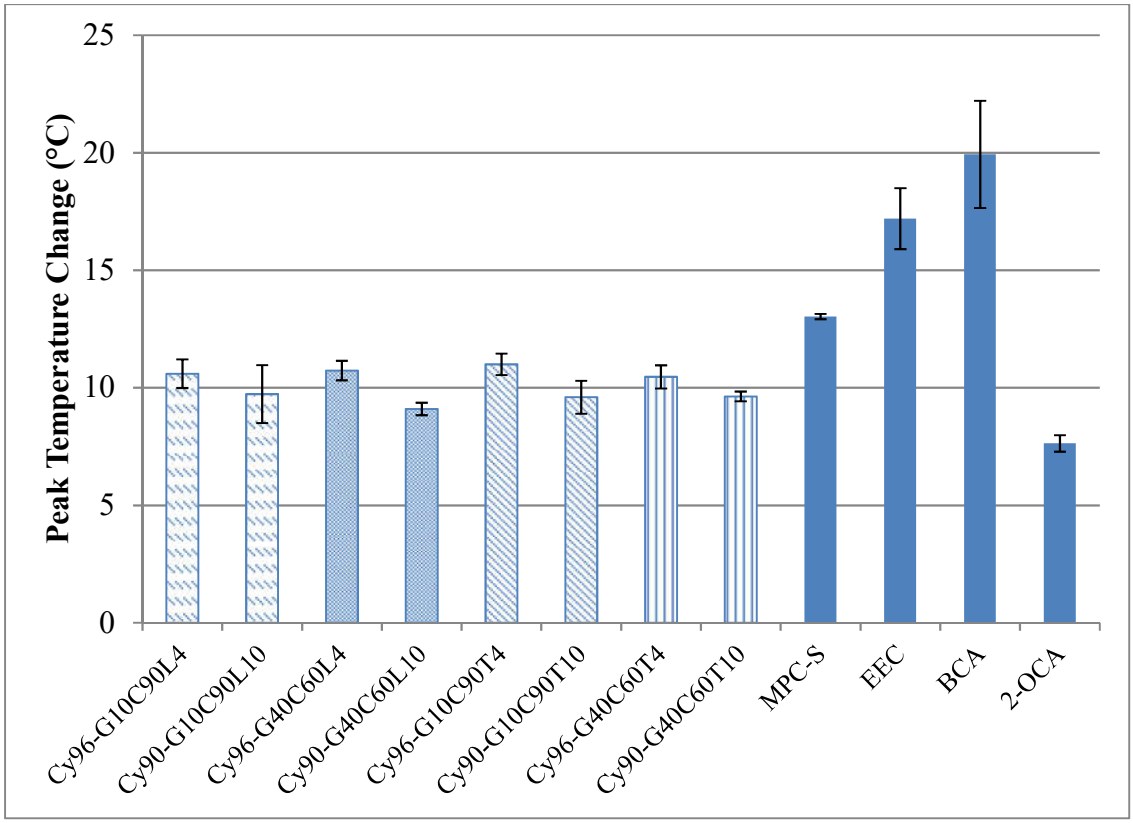


Figure 3-5: Peak Temperature Change for Adhesives by PATP Method (n=3 per adhesive, error bars = 1 standard deviation)

Figure 3-5 appears to indicate that the addition of 4 or 10 weight percent adhesive modifier to the MPC-S causes an immediate drop in the peak temperature change. The adhesive modifier thus appears to act as a heat sink, and absorb a portion of the heat from the adhesive polymerization reaction thereby decreasing the measured peak temperature change. Furthermore, as depicted in Figure 3-5, the increase from 4 to 10 weight percent appears to cause a further decrease in the measured peak temperature change for the polymerizing adhesive. This result was expected because increasing the amount of adhesive modifier would further decrease the amount of adhesive present to react, and increase the heat-sink effect of the adhesive modifier as previously discussed. In comparison to the benchmark adhesives, the research adhesives and MPC-S had a peak temperature change that was greater than 2-OCA, but less than BCA. Based on side chain length, it would be expected that MPC-S with a 3 carbon (propyl) side chain would have a greater peak temperature change than BCA with a 4 carbon (butyl) and 2-OCA 8 carbon (octyl) side chains due to a reduced steric hindrance. MPC-S, however, actually has an increased complexity due to the methoxy group in the side chain, and thus an increased steric hindrance than a typical propyl group alone. For this reason, the MPC-S adhesive has a lower peak temperature change than the BCA. Similarly, EEC with a 2 carbon (ethyl) side chain would be expected to have a greater peak temperature change than BCA; however, EEC also has a ethoxy group in the side chain, and thus an increased steric hindrance than a typical propyl group alone. EEC should thus actually have a lower peak temperature change than BCA, which was the actual result observed from this testing. When all the data was analyzed statistically using ANOVA ($\alpha$=0.05) with Tukey

multiple comparison, the result was a p-value of 0.000 with BCA > EEC > MPC-S, Cy96s, Cy90s > 2-OCA for mean peak temperature change. Although there appears to be difference between the Cy96s and Cy90s, this difference was not statistically significant.

As an additional analysis for this data, the literature peak temperature values in Table 3-1 as determined using alternative methods were compared to the values obtained using the PATP method when possible. Ethoxyethyl cyanoacrylate had a peak temperature change of 14°C in literature, and a 17°C peak temperature change using the PATP method.[16] Dermabond® (a 2-octyl cyanoacrylate product) had a peak temperature change of 11°C in literature, and using the PATP method, 2-octyl cyanoacrylate had a peak temperature change of 7°C.[16] For EEC and 2-OCA, there was only a 3-4°C difference between the literature and PATP method values, which provides evidence of the validity of the novel method for measuring the peak temperature change of polymerizing adhesives.

In terms of biocompatibility, as recorded in Figure 3-1, an increase in temperature of approximately 10°C above 37°C (increase to 47°C), would result in patient discomfort regardless of the time period tested. Based on the adhesives tested (see Figure 3-5), the 10 weight percent research adhesives (the L5 and L2 adhesives) as well as 2-octyl cyanoacrylate with mean increases in temperature of less than 10°C would produce little to no discomfort upon polymerization. In comparison, the other adhesives tested would be likely to produce a level of discomfort ranging from mild to moderate for the patient as the adhesive polymerized. Based on Figure 3-1 and Figure 3-5 the 4 weight percent research adhesives (L4 and L1 adhesives) and MPC-S with mean increases in

temperature of 10-12°C would likely produce mild discomfort, while EEC and BCA with mean increases in temperature of 17-20°C would produce moderate discomfort. Overall, based on Figure 3-1 and Figure 3-5, none of the adhesives tested would be expected to cause burns.

### 3.3.6. Adhesives Reaction Rate

In addition to increases in temperature, the adhesives also had a variable reaction rate (Figure 3-6) depending on the adhesive type.

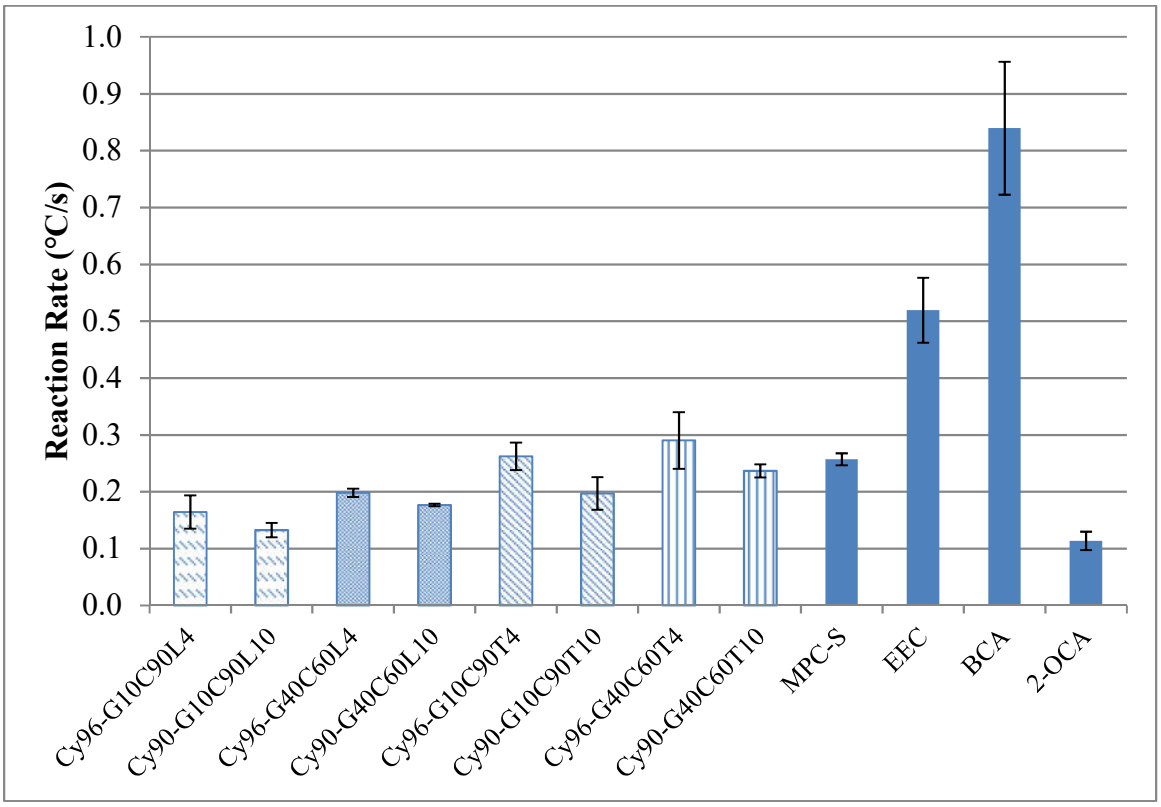


Figure 3-6: Reaction Rate for Adhesives by PATP Method (n=3 per adhesive, error bars = 1 standard deviation)

The reaction rate HOP results in Figure 3-6 are similar to the results in Figure 3-5 for peak temperature change. Specifically, the results follow a similar pattern of BCA > EEC > MPC-S, Cy96s, Cy90s > 2-OCA for mean reaction rate due to the differences in the side chain chemical structure for each adhesive type. The results also follow the pattern of adhesives with increased weight percent adhesive modifier having a decreased mean reaction rate. These relationships were previously discussed in more detail for the peak temperature change results and discussion.

There were also several inconsistencies between the reaction rate and peak temperature change results for the Cy96s, Cy90s, and MPC-S, though. First, the Cy96s and Cy90s mean reaction rates appear to increase as the Cy96s and Cy90s type increases (e.g. Cy96-G10C90L4 > Cy96-G40C60L4, and Cy90-G10C90L4 > Cy90-G40C60L4 etc.). Second, MPC-S appears to have a lower mean reaction rate than Cy96-G10C90T4 and Cy96-G40C60T4. When all the data was analyzed statistically using ANOVA ($\alpha$=0.05) with Tukey multiple comparison, the result was a p-value of 0.000 with BCA > EEC > MPC-S, Cy96s, Cy90s > 2-OCA for mean reaction rate. Based on this statistical analysis, although there appears to be a difference between the Cy96s versus MPC-S and Cy90s versus MPC-S, this difference was not significant.

For the biocompatibility, although the reaction rate was at most approximately 0.85°C/second as tested for BCA, the peak temperature change was only 20°C; thus, the adhesive's polymerization would only likely produce moderate discomfort with no burns. The other adhesives tested, with reaction rates of approximately 0.5°C/second or less, would produce little to no discomfort (Cy90 adhesives as well as 2-OCA), mild

discomfort (Cy96 adhesives as well as MPC-S), or moderate discomfort (EEC) with no burns based on the peak temperature change for each adhesive and Figure 3-1 as previously discussed.

### 3.3.7. Adhesives Heat of Polymerization

In addition to increases in temperature and variable reaction rate, the adhesives also had a variable heat of polymerization (Figure 3-7) depending on the adhesive type.

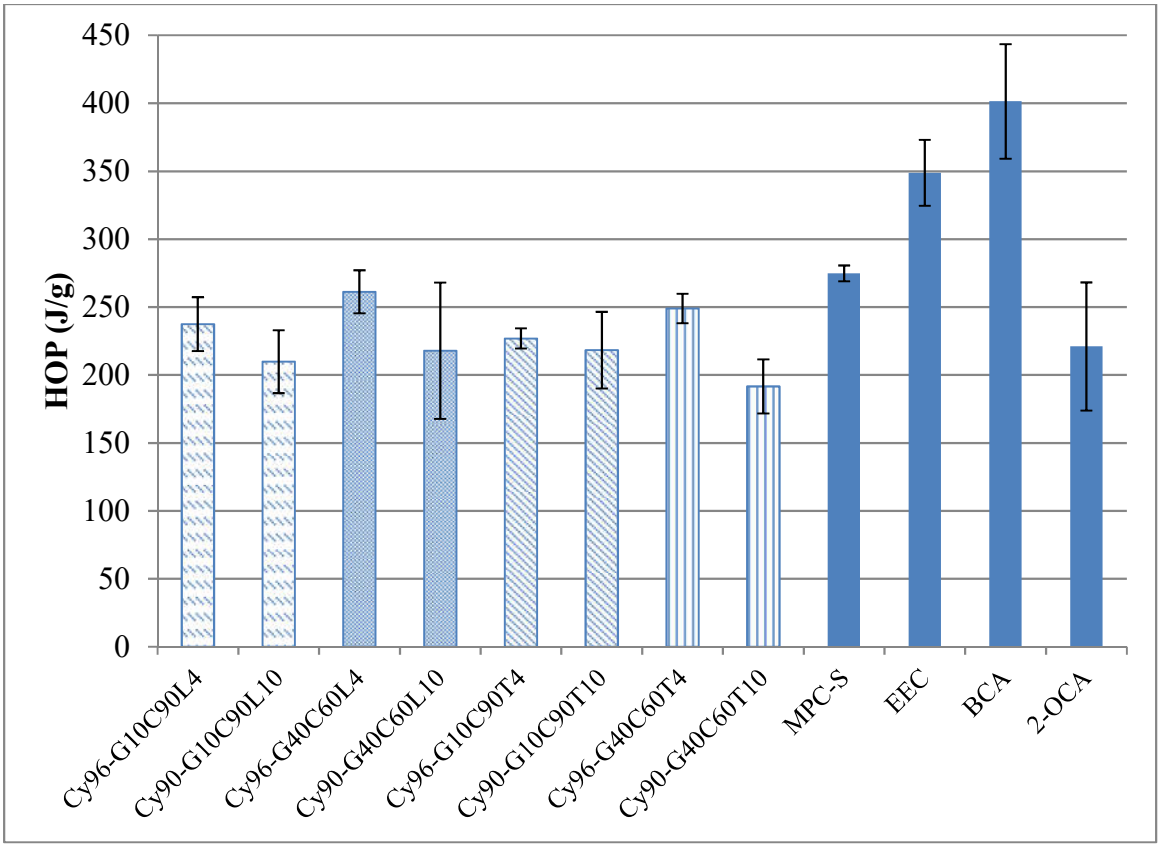


Figure 3-7: HOP for Adhesives by PATP Method (n=3 per adhesive, error bars = 1 standard deviation)

The reaction rate HOP results in Figure 3-7 are similar to the results in Figure 3-5 for peak temperature change; however, there is one difference. Specifically, the results

follow a similar pattern of BCA > EEC > MPC-S, Cy96s, Cy90s for mean HOP; however, unlike the peak temperature change and reaction rate data, the 2-OCA was measured to have a similar mean HOP as the Cy90s. Theoretically, this result would mean that although the peak temperature change was less for 2-OCA than the other adhesives, the length of time for the fully polymerization to occur would be longer resulting in a similar level of heat released as compared to the Cy90s. When all the data was analyzed statistically using ANOVA ($\alpha$=0.05) with Tukey multiple comparison, the result was a p-value of 0.000 with BCA > EEC > MPC-S, 2-OCA, Cy96s, and Cy90s for mean reaction rate. Based on this statistical analysis, although there appears to be a difference between the 4 and 10 weight percent adhesives, this difference was not significant. In addition, the statistical analysis indicates that there was no significant difference between the Cy96s versus 2-OCA and Cy90s versus 2-OCA.

For biocompatibility, as discussed in Figure 3-1 tissue response to elevated temperatures is a function of both temperature magnitude and temperature exposure time. When comparing the Cy90s and 2-OCA, the Cy90s were measured to have a greater mean peak temperature change than 2-OCA, but similar mean HOP as the 2-OCA. Based on this comparison, the Cy90s are likely to have a similar biocompatibility as the benchmark 2-OCA in terms of the thermal tissue response to the polymerizing adhesives.

### 3.3.8. PATP Method Repeatability

The developed polymerizing adhesive thermal properties test method was a new method, so its repeatability was briefly examined to determine whether the method was acceptable to measure the peak temperature change, reaction rate, and HOP data for polymerizing adhesives. The coefficient of variation (CoV = sample standard deviation / sample mean) was calculated for each data type as a measure of the repeatability for the developed test method. The CoV summary for each data type can be found in Figure 3-8.

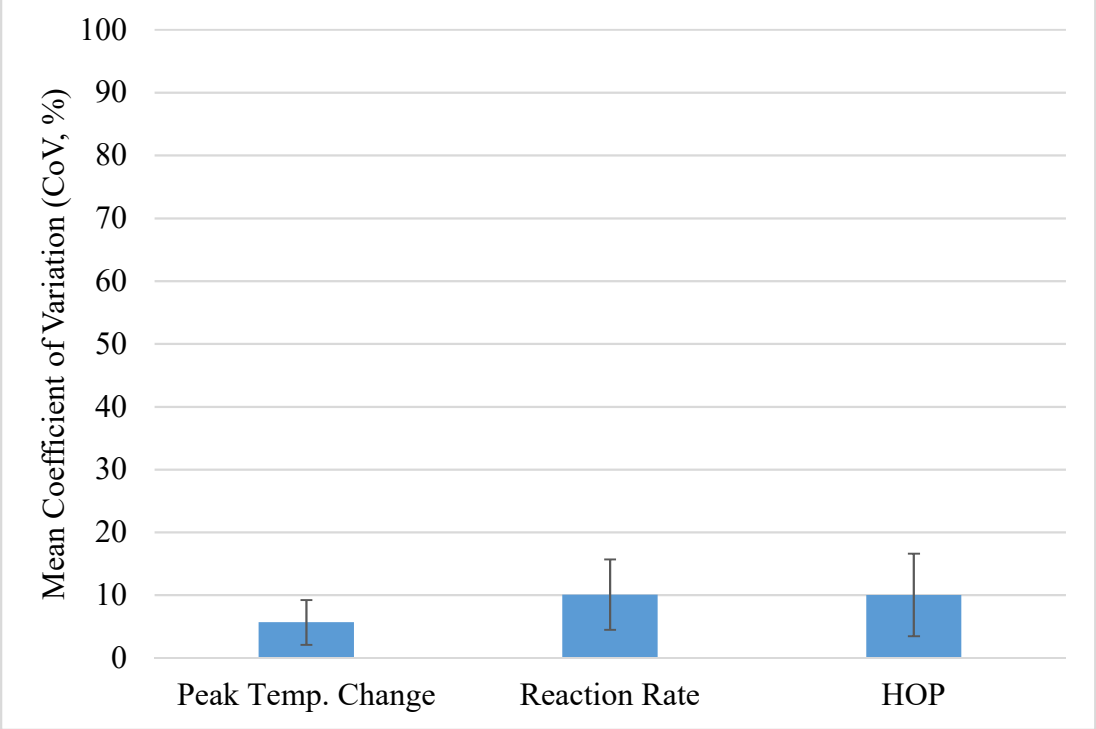


Figure 3-8: CoV Analysis for Polymerizing Adhesive Thermal Properties Test Method (all adhesives, error bars = 1 standard deviation)

Based on the CoV data in Figure 3-8, the mean CoV for each data type was less than 20% for all the adhesives tested. Using a scale of 0-100%, the developed method thus has a moderate level of repeatability. Further testing using gage R&R is recommended if one desired to qualify the test method.

## 3.4. Conclusions

In conclusion, the research completed resulted in the creation of a novel method to measure the peak temperature change, reaction rate, and heat of polymerization for cyanoacrylate adhesives. Although the method was not qualified through gage R&R or other quality metric analysis, based on the mean CoV of less than 20% for each data type tested, the developed method has a moderate level of repeatability. Using this method, an inverse relationship between adhesive modifier amount and measured peak temperature change, reaction rate, and HOP was determined. This result provides evidence that the hypothesis that that there is an inverse relationship between adhesive modifier weight percent versus adhesive reaction rate, peak reaction temperature, and heat of polymerization may be true. This relationship is expected to be due to a decrease in the amount of adhesive present as the amount of adhesive modifier increases, and due to an increase in the heat sink effect from the adhesive modifier. In addition, the data collected demonstrates that for a cyanoacrylate's side chain, there is an inverse relationship between chemical group complexity and length as compared to peak temperature change, reaction rate, and HOP. This relationship is expected to be due to a difference in steric hindrance between the adhesive types. Overall, the Cy96s and Cy90s developed were

measured to have a peak temperature change, reaction rate, and HOP less than BCA and greater than or equal to 2-OCA (the benchmark adhesives). The Cy96s and Cy90s are thus expected to have acceptable biocompatibility in terms of the tissue response to the exothermic reaction from the polymerizing adhesive.

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# CHAPTER 4

# ADHESIVES FILM FABRICATION AND MECHANICS

## 4.1. Introduction

Cyanoacrylate is typically applied during external or internal wound approximation as a thin layer that polymerizes to form a film. Although it is common to use cyanoacrylates for external wound approximation, they are not currently used extensively for internal wound approximation due to their lower burst strength and biocompatibility as compared to typical sutures (e.g. Vicryl®). By modulating the cyanoacrylate adhesive and thereby the polymerization of the adhesive (film formation), the resulting polycyanoacrylate's properties can be tailored for not only mechanical flexibility, but other properties such as improved wound approximation and biocompatibility. Wound healing in the gastrointestinal tract typically follows the standard wound healing cycle with hemostasis and wound approximation first required.[1] Previous research has demonstrated that when an adhesive is applied to internal tissue such as the small and large intestine for wound approximation, the resulting adhesion typically has reduced burst strength as compared to sutures.[2]–[5] Nevertheless, in at least one study, a BCA adhesive (Glubran® 2) produced sufficient burst strength to resist physiological pressures.[5] This property of a polycyanoacrylate is dependent on the polymerized film's flexibility and adhesion strength. In a separate study, isobutyl cyanoacrylate was used to close incisions of the small intestine with only an 8.7% complication rate.[6] The small intestine may be well suited for wound

approximation by select cyanoacrylate adhesives because the small intestine is a low pressure system, so it matches the lower burst strength for polycyanoacrylates. It is also best closed using minimal tension across the wound, which can be achieved using a flexible polycyanoacrylate such as 2-OCA.[7] Lastly, following a review of 22 articles, a group of researchers determined that inverted anastomosis with cyanoacrylate resulted in a positive outcome.[8] There is thus potential for cyanoacrylates to be used internally for wound approximation.

When cyanoacrylate monomer is applied to tissue *in vivo* for wound approximation, the adhesive will typically polymerize, changing from a liquid to a solid film. In specific cases, such as with long alkane side chains including 2-octyl, an initiator must be added to polymerize the adhesive. As previously discussed, this anionic polymerization can be initiated by water; however, there are also other types of initiators. In one study, N,N-dimethyl-p-toluidine (DMT), pyridine (Pyr), triethyl amine (Et3N), azobicyclo[2.2.2]octane (ABCO), and diazobicylo[2.2.2]octane (DABCO)] were investigated for their ability to initiate the polymerization of 2-OCA.[9] Each of the compounds could initiate the polymerization of 2-OCA with DMT demonstrated to be the weakest initiator of the five tested. Using a weaker initiator can be advantageous in order to lengthen the polymerization time, to allow the initiator to be added to the adhesive, mixed, and then poured into a mold prior to the adhesive setting (changing from a liquid to a solid) and then forming a polymerized adhesive film. The weaker initiator can also decrease the peak reaction temperature for the polymerizing adhesive, which would

enable the adhesive+initiator mixture to be handled safely by hand during the polymerization.

There are also other adhesive film fabrication methods. In one method, 10 µl of adhesive in a culture plate is exposed to ambient room conditions (temperature and humidity) for 24 hours.[10] In another method, dimethylformamide, nitromethane, THF containing triphenylphosphine, and/or 7.4 pH buffered solution are added to the adhesive to initiate its polymerization. The polymerized adhesive is then dissolved in acetone, cast onto a PET film, and then the acetone is allowed to vaporize to leave the polymerized adhesive as a film.[11] As another method, triethyl amine is added to an adhesive to polymerize it. The polymerized adhesive is then dissolved in ethyl acetate, poured into a PTFE mold, and then the ethyl acetate is allowed to vaporize to leave the polymerized adhesive as a film.[12]

The polycyanoacrylate will have variable properties depending on several factors including amount of monomers and oligomers remaining from the cyanoacrylate's polymerization, cyanoacrylate volume, polymerized shape (mold), and amount and type of adhesive modifiers contained in the adhesive formulation. The anionic polymerization of cyanoacrylates will typically result in a straight chain of connected cyanoacrylate monomers (polymer) as, ideally, one long chain with no oligomers and all monomer exhausted. Short chains of connected monomers that terminated before joining the main cyanoacrylate chain will result in oligomers. Incomplete polymerization is marked as monomer present after the polymer chain and oligomers have terminated. These oligomers and monomers are undesirable because they decrease the polymer's overall

molecular weight. They also degrade more rapidly than polymer, which can result in an increased release of formaldehyde, thereby lowering the biocompatibility for the polycyanoacrylate.[13]

As another factor affecting a cyanoacrylate's properties, the side chain for the cyanoacrylate will cause the flexibility for the polymerized film to vary. Specifically, there is a direct relationship between an adhesive's side chain length and/or complexity versus the flexibility of the polymerized adhesive.[10], [14] For this reason, 2-octyl cyanoacrylate (2-OCA) with an 8 carbon side chain is more flexible than n-butyl cyanoacrylate (n-BCA) with a 4 carbon side chain. Similarly, ethoxyethyl cyanoacrylate (EEC) is more flexible than methoxyethyl cyanoacrylate because the ethoxyethyl side chain is a more complex (bulky) group than the methoxyethyl side chain. Overall, as the side chain length and/or complexity increases, there is an increased amount of disorder for the polymerized polymer due to a reduction in intermolecular and intramolecular forces. In addition, ether groups present in the side chain such as with EEC and methoxyethyl cyanoacrylate have an increased ease of rotation due to the reduced amount of linkages for the oxygen atom as compared to a carbon atom, which makes the molecular chains more flexible.[10]

Regardless of the type, the amount of adhesive polymerized (volume) is inversely related to the flexibility of the polymerized adhesive. Specifically, as the amount of adhesive is increased, the flexibility of the polymerized adhesive decreases. This relationship is dependent on the polymerized shape (mold) because, for example, a taller mold would be needed to contain a large amount of volume. A mold that will result in a

thick polymerized film will decrease the flexibility of the polymerized adhesive. The adhesive volume and geometry affect the flexibility for the polymerized adhesive because it affects the polymerized adhesive's cross-sectional area ($A_0$). This property of the polymerized adhesive is related to the modulus of elasticity (E) according to the equation $E=\sigma/\epsilon$ where $\sigma$ is stress ($\sigma=F/A_0$) and $\epsilon$ is strain ($\epsilon=\Delta l/l_0$).[15] The equation for E demonstrates that $A_0$ and E are inversely related.

Similar to the type of cyanoacrylate, the amount and type of adhesive modifiers contained in the adhesive formulation will also affect the flexibility of the polymerized adhesive. Adhesive modifiers with an increased amount of disorder or ease of rotation such as polycaprolactone will naturally have an increased amount of flexibility. In comparison, adhesive modifiers with a decreased amount of disorder such as polyglycolide will naturally have a decreased amount of flexibility. The flexibility of the adhesive modifiers can thus add to or take away from the flexibility of the polymerized adhesive. There are also examples of modifiers that are directly incorporated into the polycyanoacrylate chain such as polyethylene glycol (PEG) modified polycyanoacrylate adhesives. These specific PEG based adhesives had an increased flexibility when compared to BCA.[16]

Based on this background, it was desirable to fabricate polymerized films of the research and benchmark adhesives in order to study the films' dimensions, mechanical strength, and mechanical flexibility. Polymerized films have a three dimensional shape with a specific importance on the film thickness. In particular, strength and flexibility can provide an estimate of the expected burst strength for the polymerized adhesive. This

evaluation would allow one to test the hypothesis that there is a direct relationship between adhesive modifier weight percent versus polymerized adhesive flexibility. In addition, the hypothesis that there is a direct relationship between adhesive modifier ε-caprolactone molar percent versus polymerized adhesive flexibility could also be tested by this evaluation.

## 4.2. Materials and Methods

### 4.2.1. Adhesives Polymerization Method Development

Water, DMT, and benzalkonium chloride were first tested as polymerization initiators for methoxypropyl cyanoacrylate (MPC) in varying amounts of both initiator and adhesive as included in Table 4-1.

Table 4-1: MPC and Initiator Volumes for Each Initiator Test

| Initiator Type | Initiator Volume (µl) | MPC Volume (µl) |
|---|---|---|
| Water | 2000 | 40 |
| DMT | 2.5 | 3000 |
| BZC | 30 mg BZC in 60 µl Acetone | 6000 |

For these initial tests, MPC was first measured and dispensed using a pipette into a polypropylene (PP) tube. Next, an initiator was then measured and added into the adhesive filled tube. The tube was then briefly, rapidly agitated using a vortex mixer. It was then determined if the adhesive polymerized by visually inspecting the contents of the tube.

Subsequent to this initial testing, the initiator:adhesive ratio for MPC-S (base cyanoacrylate for the research adhesives) as well as mold type were determined. DMT volumes of 4, 8, and 12 µl were first attempted with 6000 µl MPC-S. A release paper folded tray with dimensions of 3”x3”x0.5” (LxWxH) was then tested as a potential adhesive mold. The release paper used was obtained from the Paul N. Gardner Company, Inc. (Gardco). It was a 0.13 mm thick paper with a glossy side containing a silicone finish and an opposite matte finish side. PTFE film trays were next tested. The trays were cut and folded to dimensions of 1”x1”x0.5” (LxWxH) and 0.5”x2”x0.5” (LxWxH) to fabricate test specimens for hydrolysis and 3 point bend testing respectively.

An initiator:adhesive ratio specific for these small trays was then determined. The adhesive amount was first decreased from 6 ml to 1 ml. A DMT initiator amount needed to polymerize the 1 ml aliquot of adhesive was then selected. For this initial evaluation and subsequent film fabrication, the 15 ml PP centrifuge tubes were replaced with 5 ml PP centrifuge tubes. Using these new tubes, DMT volumes of 3, 2.5, 2, 1.75, 1, and 0.75 µl were tested with 1 ml aliquots of MPC-S. Other DMT volumes were also tested due to the different types of research and benchmark adhesives to be polymerized. The goal with each initiator amount for the adhesives polymerized was to have the adhesive set in approximately 60 seconds as previously discussed. The final DMT amounts per adhesive polymerized can be found in Table 4-2.

Table 4-2: Adhesives Polymerized and Initiator Amounts

| Adhesive | DMT Volume (µl) |
|---|---|
| Cy96-G10C90L4 | 1 |
| Cy90-G10C90L10 | 2 |
| Cy96-G40C60L4 | 1 |
| Cy90-G40C60L10 | 1.5 |
| Cy96-G10C90T4 | 1 |
| Cy90-G10C90T10 | 1.5 |
| Cy96-G40C60T4 | 1 |
| Cy90-G40C60T10 | 1.75 |
| MPC-S | 1 |
| EEC | 0.7 |
| BCA | 0.6 |
| 2-OCA | 60 |

### 4.2.2. Final Adhesives Polymerization Method

The final method for preparing the polymerized films was to first measure and dispense 1 ml adhesive into a 5 ml PP centrifuge tube using a pipette. DMT (see Table 4-2) was then measured by pipette and dispensed into the tube. A vortex mixer was next used to mix the tube's contents for three seconds. The mixed material was then poured into a PTFE tray. The mixture was next uniformly spread by slightly tilting the tray to spread it before allowing it to set for approximately 60 seconds. Each adhesive was set once it changed from a liquid to a solid. The set films were then left in a chemical fume hood with a piece of Gardco release paper placed on top of the PTFE trays. Each tray was left for at least 24 hours for the adhesive to fully polymerize. The films were prepared in triplicate by tray size (1"x1"x0.5" and 0.5"x2"x0.5") and adhesive type. After at least 24 hours, the polymerized films were removed from the PTFE trays, and then placed into re-

closable polyethylene bags. The filled bags were then placed into a refrigerator at approximately 4°C for storage.

### 4.2.3. Polymerized Adhesive Dimensional Analysis

At a later date, the adhesive films were removed from storage, and placed at room temperature until their temperature had equilibrated with the room temperature. Each of the 1”x1” films were then broken into nine approximately 0.33”x0.33” pieces. Six pieces that had the most similar shape to each other of the set of nine were then imaged against a ruler as a top view and then a side view. The images were then analyzed using ImageJ software to determine the top plane area and thickness for each imaged polymerized adhesive piece.[17] For this analysis, the ruler in the image was used to set the scale for the image in the software. Thickness was measured by drawing a line for the height of each piece using the side view images. Top plane area was measured by tracing the perimeter for each piece using the top view images.

### 4.2.4. Polymerized Adhesive Composition

Select polymerized adhesives were analyzed using proton nuclear magnetic resonance ($^{1}$H NMR) to determine the polymerized composition and extent of polymerization by monomer remaining. Proton nuclear magnetic resonance (NMR) was conducted using a JEOL 300 ECX spectrometer. Samples were dissolved in deuterated chloroform at concentrations of 20 mg/mL, and data was collected with a 16-scan profile. The composition for the tested film pieces was determined based on the peak locations on

the NMR spectrum result. This composition included weight percent of polymerized MPC-S, weight percent of MPC-S monomer, and weight percent of adhesive modifier polymer. The extent of polymerization by monomer remaining was defined by the calculated weight percent of MPC-S monomer from the NMR testing results.

### 4.2.5. Polymerized Adhesive Flexibility

The 0.5”x2” films were then tested to determine their modulus of elasticity (flexibility) using a 3-point bend method. This testing was performed using an MTS Synergie 200 with a 1 kN load cell, TestWorks® software, and two grips. Briefly, these grips included two bottom support bars as one grip with a gap separation of 17.2 mm between them. In addition, a top bar as a second grip was held above the two bottom bars at the center of their gap. Prior to each test, the width (W) and thickness (Th) of a film was measured using digital calipers and input into the TestWorks software. The measured film was then centered on the bottom bars. The top bar was then lowered until it just contacted the film as measured by a change in load of approximately 0.1 N or less. During each test, the top bar was lowered at 2 mm/minute while the software recorded the change in load and change in height of the top bar (extension). Each test was ended when the film fractured or bent into a V shape causing it to fall off the two bottom supports. The modulus of elasticity was then calculated by the TestWorks software according to the equation $E=\sigma/\epsilon$ as previously discussed. The software used the input width and thickness values, as well as the measured change in load and extension for the

initial linear portion of the load versus extension curve results to calculate the stress, strain, and lastly modulus of elasticity for the film.

## 4.3. Results and Discussion

### 4.3.1. Adhesives Polymerization Method Development

The research adhesives (Cy96s and Cy90s) had been formulated (MPC-S + novel polyester adhesive modifier), but now a consistent method needed to be determined to polymerize them. Literature research indicated that water, DMT, and benzalkonium chloride (BZC, initiator for Dermabond®) were known cyanoacrylate initiators, so these initiators were first tested to determine their ability to polymerize MPC.[18] MPC was used instead of MPC-S for this testing in order to conserve the amount of MPC-S in stock. The results of testing the initiators can be found in Table 4-3.

Table 4-3: Cyanoacrylate Polymerization Initiator Tests Results

| Initiator Type | Mixture Result |
|---|---|
| Water | Mixture could not be dispensed from mixing tube. Adhesive swelled 3x its initial area. All of the adhesive set within 60 s. |
| DMT | Mixture could be dispensed from mixing tube. Adhesive did not swell. All of the adhesive set within 60 s. |
| BZC in Acetone | Mixture could not be dispensed from mixing tube. Adhesive did not swell. Partial adhesive polymerization at the MPC-initiator interface only. |

In general, water polymerized the adhesive too rapidly and with swelling. Also, BZC only polymerized the adhesive at the interface between the BZC and the adhesive only (no bulk polymerization). In comparison, DMT could be added, mixed into the adhesive, and the resulting mixture could be poured out on to release paper prior to setting. For this reason, DMT was selected as the initiator for the adhesive polymerization for this research.

When DMT was used with the anionically stabilized MPC (MPC-S), the adhesive set in approximately 30 minutes, which was much greater than the 60 seconds observed with MPC. The anionic stabilization of the MPC was the expected reason for the longer set time for MPC-S as compared to MPC when the same amount and type of initiator as well as adhesive volume were used. For this reason, the optimal amount of DMT needed to set MPC-S had to be determined especially because MPC-S was the base adhesive for the research adhesives. When DMT volumes of 4, 8, and 12 µl were first attempted with 6000 µl MPC-S, the 12 µl volume was selected as the optimum volume because it set the adhesive in approximately 30 seconds.

Next, the mold type (material and size) had to be selected. The Gardco paper was first tested with the plan to polymerize the adhesive on the glossy side, and then easily peel it off. When the adhesive polymerized in these paper trays, however, the large amount of heat from the adhesive's polymerization caused an increased stiffness in several areas of the film. The matching area on the paper of these films was no longer glossy, so it was assumed there was a transfer of material from the paper to the film. In addition, in several of these cases, the paper adhered to the film, so it could not be readily

removed. For this reason, PTFE film trays were tested as an alternative material. The PTFE trays did not result in the areas of increased stiffness when initially tested, so this material type was selected for the fabrication of the films for this research. For the trays dimensions, the 9 inch$^2$ film size was initially selected to be able to cut several of the films into 9 1”x1” test specimens for hydrolysis testing and 3 1”x3” test specimens for 3 point bend testing. These 3”x3” test films were brittle, so they could not be cut into the 1”x1” and 1”x3” sizes, however. For this reason new trays were cut and folded to dimensions of 1”x1”x0.5” (LxWxH) and 0.5”x2”x0.5” (LxWxH) to fabricate test specimens for hydrolysis and 3 point bend testing respectively.

The smaller size trays required less adhesive to achieve a thin film, so a new initiator:adhesive ratio had to be determined. The adhesive amount was first decreased from 6 ml to 1 ml. This new volume would result in a film that was approximately 1 cm$^3$ (0.061 in.$^3$) assuming full transfer of the 1 ml adhesive to the PTFE tray following initiator addition and mixing. Based on the dimensions of the smaller trays, the expected film thickness (with the previously discussed assumption) would be 0.061 inch (1.55 mm). Based on the initial MPC-S tests, not all the adhesive would transfer from the mixing tube to the tray. The 1 ml volume was thus expected to result in a polycyanoacrylate film of 1-1.5 mm thickness.

For the film fabrication, the PP centrifuge tube size was decreased from 15 ml to 5 ml. This change was done to decrease the surface area in the tube to allow for better mixing and potentially to increase the amount of mix material transferred from the tube to the PTFE tray. Using this new tube size, it was determined that of the DMT volumes

tested, only 1.75, 1, and 0.75 µl were able to added to the adhesive, mixed with the adhesive in the tube for three seconds, and then dispensed into the PTFE tray. For these three volumes, 1 µl was the optimum amount tested to allow a set time closest to 60 seconds to mimic the typical set time of Dermabond®, so this DMT amount was used as the base amount for polymerizing the adhesives for this research.[19]

The 1 µl DMT volume had to be further increased or decreased due to the type and amount of adhesive polymerized. For example, the DMT volume had to be decreased for EEC because it more readily polymerizes than the MPC-S due to a decrease in side chain length and thus less shielding of the cyanoacrylate's electronegative groups. In comparison, the DMT volume had to be increased for 2-OCA because it does not readily polymerize due to an increase in side chain length and thus increased shielding of the cyanoacrylate's electronegative groups. Lastly, the Cy90 adhesives required more DMT to set in a similar amount of time as the other adhesives. This change was assumed to be due to the increased amount of adhesive modifier present interacting with the DMT, and thereby decreasing the amount of DMT available to interact with the MPC-S present in each Cy90 adhesive. The goal with each initiator amount for the adhesives polymerized was to have the adhesive set in approximately 60 seconds as previously discussed. After setting, each tray was left for 24 hours for the adhesive to fully polymerize with the full polymerization time determined by the MPC, EEC, BCA, and 2-OCA manufacturers.

### 4.3.2. Polymerized Adhesives Dimensional Analysis

The results from the top plane area and thickness analysis for each 0.33”x0.33” test specimen are included in Figure 4-1 and Figure 4-2.

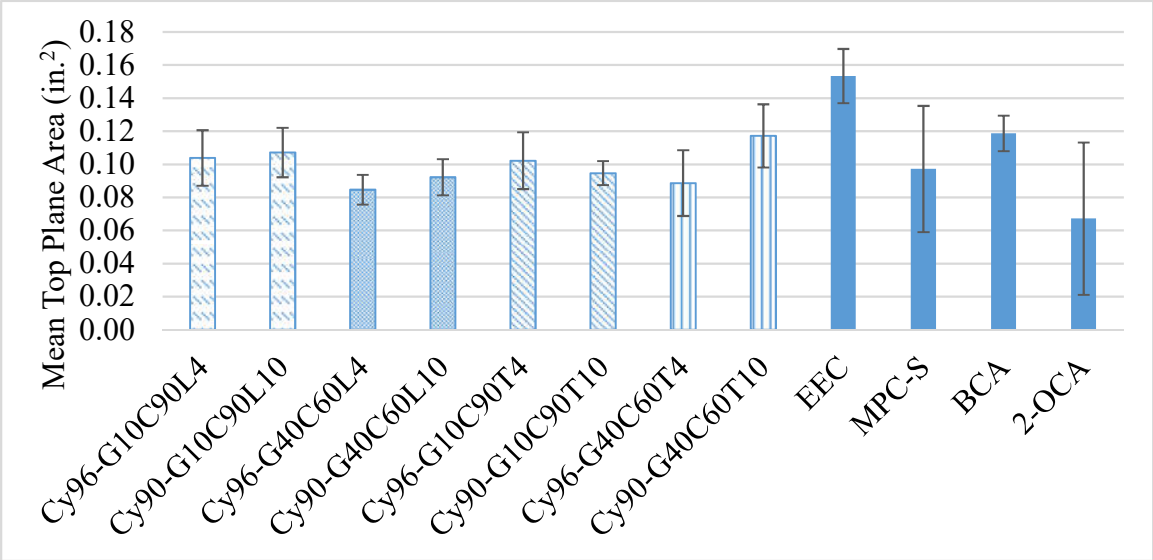


Figure 4-1: Mean Top Plane Area for Polycyanoacrylate 0.33”x0.33” Test Specimens (n=3 per adhesive, error bars = 1 standard deviation)

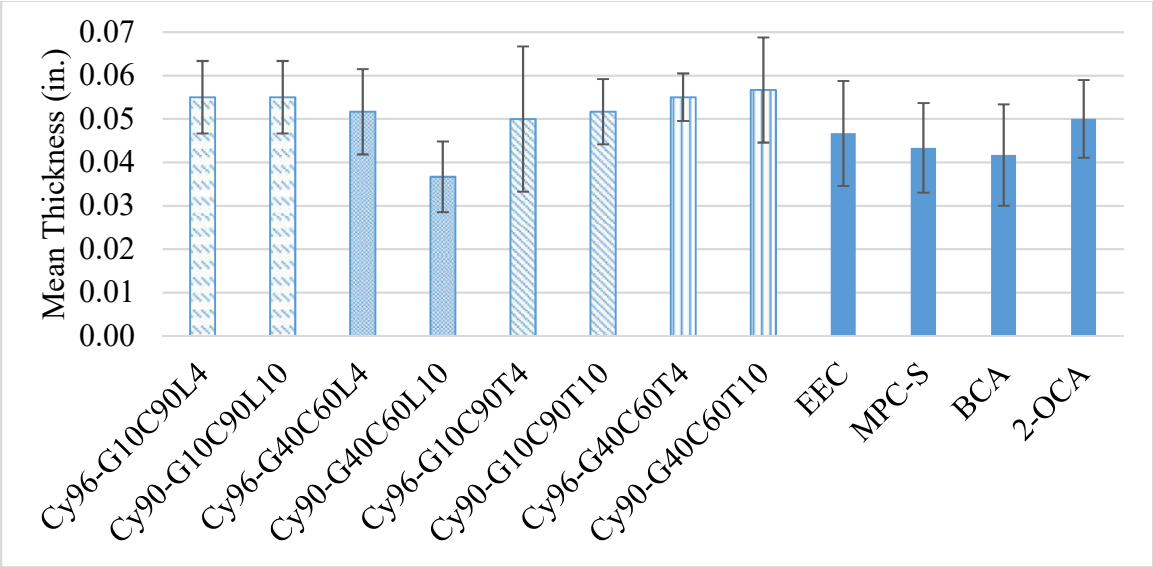


Figure 4-2: Mean Thickness for Polycyanoacrylate 0.33”x0.33” Test Specimens (n=3 per adhesive, error bars = 1 standard deviation)

Based on a review of the summarized data in Figure 4-1 and Figure 4-2, the area data and the thickness data for all the adhesives tested appear to be similar by data type. Although the area and thickness data vary for each adhesive, this variation is expected to be due to the known variability in the method to break the 1”x1” polymerized films into the 0.33”x0.33” test specimen. Specifically, because most of the films were brittle, when the films were broken into pieces, they broke like a piece of glass forming variable sized shards instead of the ideal 0.33”x0.33” size. The EEC and 2-OCA films were flexible enough to be cut into pieces using scissors rather than being broken into pieces; however, the cutting method also appeared to produce variability for the specimen sizes. The thickness appears to be less variable between the adhesives; however, similar to the area data, there appears to be a high variability for the thickness for the test specimens for each adhesive. This variation is likely because although the trays were shaken to spread the adhesive evenly in them as it set, the adhesive still naturally preferentially flowed to the corners and edges of the tray and away from the center of the tray. For this reason, the adhesive specimens were slightly thicker on the corners and edges of the trays and slightly thinner in the center of the tray. Although there was variation present, the sizes were deemed similar enough in terms of their surface area for this property to be a minimal factor in the variation of the release testing properties for the polycyanoacrylates.

### 4.3.3. Polymerized Adhesives Composition

The composition and extent of polymerization by monomer remaining were determined for select 0.33”x0.33” pieces of polymerized adhesive by NMR. The results from this testing can be found in Table 4-4.

Table 4-4: Polymerized Adhesives Total Composition

| | Poly(MPC-S) Weight Percent* | | Polyester Adhesive Modifier Weight Percent | | MPC-S Monomer Weight Percent | |
|---|---|---|---|---|---|---|
| **Polymerized Adhesive** | **Theoretical (%)** | **Actual (%)** | **Theoretical (%)** | **Actual (%)** | **Theoretical (%)** | **Actual (%)** |
| Cy96-G40C60L4 | 96 | 97 (98) | 4 | 2 | 0 | 1 |
| Cy90-G40C60L4 | 90 | 88 (89) | 10 | 11 | 0 | 1 |
| Cy96-G10C90T4 | 96 | 96 (98) | 4 | 2 | 0 | 2 |
| MPC-S^ | 100 | 100 | 0 | 0 | 0 | 0 |

*The values in parentheses are the weight percent for the adhesive composition calculated as polymer only.
^There was no monomer or other compounds detected for poly(MPC-S), so the composition was 100% poly(MPC-S).

For the previously discussed NMR testing, Cy96-G40C60L4 and Cy96-G10C90T4 were selected for testing to determine whether there was a difference in polymerization for the two types of adhesive modifiers used for this research (linear versus triaxial polymer chain). There was not expected to be a difference because the adhesive modifier was expected to be physically polymerized within the adhesive, but not chemically part of the polycyanoacrylate chain because the adhesive modifiers were not functionalized (open end groups attached). Based on the results in Table 4-4, there was nearly no difference between the two polymerized adhesives by composition. The results

also demonstrate that there was only two weight percent adhesive modifier instead of four weight percent. This result was unexpected because of the known charges added to prepare the monomer adhesives as discussed in Chapter 2. This unexpected result could be due to non-homogenous mixing of the adhesive modifier into the MPC-S. In comparison, the actual results for the composition of the polymerized Cy90-G40C60L4 adhesive nearly matched the theoretical results in Table 4-4. These differences in composition deviation for the Cy96s and Cy90s could be due to variability in the mixing method and NMR measurement method. Overall, the difference between the actual and theoretical polymer only composition for Cy96-G40C60L4 and Cy96-G10C90T4 was only two weight percent as compared to Cy90-G40C60L10 with a difference of one weight percent; thus, the composition variation was deemed acceptable because the variation was minimal.

In addition, several of the hypothesis statements for this dissertation as included in Chapter 1 include comparing polymerized adhesives with a small amount and large amount of adhesive modifier. The adhesive composition results included in Table 4-4 indicate that the polymerized adhesives contained two weight percent or 11 weight percent adhesive modifier; thus, the hypothesis statements for this dissertation were able to be tested. Lastly, based on the MPC-S monomer remaining in the adhesive of two weight percent or less (with one weight percent or less preferred); the polycyanoacrylates were deemed sufficiently polymerized.[20] This extent of polymerization was important because a large amount of monomer would likely increase the hydrolysis rate yielding false results for the polycyanoacrylate's degradation as discussed in the Introduction

section for this chapter. Although the other adhesives were not tested for composition and extent of polymerization, these properties for the other adhesives were expected to be similar to the NMR results collected and analyzed for these select adhesives due to the use of a similar polymerization for the other adhesives.

### 4.3.4. Polymerized Adhesives Flexibility

Other than the 1"x1" films, testing was also performed for the 0.5"x2" films. These latter films were 3-point bend tested as previously discussed, with the mean modulus of elasticity values for each polymerized adhesive found as Figure 4-3.

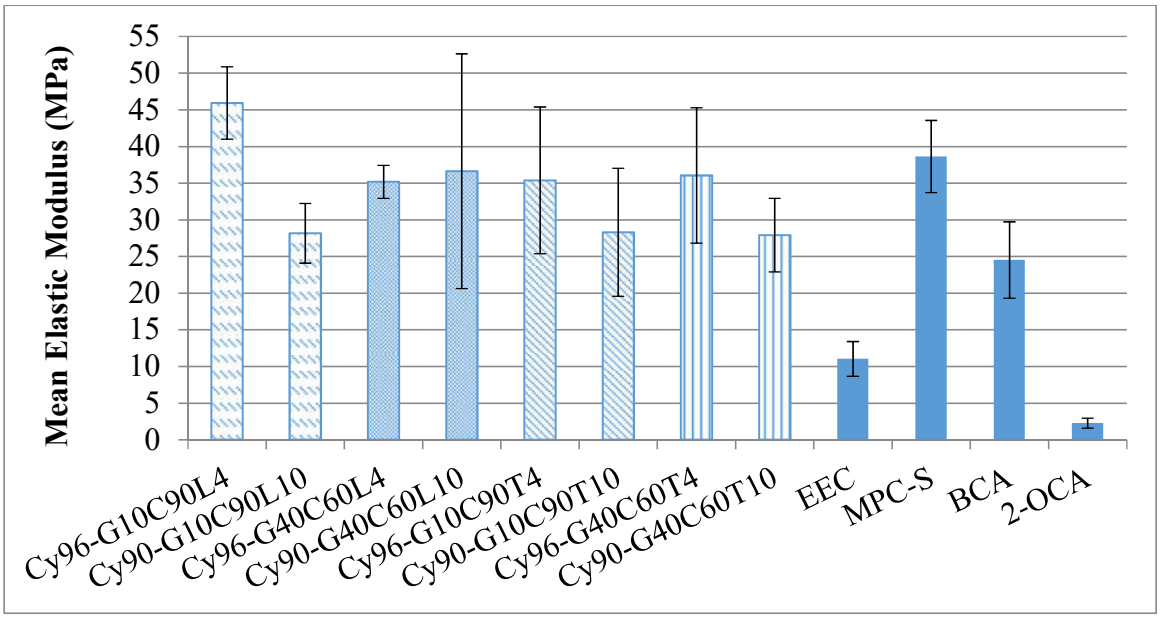


Figure 4-3: Mean Modulus of Elasticity for Each Polymerized Adhesive (n=3 per adhesive, error bars = 1 standard deviation)

It was hypothesized that the increased adhesive modifier amount for the Cy90s as compared to the Cy96s would result in a decreased modulus of elasticity (increased

flexibility) for the polymerized adhesive. The data in Figure 4-3 provides evidence that this hypothesis may be true except for Cy90-G40C60L10. The adhesive modifiers likely acted as plasticizers based on their known large amount of ε-caprolactone and low molecular weight as discussed in Chapter 2. The data for Cy90-G40C60L10 had a larger amount of variation as compared to the other adhesives. This increased variation may potentially be due to differences the 0.5”x2” films tested between adhesive types. This reason may be why the adhesive did not have a lower elastic modulus than Cy96-G40C60L4.

It was also hypothesized that there is a direct relationship between adhesive modifier ε-caprolactone molar percent versus polymerized adhesive flexibility. The data in Figure 4-3 fails to provide evidence that this hypothesis is true. The reason for this unexpected result may be a result of the apparent amorphous state for both the G10C90 and G40C60 polymers synthesized thus providing a similar level of flexibility. MPC-S was expected to have a larger modulus of elasticity than the Cy96s and Cy90s because it did not contain any adhesive modifier. Due to the decreased intermolecular and intramolecular forces from the longer side chain, BCA and 2-OCA were expected to have a lower modulus of elasticity than MPC-S. Similarly, EEC was expected to have a lower modulus of elasticity than MPC-S due to its increased alkoxy side chain length and thus increased ease of rotation for the side chain.

Based on a review of the modulus of elasticity results in Figure 4-3, the adhesives appear to be in three groups consisting of the Cy96s, Cy90s, MPC-S, and BCA as group 1, EEC as group 2, and 2-OCA as group 3 with group 1 < group 2 < group 3 in terms of

flexibility. The means for this data were statistically analyzed using Minitab 17 by ANOVA with Tukey multiple comparison ($\alpha$=0.05) to determine whether there were any statistically significant differences present. The resulting p-value of 0.000 indicated that there were statistically significant differences present. The Tukey multiple comparison results were confounded because they demonstrated that all the adhesives except 2-OCA overlapped in two groups. This result indicates that there was too much variability in the test results to indicate statistically significant differences except that 2-OCA had a lower modulus of elasticity than all the adhesives tested.

Nevertheless, examining the means only, except for Cy90-G40C60L10 with its excess variability, there appears to be a pattern with the adhesives containing four weight percent adhesive modifier having a larger modulus of elasticity (increased stiffness) as compared to the adhesives containing 10 weight percent adhesive modifier (increased flexibility). In addition, the adhesives containing no adhesive modifier appear to be ordered in terms of most stiff to most flexible as MPC-S > BCA > EEC > 2-OCA. This result may indicate that decreased intermolecular and intramolecular forces from an increased alkane side chain length (e.g. BCA) may contribute less to flexibility for a polymerized adhesive as compared to increased ease of rotation from an increased alkoxy side chain length (e.g. EEC). In comparison, the extent of rotation from the ethoxy group for the EEC side chain was greater than the decreased in intermolecular and intramolecular forces from the n-butyl group for the BCA side chain. Lastly, the much longer 2-octyl side chain for 2-OCA resulted in the lowest intermolecular and intramolecular forces for the resulting polycyanoacrylate of the adhesives tested, in turn

resulting in the lowest modulus of elasticity (greatest flexibility). In terms of clinical relevance, 2-OCA and EEC would be expected to have the largest burst strength as indicated by the measured flexibility of the adhesives tested. Also, the adhesives containing 10 weight percent adhesive modifier would be expected to have an improved burst strength in terms of flexibility in comparison to the adhesives containing four weight percent adhesive modifier.

## 4.4. Conclusions

Through the completion of this work, polymerized adhesives of variable types and weight percent adhesive modifier could be fabricated for initial dimensions analysis, 3-point bend analysis, composition and extent of reaction testing by NMR, and later testing for release properties through a hydrolysis study. Although there was variation present in the prepared polymerized adhesive sizes, the sizes were deemed similar enough in terms of their top planar area and thickness (volume) for this property to be a minimal factor in the degradation rate of the polycyanoacrylates.

For the composition of these polymerized cyanoacrylates, the NMR results confirmed that one of the goals for this research was enabled, which was to compare adhesives with a small amount and large amount of adhesive modifier. Specifically, the NMR results demonstrated that two of the adhesives contained two weight percent adhesive modifier with four weight percent expected and one of the adhesives contained 11 weight percent adhesive modifier with 10 weight percent expected. Based on the

MPC-S monomer remaining in the adhesives analyzed of two weight percent or less, the polycyanoacrylates fabricated were deemed sufficiently polymerized.

When the polymerized adhesives were analyzed for their modulus of elasticity through 3-point bending, the results mostly indicated that there was too much variability in the test results to indicate statistically significant differences. From examining the means only, there appeared to be a pattern with the adhesives containing four weight percent adhesive modifier having increased stiffness as compared to the adhesives containing 10 weight percent adhesive modifier having increased flexibility. The adhesives containing no adhesive modifier also appeared in terms of most stiff to most flexible as MPC-S > BCA > EEC > 2-OCA. This result provided additional information regarding the relationship between the cyanoacrylate chain complexity and length versus the resulting polycyanoacrylate flexibility. Specifically, the increased chain complexity and length increased the flexibility of the resulting polymerized cyanoacrylate adhesive.

Lastly, in terms of predicted clinical relevance, 2-OCA had the greatest flexibility of the adhesives tested, as expected. There was also evidence presented that demonstrated the ability for the adhesive modifiers to increase the flexibility of polymerized cyanoacrylate. This ability may also indicate an improved burst strength for the Cy96s and Cy90s adhesives as compared to MPC-S, and thus an improved biocompatibility for these adhesives if they were used internally for applications such as small intestine wound approximation.

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# CHAPTER 5

# HYDROLYSIS OF POLYMERIZED ADHESIVES FILMS

## 5.1. Introduction

When cyanoacrylates are applied externally on stratified squamous, keratinized epithelial tissue (skin epidermis), they will polymerize, and then typically slough off as skin naturally sheds over several days.[1]–[5] The polycyanoacrylates are thus naturally removed with typically little effect from their hydrolysis and degradation. The cyanoacrylate and polycyanoacrylate do still have an effect on the epithelial tissue due to their properties such as wound approximation ability (t-peel load and flexibility) and thermal changes due to polymerization including change in temperature, reaction rate, and heat of polymerization. Due to their ability to affect tissue, only the longer side chain types of cyanoacrylates including n-butyl cyanoacrylate (BCA) and 2-octyl cyanoacrylate (2-OCA) marketed as products such as Histoacryl® and Dermabond® respectively are FDA approved for external medical use. Briefly, these longer side chain cyanoacrylates produce a more flexible yet adherent bond to achieve appropriate burst strength to support external loads for wound approximation. In addition, the longer side chain cyanoacrylates reduce the reaction rate, change in temperature, and heat of polymerization for the polymerizing adhesive. Overall, the inter- and intra-molecular forces are reduced for these cyanoacrylates due to their extended side chain length, which subsequently results in the aforementioned mechanical and thermal properties for these adhesives. In addition, these extended side chains reduce the degradation rate for the

adhesive due to a shielding of the alkane bonds (steric hindrance) present within the polycyanoacrylate repeating unit by the long side chains.

Although there has been progress in the field of internal use cyanoacrylates with Omnex™, a 2-OCA based product, and Trufill®, a BCA based product, these materials still hydrolyze slowly into byproducts including formaldehyde, a known cytotoxic chemical. Due to the natural slow degradation of these long side chain polycyanoacrylates, the formaldehyde released is at low levels resulting in mild inflammation, which is one of the reasons why only these types of cyanoacrylates are approved for internal use.[6] There is thus a potential that if other types of cyanoacrylates could be formulated either by synthesis or through the addition of adhesive (rheological) modifiers to release similar levels of formaldehyde or lower, then these new cyanoacrylates may have similar or better levels of acceptable biocompatibility for internal use than the currently marketed products.

Cyanoacrylates degrade through several mechanisms including: 1) unzipping (depolymerization) through a reverse Knoevenagel reaction resulting in the hydrolysis of the polycyanoacrylate repeating unit, 2) a side-chain scission reaction resulting in the hydrolysis at the ester group in the polycyanoacrylate repeating unit's side chain, and 3) esterase enzymatic degradation.[2], [7]–[10],[11] The unzipping reaction results in the formation of formaldehyde and alkyl cyanoacetate as illustrated in Figure 5-1. The side-chain scission reaction (see Figure 5-2) and enzymatic reaction (see Figure 5-3) result in the formation of cyanoacrylic acid and alcohol.[8]

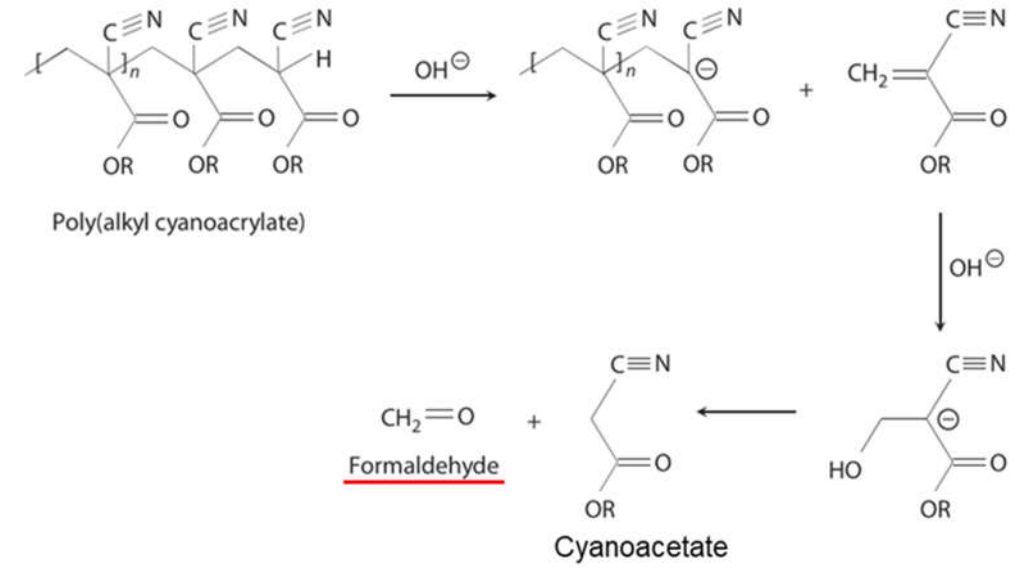


Figure 5-1: Polycyanoacrylate Degradation by Unzipping[2]

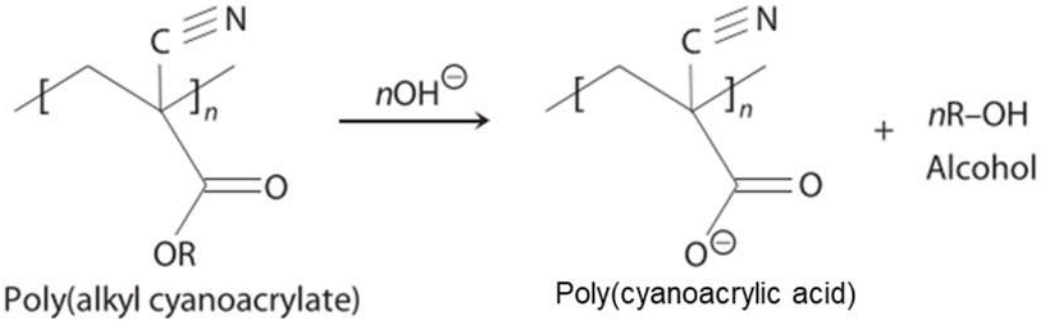


Figure 5-2: Polycyanoacrylate Degradation by Side Chain Scission[2]

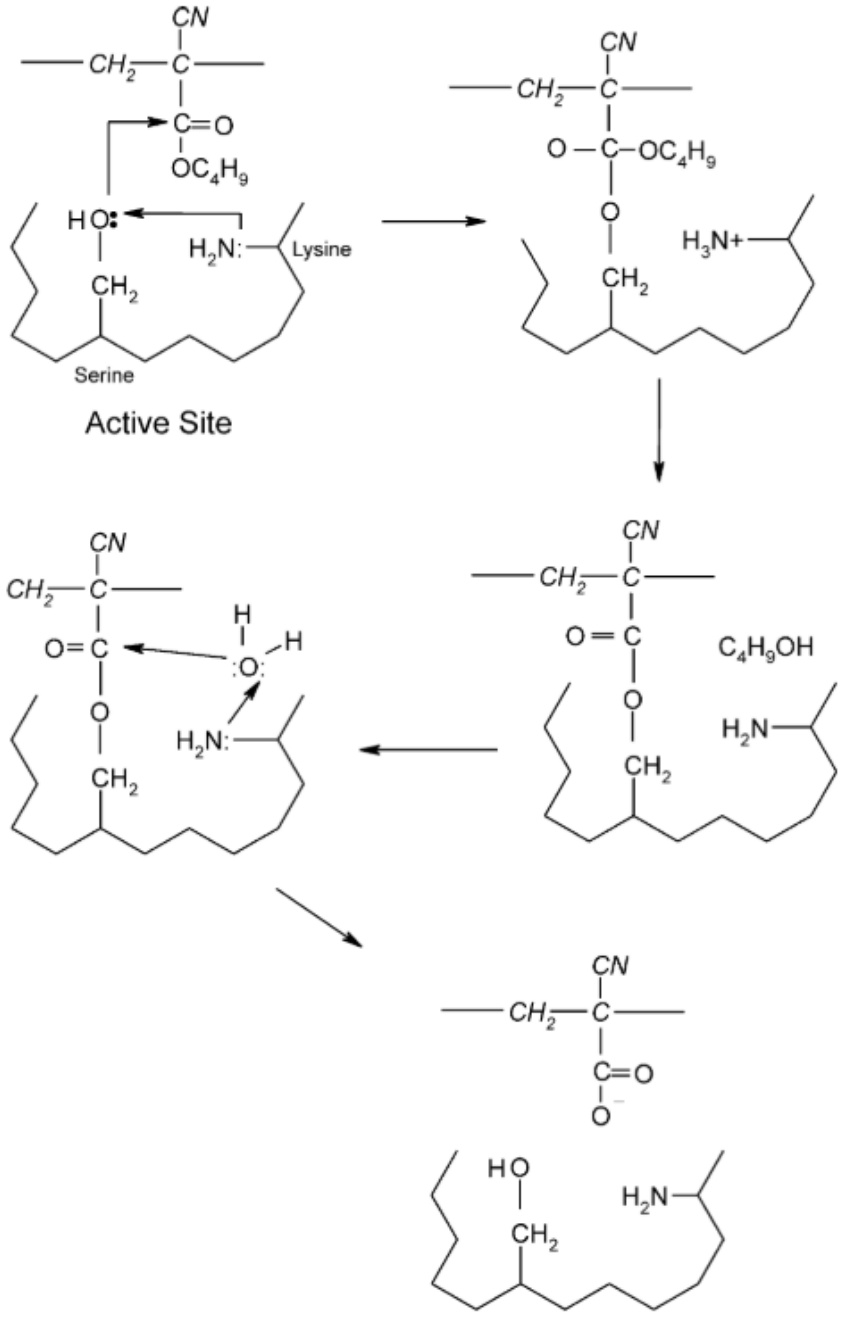


Figure 5-3: Polycyanoacrylate Degradation by Enzymatic Reaction[10]

Multiple studies have demonstrated that basic pH solutions direct polycyanoacrylates to degrade by the unzipping reaction resulting in formaldehyde as depicted in Figure 5-4 as an example.[8], [11]–[13]

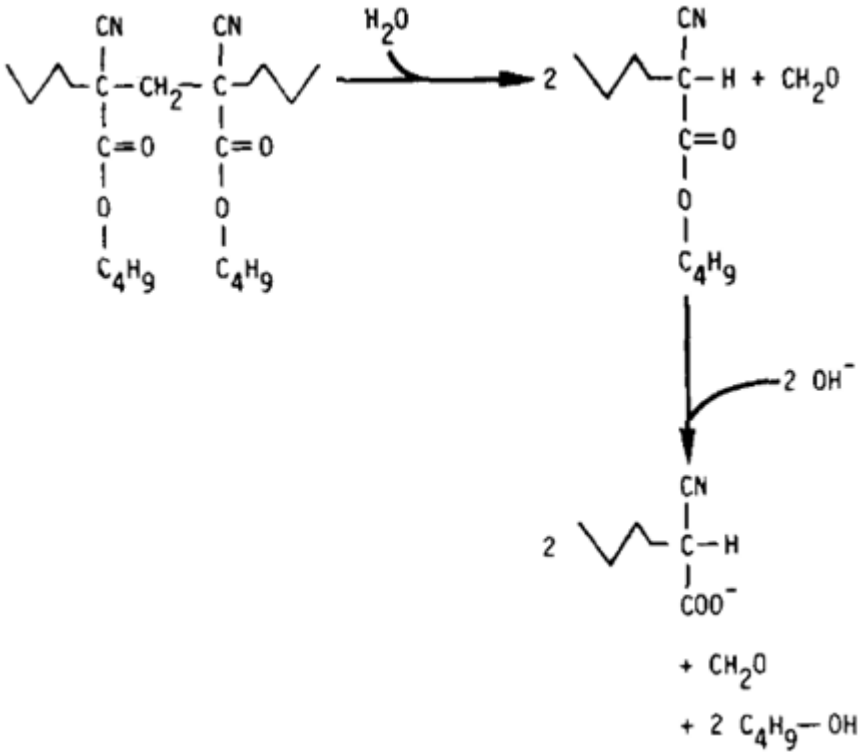


Figure 5-4: Poly(isobutyl cyanoacrylate) Degradation by Unzipping (Basic Solution)[12]

In contrast, several studies have focused on the effect of acidic pH solutions on the degradation of polycyanoacrylates. These studies have demonstrated that acidic pH solutions result in a slower degradation rate due to the lower amount of hydroxide ions present as compared to a basic pH solution.[13], [14] In an acidic solution, the excess protons in the solution bond with the polycyanoacrylate (see Figure 5-5), thereby inhibiting degradation of the polycyanoacrylate and generating a “dormant polymer.”[15] The minimized degradation maintains the polymerized adhesive backbone thereby

inhibiting degradation of the adhesive by unzipping, and thus maximizing side-chain scission degradation of the polymerized adhesive.[16]

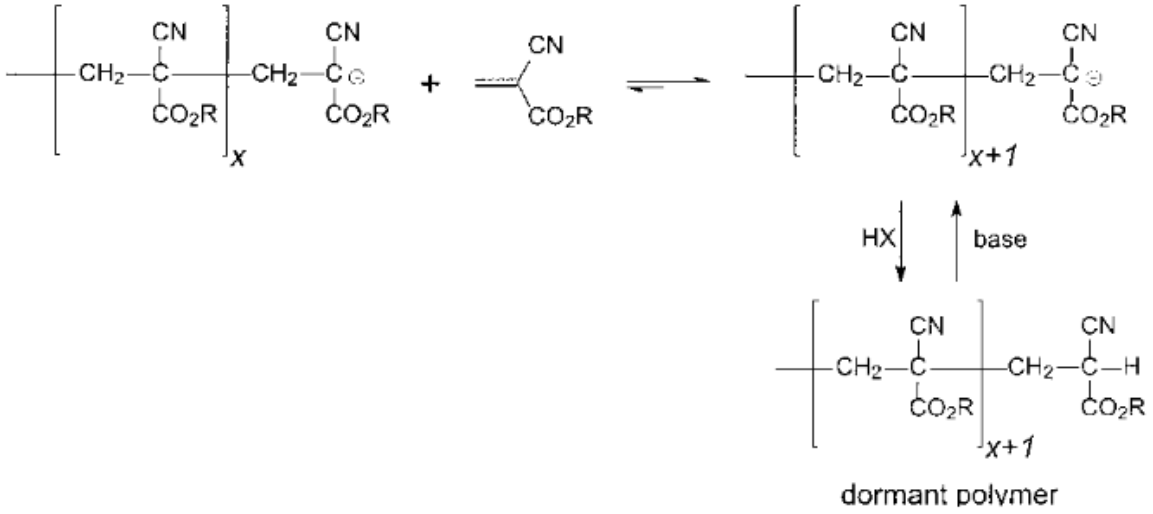


Figure 5-5: Polycyanoacrylate Acidic Stabilization and Unzipping Degradation Inhibition

One of the byproducts from the unzipping degradation reaction for polycyanoacrylates, formaldehyde, is a known cytotoxic, mutagenic, pro-allergenic compound with several studies demonstrating carcinogenic effects from the compound. For this reason, the EPA classified formaldehyde as a probable carcinogen. Formaldehyde is a small molecule water soluble gas that can easily penetrate tissues. It is very reactive, so when exposed to growth medium, this compound will immediately bind extracellular protein components such as amino and sulfhydryl groups. Studies have indicated that formaldehyde can cause cellular transformation, DNA crosslinking and repair interference, sister chromatid exchange, and chromosome aberrations. Excess

formaldehyde was also associated with human aortic smooth muscle cells death. Overall, an inverse relationship has been observed between cyanoacrylate toxicity and formaldehyde release rate.[9], [17]–[20]

In contrast, the cyanoacrylic acid and alcohol, released as byproducts from side chain scission polycyanoacrylate degradation can be less cytotoxic depending on the polycyanoacrylate type degrading. These compounds are also soluble in water, and can be excreted by kidney filtration.[8] One study demonstrated that when polycyanoacrylates with an alkoxy side chain such as poly(ethoxyethyl cyanoacrylate) hydrolyze by chain scission, the alcohol released as a byproduct is non-toxic. This study also demonstrated that when polycyanoacrylates with a long alkyl side chain such as poly(2-octyl cyanoacrylate) hydrolyze by chain scission, the alcohol released as a byproduct is cytotoxic because it is more lipid soluble.[11]

Based on the information collected, it is hypothesized that if a polycyanoacrylate with an alkoxy side chain were to degrade in an acidic instead of basic solution, then the formaldehyde levels released should be minimized, and the alcohol and cyanoacrylic acid released should be less toxic; thus, rendering the polycyanoacrylate potentially safe for internal use. Methoxypropyl cyanoacrylate (MPC) meets the requirement of a cyanoacrylate with an alkoxy side chain. Typical body fluid and tissue including at wound areas has a local pH of 7.2-8.0.[21] The pH would thus need to be lowered by a method in order to create an acidic pH local environment to meet the previously discussed requirement to potentially render a polycyanoacrylate safe for internal use.

As previously discussed, it is possible to mix materials (adhesive modifiers) into cyanoacrylates to modulate their properties. These materials can also be bioresorbable polyesters. This family of materials is known to hydrolyze into non-toxic acidic byproducts. Polyglycolide especially is known to be capable of reducing the pH of a 7.4 pH phosphate buffer fluid to as low as 2.[22] Polymer crystallinity also affects the degradation rate for polyesters, with a direct relationship between crystallinity and degradation rate.[22] Lastly, it can be advantageous to also utilize a slow degrading polymer such as ε-caprolactone (PCL) for a system to tune the degradation of the polyglycolide (PG) within the system as a PGCL copolymer.

Based on these properties, mixing PGCL as an amorphous polymer into cyanoacrylate, polymerizing the cyanoacrylate, and then allowing the polycyanoacrylate to degrade in water or a phosphate buffered saline was hypothesized to allow the adhesive modifier contained within the polycyanoacrylate to also hydrolyze and self-modulate the pH of the surrounding local environment to an acidic level. MPC with an adhesive modifier as described could thus potentially meet the two previously discussed requirements to render the cyanoacrylate safe for internal use. Cytotoxicity testing as directed by ISO 10993-5 can also be used to measure the cytotoxicity of each polymerized adhesive as it hydrolyzes and thus potentially releases formaldehyde byproducts.[23] Theoretically, if the adhesive is degrading mostly by side-chain scission, it will produce less formaldehyde than normal, which can result in a more biocompatible (less cytotoxic) polymerized adhesive. Other than the previously stated hypothesis, there

were also several other hypotheses included in Chapter 1 and as follows for the results from the degradation of the adhesives.

1. There is a direct relationship between adhesive modifier weight percent versus polymerized adhesive degradation rate.
2. There is a direct relationship between adhesive modifier glycolide molar percent versus polymerized adhesive degradation rate.
3. There is an inverse relationship between adhesive modifier weight percent versus polymerized adhesive eluent pH, formaldehyde release amount, and cytotoxicity.
4. There is an inverse relationship between adhesive modifier glycolide molar percent versus polymerized adhesive eluent pH, formaldehyde release amount, and cytotoxicity.

## 5.2. Materials and Methods

### 5.2.1. Hydrolysis Study Method, Mass Loss, and Eluent pH

Polycyanoacrylate film sections for the research and benchmark adhesives were first obtained in groups of six per adhesive type, and weighed to obtain their initial mass. The pieces were then placed in 15 ml polypropylene centrifuge tubes. Phosphate buffered saline (Gibco™ PBS at 7.4 pH, 0.2 M) as obtained from Fisher Scientific was next measured and added to each tube as well as six empty tubes (no test specimens) with 2 ml per tube. The filled tubes were then placed in an incubator at 50°C with no oscillation.

The tubes were pulled in triplicate (specimens 1-3) at 11.8 days and triplicate (specimens 4-6) at 37.6 days for each adhesive. After the removal from 50°C at each time point, the specimens in the tubes were then moved to new 15 ml polypropylene tubes filled with 4 ml deionized water. The eluent in the original tubes was left in the original tubes for each time point. These eluent tubes were then placed at 4°C for temporary storage until later testing.

The new water filled tubes were then placed in a 37°C incubator with a slowly oscillating table for one week for each time point. At the end of the one week for the salts removal method, the water tubes were moved to room temperature, and the eluent in the tubes was fully decanted without removing the specimens from the tubes. The tubes containing only the test specimens were then placed in a room temperature vacuum oven for at least four days for each time point. Each specimen was then removed and weighed to obtain its final mass value. Using the initial and final mass values, the percent mass loss (%ML) for each specimen was then calculated for each time point using the equation %ML = [(initial mass – final mass)/initial mass *100].

The pH for each of the original eluents for both time points was also obtained. The original tubes were first moved from the refrigerator and allowed to equilibrate to room temperature. A pH meter with a glass probe was then initialized and calibrated as a two-point calibration using pH 4 and pH 7 certified buffers. The pH for each eluent was then measured. A rinse with deionized water was performed before and between each measurement to reduce any cross contamination between the eluents.

### 5.2.2. Hydrolysis Study Formaldehyde Release

The formaldehyde content for the each of the original eluents for both time points was measured using a QuantiChrom™ formaldehyde assay kit as obtained from BioAssay Systems. The procedure for this kit included first diluting a formaldehyde standard included in the kit using deionized water to concentrations of 100 μM, 60 μM, 30 μM, and 10 μM to later generate a standard curve. Each of the original test specimen eluents, a blank of Gibco™ PBS only, a blank of deionized water only, and the prepared formaldehyde standards were then reacted using the assay kit for 30 minutes. During this reaction formaldehyde was chemically changed with acetoacetanilide in the presence of ammonia. Three 96-well well plates were needed to fit all the test specimens, so the two blanks and formaldehyde standards were included on each well plate. The product of the assay reaction was then read as two wells per specimen using a fluorescence plate reader with an excitation wavelength of 370 nm and emission wavelength of 470 nm.

Each of the resulting relative fluorescence units (rfu) data were then normalized by subtracting out the mean rfu value for the water blank and mean rfu value for the Gibco™ PBS blank. The mean rfu values for the formaldehyde standards versus the formaldehyde concentrations were then plotted on a scatter plot. A line of best fit was then calculated for the standard curve for each well plate. The slope from each standard curve was then used to convert the rfu values for the test specimens' eluents to actual formaldehyde concentrations by dividing each rfu with the two blanks subtracted out by the slope. The formaldehyde concentration values were then divided by the respective initial cyanoacrylate mass values. For the Cy96s and Cy90s, the cyanoacrylate mass was

calculated based on the mass measured and the weight percent of cyanoacrylate as compared to adhesive modifier in each Cy96 and Cy90 formulation.

### 5.2.3. Adhesives Cytocompatibility

Several of the other pieces of the polymerized adhesives were also tested for cytotoxicity by Nelson Laboratories according to ISO 10993. For this testing, three film pieces per adhesive type were placed on a layer of agarose on top of a monolayer of L929 cells in a well with one well per film piece. The wells were then incubated for 24-26 hours at 37±1°C in 5±1% $CO_2$. The zone of cell destruction was then measured and scored using a scale from 0 to 4 according to ISO 10993 to determine their level of cytotoxicity.

## 5.3. Results and Discussion

### 5.3.1. Summary of Adhesives

For this section, a summary of the main description factors for the Cy96s and Cy90s and benchmark adhesives was included as Table 5-1 and Table 5-2. Additionally, although the adhesives may not be identified as polymerized adhesives (e.g. poly-(Cy96-G10C90L4)), unless otherwise stated, the results and discussion for this chapter are for the evaluation of the polymerized adhesives.

Table 5-1: Description Factors for Cy96 and Cy90 Adhesives

| Adhesive | Adhesive Modifier Initiator Chemical Structure (Initiator) | Adhesive Modifier Weight Percent (Wt. %) | Adhesive Modifier Glycolide Molar Percent (Gly Mol %) | Hydrolysis Time in Days (Stability Time) |
|---|---|---|---|---|
| Cy96-G10C90L4 | Linear | 4 | 10 | 12 and 38 |
| Cy90-G10C90L10 | Linear | 10 | 10 | 12 and 38 |
| Cy96-G40C60L4 | Linear | 4 | 40 | 12 and 38 |
| Cy90-G40C60L10 | Linear | 10 | 40 | 12 and 38 |
| Cy96-G10C90T4 | Branched | 4 | 10 | 12 and 38 |
| Cy90-G10C90T10 | Branched | 10 | 10 | 12 and 38 |
| Cy96-G40C60T4 | Branched | 4 | 40 | 12 and 38 |
| Cy90-G40C60T10 | Branched | 10 | 40 | 12 and 38 |

Table 5-2: Description Factors for EEC, MPC-S, BCA, and 2-OCA Adhesives

| Adhesive | Alkane Side Chain Length | Alkoxy Chain Length | Hydrolysis Time in Days (Stability Time) |
|---|---|---|---|
| EEC | 2 | 2 | 12 and 38 |
| MPC-S | 3 | 1 | 12 and 38 |
| BCA | 4 | 0 | 12 and 38 |
| 2-OCA | 8 | 0 | 12 and 38 |

### 5.3.2. Polymerized Adhesives Hydrolysis Study Overview

Based on a review of the initial mass values for the six polycyanoacrylate film pieces for each film type as included in Figure 5-6, the prepared polycyanoacrylate specimens were found to have a sufficient similarity within and between the adhesive types (0.1 g or less variation).

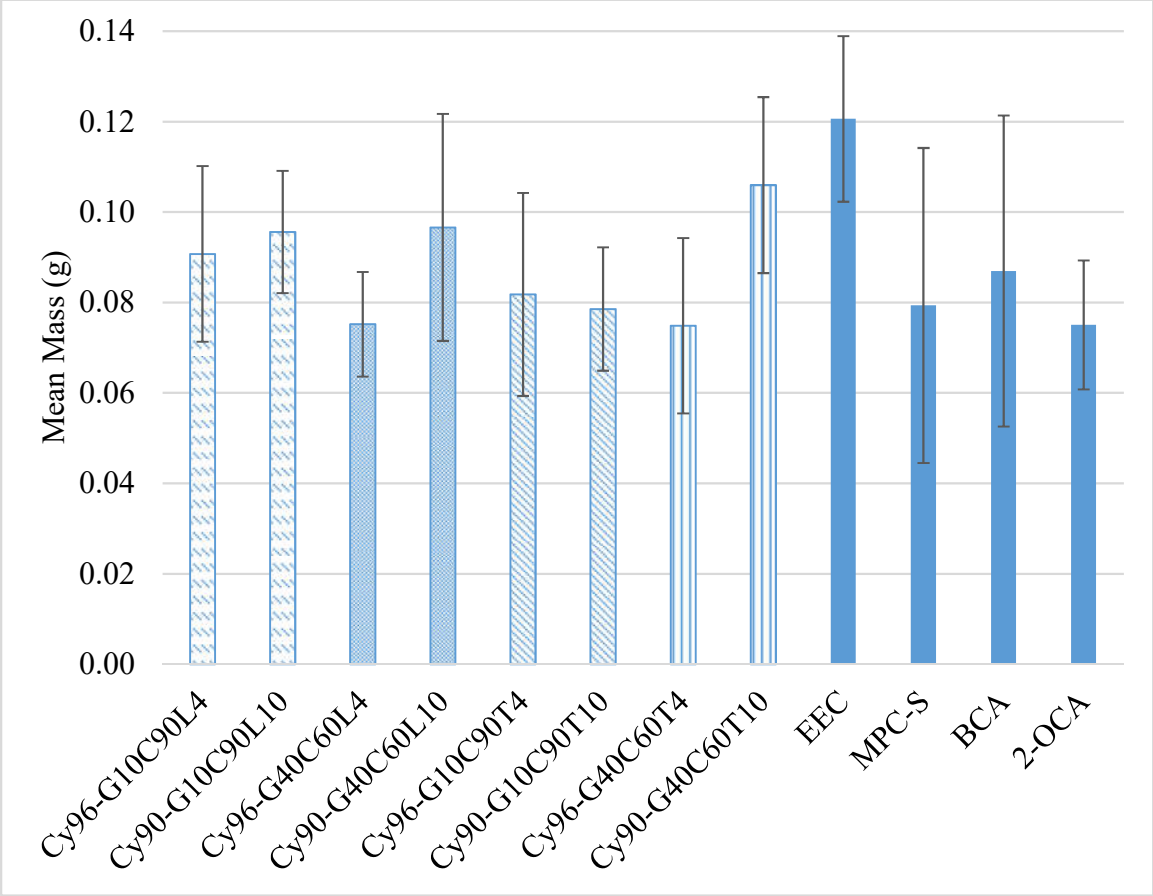


Figure 5-6: Polymerized Adhesives Pieces Initial Masses

These film pieces were placed in 2 ml PBS after their initial mass measurement. This PBS volume was selected in order to have a sufficient amount to cover the specimen in the tube, but not too much to over-dilute the potential formaldehyde to be released

from the film during hydrolysis. Excess dilution could render formaldehyde undetectable by the formaldehyde assay kit previously discussed due to the kit's 1.5 μM minimum detectable formaldehyde concentration.

The films were removed from hydrolysis after 11.8 days (specimens 1-3) and 37.6 days (specimens 4-6). These two time points were selected because they are approximately equivalent to 1 month and 3 months at 37°C (body temperature) respectively by the Arrhenius equation.[24] At each time point, after moving the tubes to room temperature, the specimens were observed to have changed in appearance from clear to white or opaque. This change in appearance was thought to be due to an uptake in salts from the Gibco™ PBS, so the films were then subjected to a deionized water soak as previously stated. The water soak was selected to pull the salts out of the specimens through diffusion, moving with the concentration gradient (salts removal method). The 4 ml volume was picked because it was twice the original Gibco™ PBS volume of 2 ml. This additional water soak may have resulted in additional mass loss for the polymerized cyanoacrylate films; however, it would not affect the eluent pH and formaldehyde release for the 11.8 or 37.6 days initial eluents because each initial eluent was retained prior to the water soak. Additionally, the same degradation method was used for each test specimen, so the degradation study results can be compared between all the adhesives (research and benchmark).

### 5.3.3. Polymerized Adhesives Mass Loss

After subjecting the polycyanoacrylate films to the hydrolysis study previously outlined, the mass loss from the films was determined. A summary of the mass loss results is in Figure 5-7.

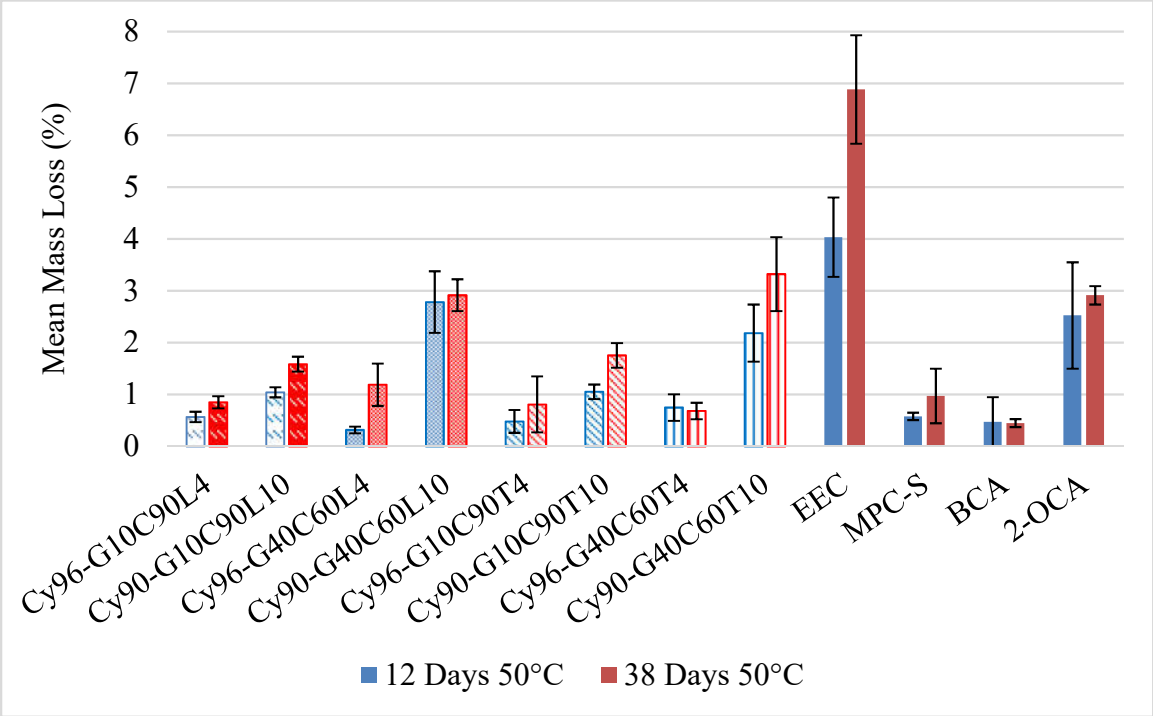


Figure 5-7: Adhesives Mass Loss Results

Based on Figure 5-7, for the Cy96s and Cy90s, there appears to be a direct relationship between weight percent adhesive modifier and mass loss regardless of the adhesive modifier type. This relationship matches the expected result that the adhesive modifier would hydrolyze rapidly, so increasing the amount within a polymerized adhesive would cause the adhesive formulation to lose mass more rapidly. The Cy96s and Cy90s containing a greater amount of glycolide also hydrolyzed more rapidly as expected. In addition, for all the Cy96s and Cy90s except Cy96-G40C60T4, there

appears to be a direct relationship between days in hydrolysis and mass loss. This relationship matches the expected result that the adhesive modifier and adhesive would degrade when placed in Gibco$^{TM}$ PBS (hydrolyze), so increasing the amount of time the polymerized adhesive formulation was in Gibco$^{TM}$ PBS would cause the adhesive formulation to lose mass more rapidly. Cy96-G40C60T4 appears to have lost a similar amount of mass for each time point, and a greater amount than Cy96-G40C60L4 at the 12 days time point. The adhesive modifier for Cy96-G40C60T4 was G40C60T, which had a 40/60 molar ratio glycolide/ε-caprolactone and trimethylolpropane initiator. The high amount of glycolide present in the polymer would be expected to increase the mass loss rate for the polymer. The branched initiator would be expected to decrease the crystallinity for the polymer as compared to its linear initiator polymer analog (G40C60L, see Chapter 2) used for Cy96-G10C90L4 and Cy90-G10C90L10. The lower crystallinity adhesive modifier in Cy96-G40C60T4 was thus assumed to have caused the polycyanoacrylate formulation to hydrolyze at a different rate than the Cy96-G40C60L4 polymerized adhesive formulation. Although this difference due to the initiator types (linear versus branched) appeared to cause a change in the hydrolysis for these two polymerized adhesive formulations, overall, as depicted in Figure 5-7, the change in initiator type did not appear to cause a significant change between the Cy96s and Cy90s. Increasing the amount of branched initiator for the synthesis of the triaxial polymers (G10C90T and G40C60T) would likely further decrease the crystallinity of the resulting polymers. If these new polymers were mixed into adhesive, they could cause a significant

difference for the polymerized adhesive formulations due to the reduced chain lengths for the triaxial adhesive modifiers.

The mass loss results for the polymerized tested adhesives that did not contain adhesive modifiers (MPC-S, EEC, BCA, and 2-OCA) were also summarized in Figure 5-7. These results appear to indicate a direct relationship between alkoxy side chain length and mass loss because EEC had a greater mass loss than MPC-S. This result was expected because the longer alkoxy side chain for EEC would result in a decreased steric hindrance and increased ease of rotation for the polymerized EEC, in turn allowing an increased mass loss rate. There also appears to be an inverse relationship between alkane side chain length and mass loss when comparing polymerized MPC-S, EEC, and BCA. This result was expected because there is a direct relationship between alkane side chain length and steric hindrance for polymerized adhesives, which in turn causes a decreased degradation (mass loss) rate. 2-OCA was expected to have the least mass loss due to having the longest alkane side chain (8 carbons) of the adhesives tested; however, this adhesive was measured to have a greater mass loss than both BCA (4 carbon side chain) and MPC-S (3 carbon side chain). Potential reasons for this unexpected result are discussed later with the formaldehyde results discussion.

The mass loss results were further analyzed using statistics to determine whether there were any significant differences between the means for all the polymerized adhesives tested at each time point. ANOVA with Tukey multiple comparison ($\alpha$=0.05) was performed for the data using Minitab 17 software, with a resulting p-value of 0.000 indicating there was a statistically significant difference between the means. The Tukey

multiple comparison demonstrated that the adhesives were split into seven groups although there was overlap across several groups for several of the adhesives. Other than EEC at 38 weeks hydrolysis having the greatest mass loss, significant differences between the adhesives in the other groups could not be distinguished. For this reason, the data was subjected to further statistical analysis through regression analysis to determine whether the factors that describe the adhesives significantly affected the mass loss results for the adhesives. From this data analysis, a p-value was calculated to identify the significance of each factor (see Table 5-1 and Table 5-2) as included in Table 5-3 and Table 5-4. Main effects plots (see Figure 5-8 and Figure 5-9) were also generated to display the relationships between the means from the mass loss for the adhesives and the factors that describe the adhesives.

Table 5-3: Factors Significance for Cy96 and Cy90 Adhesives for Mass Loss

| | **Adhesive Modifier Initiator Chemical Structure (Initiator)** | **Adhesive Modifier Weight Percent (Wt. %)** | **Adhesive Modifier Glycolide Molar Percent (Gly Mol %)** | **Hydrolysis Time in Days (Stability Time)** |
|---|---|---|---|---|
| **Significance by p-value** | 0.858 (p-value>α of 0.05, so factor not significant) | 0.000 (p-value<α of 0.05, so factor significant) | 0.000 (p-value<α of 0.05, so factor significant) | 0.002 (p-value<α of 0.05, so factor significant) |

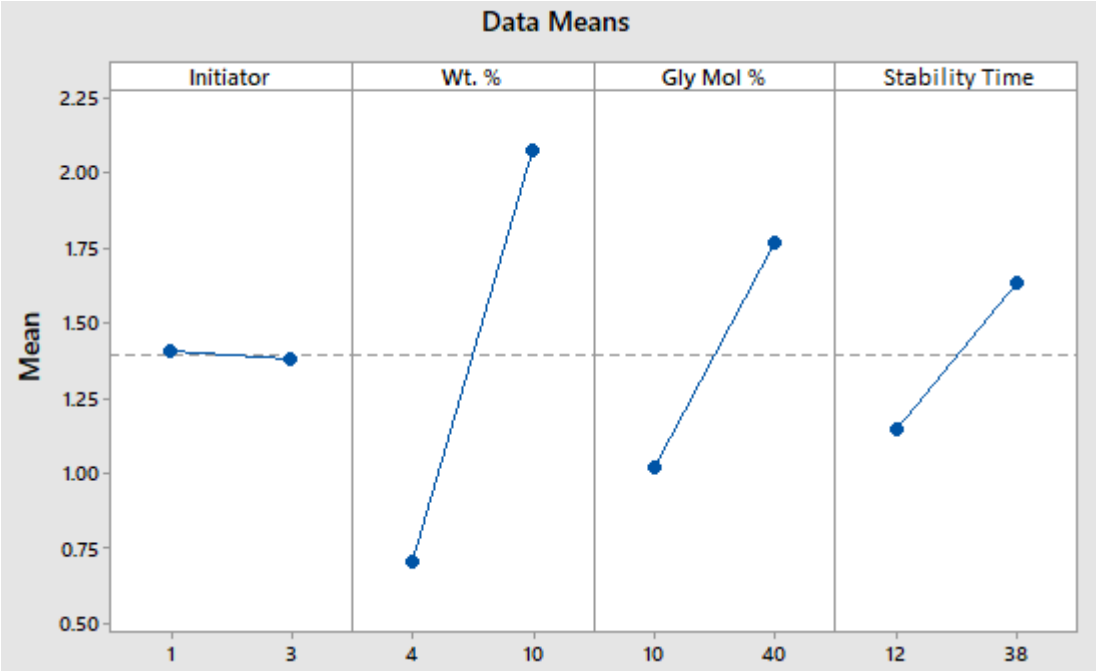


Figure 5-8: Main Effects Plot for Cy96 and Cy90 Adhesives Mass Loss

The results in Table 5-3 and Figure 5-8 confirm that there are direct relationships between adhesive modifier weight percent, glycolide content in adhesive modifier, and stability time versus mass loss. The main effects plot and regression analysis results also confirm the lack of significance of the initiator type (linear versus branched) on the mass loss of the resulting polymerized adhesive formulation.

Table 5-4: Factors Significance for EEC, MPC-S, BCA, and 2-OCA Adhesives Mass Loss

| | **Alkane Side Chain Length** | **Alkoxy Chain Length** | **Hydrolysis Time in Days (Stability Time)** |
|---|---|---|---|
| **Significance by p-value** | 0.000 (p-value<α of 0.05, so factor significant) | 0.000 (p-value<α of 0.05, so factor significant) | 0.103 (p-value>α of 0.05, so factor not significant) |

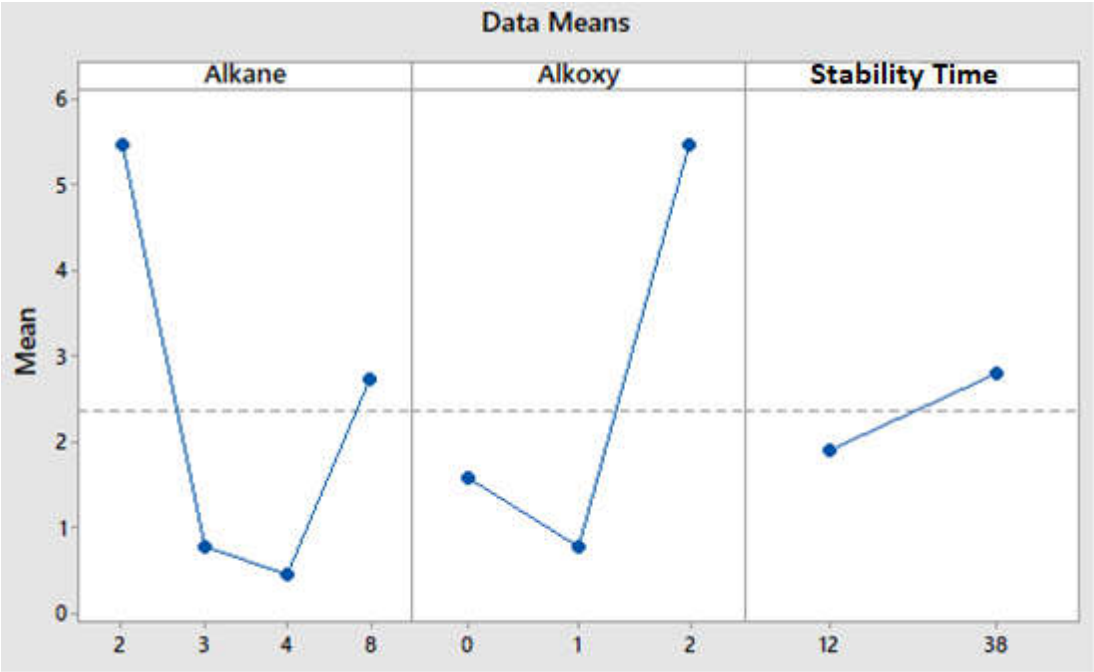


Figure 5-9: Main Effects Plot for EEC, MPC-S, BCA, and 2-OCA Adhesives Mass Loss

Other than the 2-OCA unexpected result to be further discussed, the results in Table 5-4 and Figure 5-9 mostly confirm the relationships observed. Specifically, an inverse relationship is demonstrated between side chain alkane length and mass loss. A direct relationship is also demonstrated between alkoxy side chain length and stability time versus mass loss for the main effects plot; however, the regression analysis results indicate that the stability time was not a significant factor. This discrepancy between the main effects plot and regression analysis results is likely because these four adhesives tested did not contain any adhesive modifier; thus, their degradation path is limited to only the adhesive itself, which will typically more slowly degrade than polyglycolide based polyesters. As an additional clarification, the slight drop for mass loss as the alkoxy side chain length is increased from 0 to 1 is due to the unexpected 2-OCA result.

### 5.3.4. Polymerized Adhesives Eluent pH

The eluents obtained from the initial hydrolysis in Gibco™ PBS were retained for pH testing and to determine the formaldehyde content in them. The results from the pH analysis are summarized in Figure 5-10.

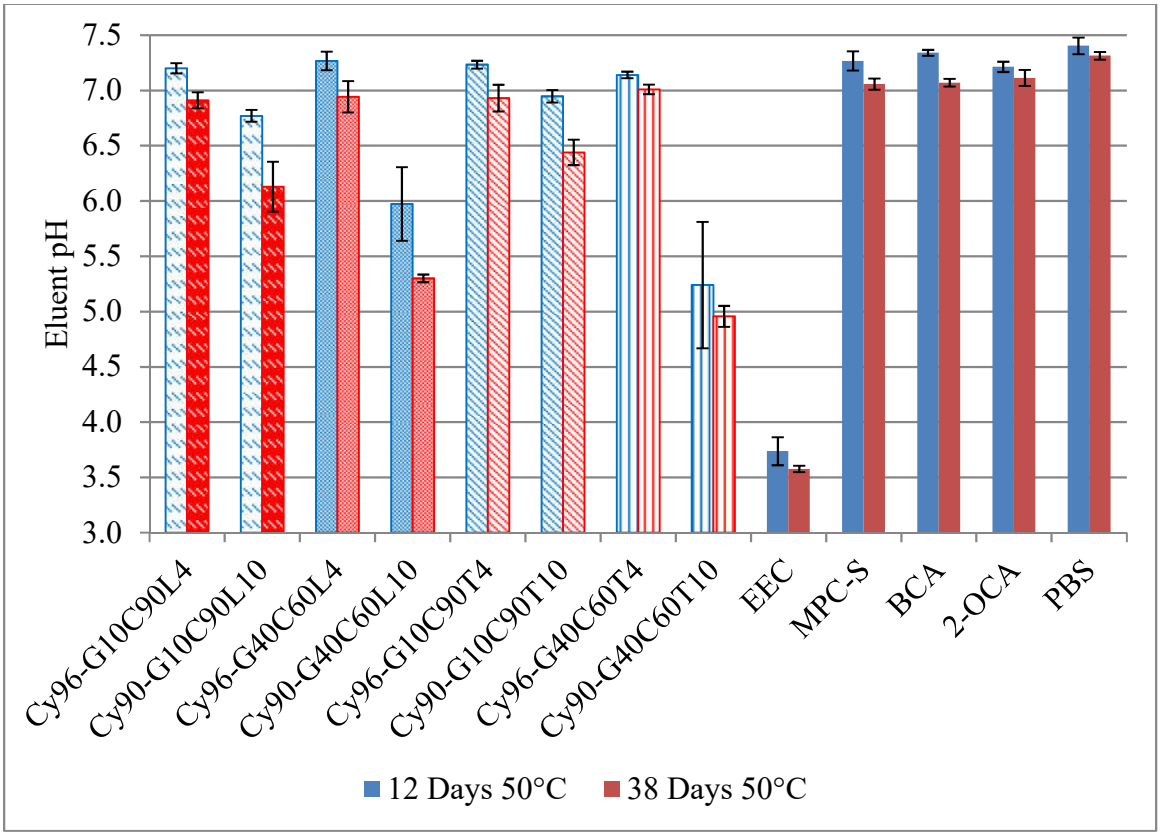


Figure 5-10: Adhesives and Gibco™ PBS Eluents pH Results

Based on Figure 5-10, for the Cy96s and Cy90s, there appears to be an inverse relationship between weight percent adhesive modifier and pH regardless of the adhesive modifier type. This relationship matches the expected result that the polyester adhesive modifier would hydrolyze rapidly, so increasing the amount within a polymerized

adhesive would cause the adhesive formulation to lose mass more rapidly resulting in a more rapid decrease in pH (polyester degrades into acidic byproducts). Furthermore, increasing the amount of glycolide for the Cy96s and Cy90s resulted in a greater decrease in pH as expected due to the expected increased hydrolysis rate for glycolide. In addition, for all the Cy96s and Cy90s there appears to be an inverse relationship between days in hydrolysis and pH. This relationship matches the expected result that the adhesive modifier and adhesive would degrade when placed in Gibco™ PBS (hydrolyze), so increasing the amount of time the polymerized adhesive formulation was in Gibco™ PBS would cause the adhesive formulation to lose mass more rapidly, and thus result in a more rapid decrease in pH. Lastly, the initiator type (linear versus branched) appears to have resulted in a larger decrease in pH, likely due to the expected reduced crystallinity for the adhesive modifier and thus faster degradation.

The pH results for the polymerized tested adhesives that did not contain adhesive modifiers (MPC-S, EEC, BCA, and 2-OCA) were also summarized in Figure 5-10. These results appear to indicate an inverse relationship between alkoxy side chain length and pH because EEC had a greater pH decrease than MPC-S. This result provides evidence that EEC degrades primarily by the side chain scission polycyanoacrylate degradation pathway resulting in cyanoacrylic acid and alcohol with the acid lowering the pH of the eluent environment below the initial 7.4 pH for the Gibco™ PBS. There also appears to be a direct relationship between alkane side chain length and pH. This result was expected because there is a direct relationship between alkane side chain length and steric

hindrance for polymerized adhesives, which in turn causes a decreased degradation (mass loss) rate and thus less change in pH from any side chain scission degradation.

The pH results were further analyzed using statistics to determine whether there were any significant differences between the means for all the polymerized adhesives tested at each time point. ANOVA with Tukey multiple comparison ($\alpha$=0.05) was performed for the data using Minitab 17 software, with a resulting p-value of 0.000 indicating there was a statistically significant difference between the means. The Tukey multiple comparison demonstrated that the adhesives were split into seven groups in order of lowest to highest pH value. Group 1 included EEC at both time points. Group 2 included Cy90-G40C60L10 at the 38 days time point and Cy90-G40C60T10 at both time points. Group 3 included Cy90-G10C90L10 at the 38 days time point, Cy90-G40C60L10 at the 12 days time point, and Cy90-G10C90T10 at the 38 days time point. Groups 4-7 had too much overlap across several groups for several of the adhesives, so significant differences between the adhesives in the other groups could not be distinguished. For this reason, the data was subjected to further statistical analysis through regression analysis to determine whether the factors that describe the adhesives significantly affected the pH results for the adhesives. From this data analysis, a p-value was calculated to identify the significance of each factor (see Table 5-1 and Table 5-2) as included in Table 5-5 and Table 5-6. Main effects plots (see Figure 5-11 and Figure 5-12) were also generated to display the relationships between the means from the pH for the adhesives and the factors that describe the adhesives.

Table 5-5: Factors Significance for Cy96 and Cy90 Adhesives pH

| | **Adhesive Modifier Initiator Chemical Structure (Initiator)** | **Adhesive Modifier Weight Percent (Wt. %)** | **Adhesive Modifier Glycolide Molar Percent (Gly Mol %)** | **Hydrolysis Time in Days (Stability Time)** |
|---|---|---|---|---|
| **Significance by p-value** | 0.526 (p-value>α of 0.05, so factor not significant) | 0.000 (p-value<α of 0.05, so factor significant) | 0.000 (p-value<α of 0.05, so factor significant) | 0.002 (p-value<α of 0.05, so factor significant) |

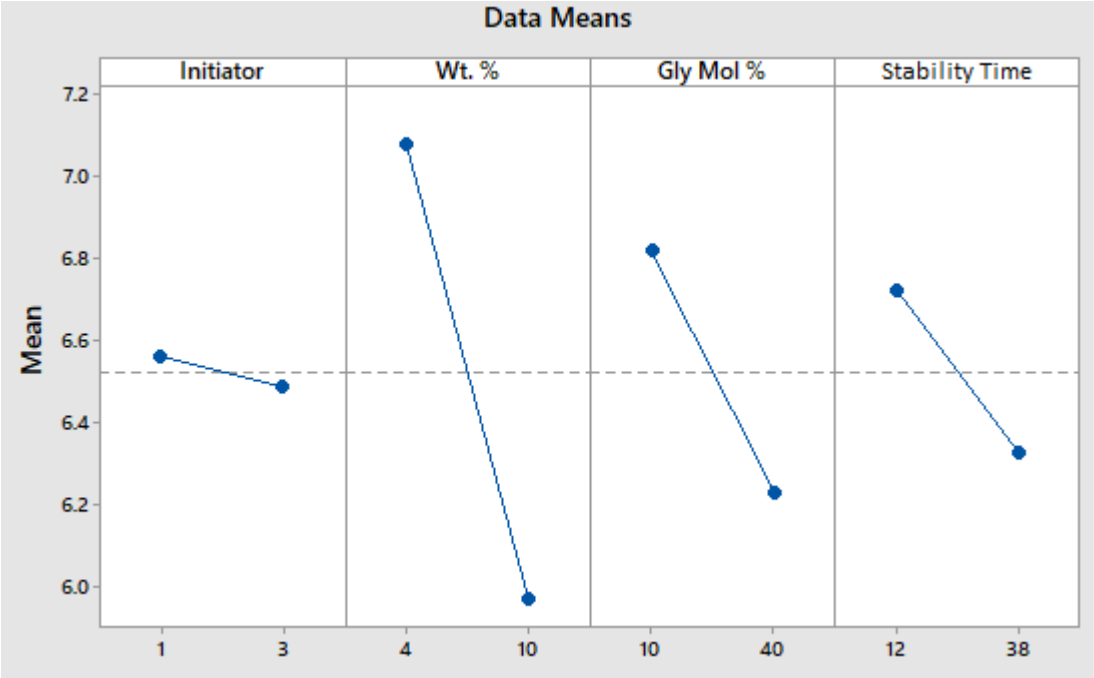


Figure 5-11: Main Effects Plot for Cy96 and Cy90 Adhesives pH

The results in Table 5-5 and Figure 5-11 confirm that there are inverse relationships between adhesive modifier weight percent, glycolide content in adhesive modifier, and stability time versus pH. The main effects plot and regression analysis

results also confirm the lack of significance of the initiator type on the pH of the resulting polymerized adhesive formulation.

Table 5-6: Factors Significance for EEC, MPC-S, BCA, and 2-OCA Adhesives pH

| | **Alkane Side Chain Length** | **Alkoxy Chain Length** | **Hydrolysis Time in Days (Stability Time)** |
|---|---|---|---|
| **Significance by p-value** | 0.165 (p-value>α of 0.05, so factor not significant) | 0.000 (p-value<α of 0.05, so factor significant) | 0.566 (p-value>α of 0.05, so factor not significant) |

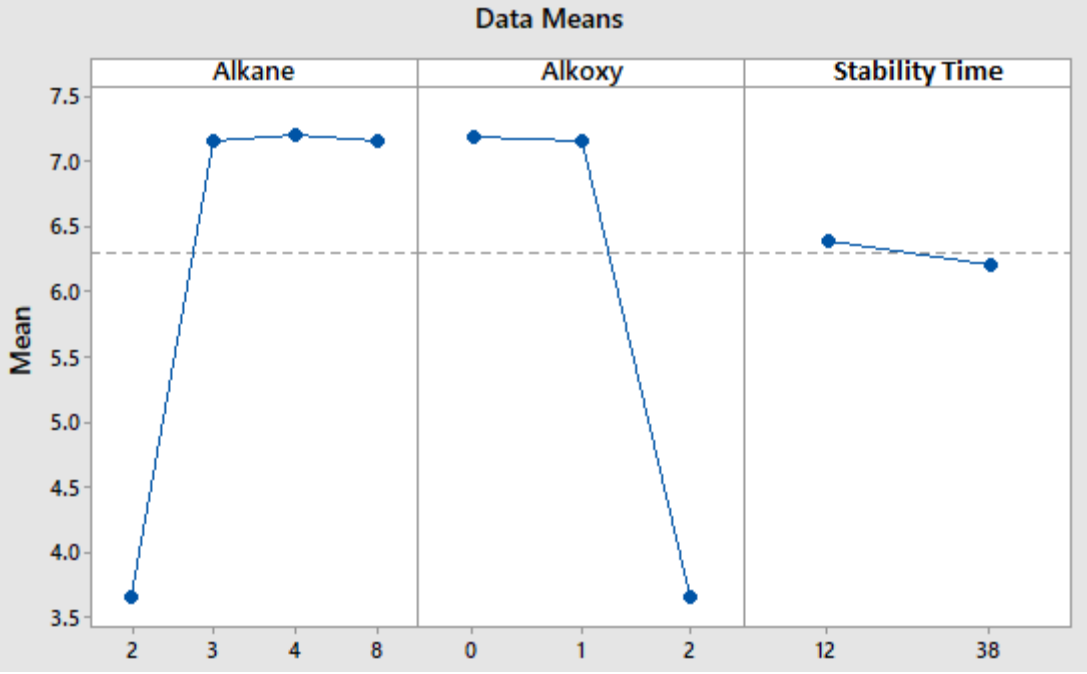


Figure 5-12: Main Effects Plot for EEC, MPC-S, BCA, and 2-OCA Adhesives pH

The results in Table 5-6 and Figure 5-12 partially demonstrate the relationships observed. Specifically, a direct relationship is demonstrated between side chain alkane

length and pH going from a length of 2 to 3 for the main effects plot; however, the regression analysis results demonstrate that the alkane side chain length was not a significant factor. There was only a significant change in pH when the alkane side chain length was increased from 2 to 3, which may be a cause of this discrepancy. This result indicates that an alkane length of 3 and greater has a similar level of pH change for the hydrolysis conditions used for this study. An inverse relationship was also confirmed between alkoxy side chain length and pH. Lastly, both the main effects plot and regression analysis results demonstrate that stability time did not significantly affect the pH. This result is likely because these four adhesives tested did not contain any adhesive modifier; thus, their degradation path is limited to only the hydrophobic adhesive itself, which will typically more slowly degrade than hydrophilic polyglycolide based polyesters.

### 5.3.5. Polymerized Adhesives Formaldehyde Release

As discussed in the Materials and Methods section, the formaldehyde assay first required the generation of a standard curve for each 96-well well plate used for testing the adhesives eluents. The standard curves can be found as Figure 5-13.

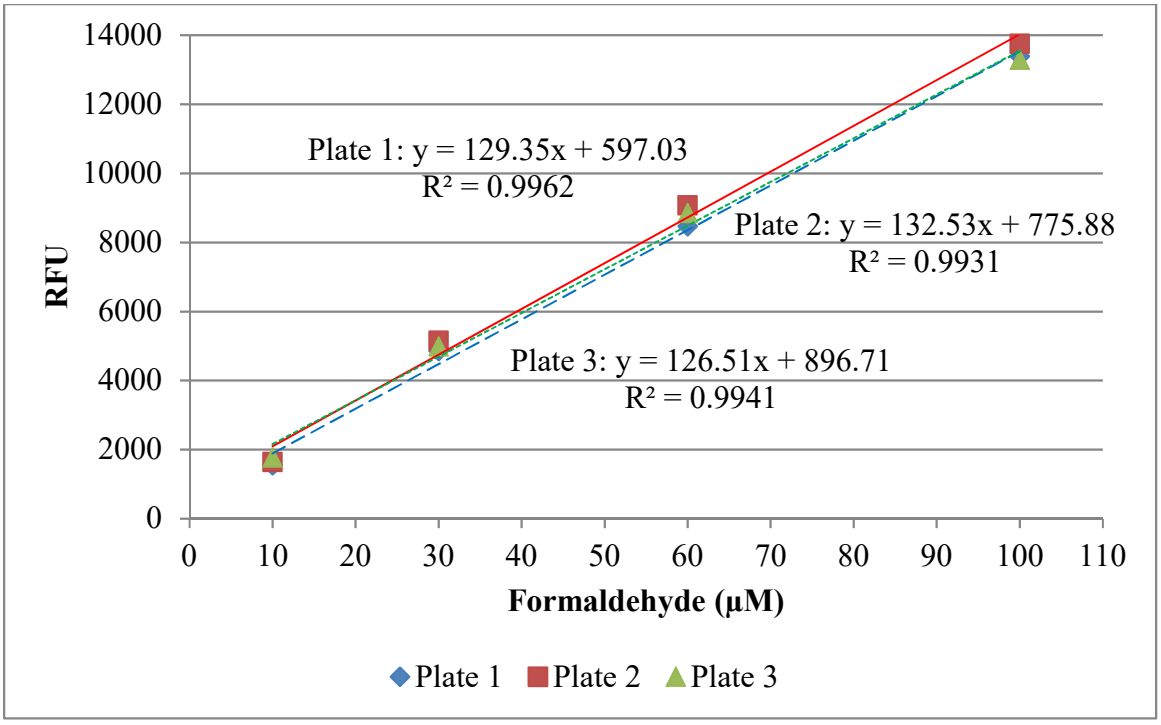


Figure 5-13: Formaldehyde Assay Standard Curves

The three curves have a nearly identical slope, y-intercept, and correlation coefficient ($R^2$), which indicates the precision for the formaldehyde assay. Using the slope values from the standard curves in Figure 5-13, the RFU values from testing the specimens' eluents were then converted to formaldehyde concentrations. These concentrations were further normalized using the initial cyanoacrylate mass values as previously discussed. Figure 5-14 includes a summary of the calculated, normalized formaldehyde concentrations by initial adhesive mass for the specimens' eluents.

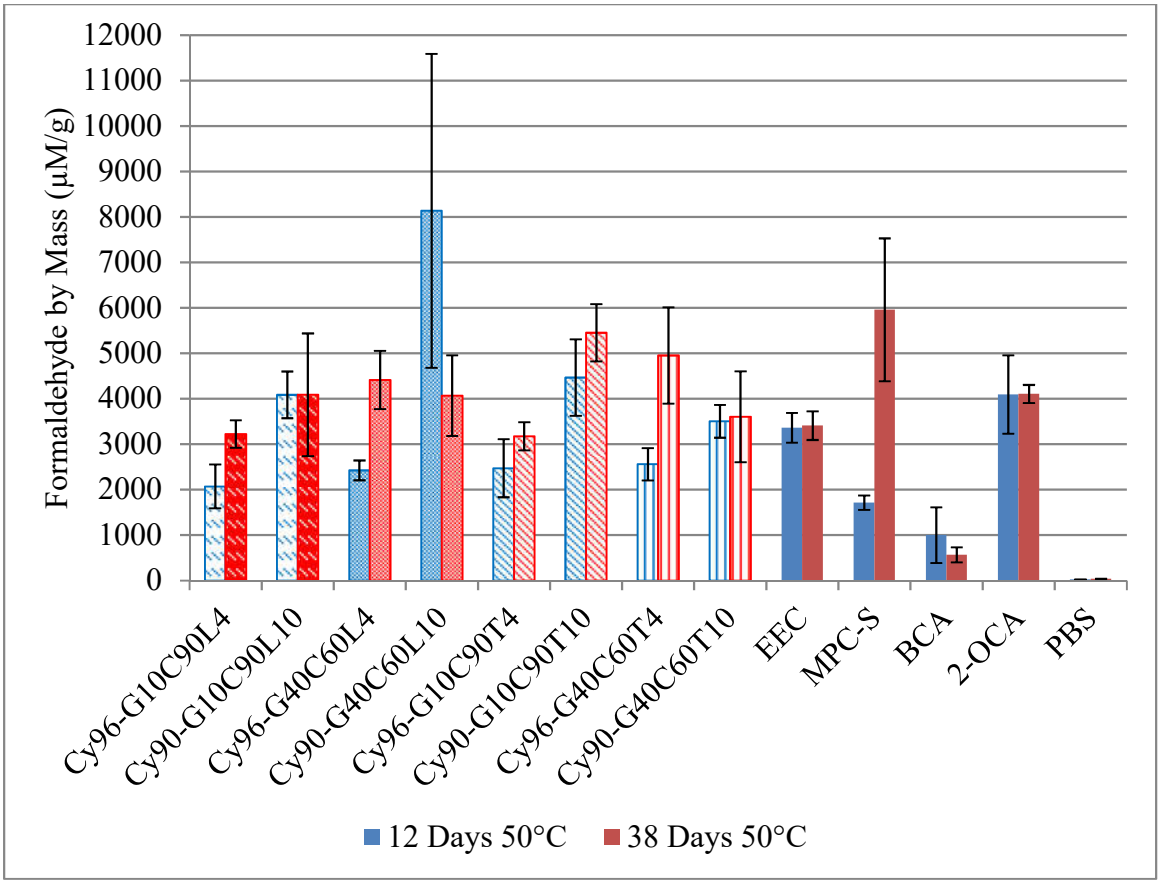


Figure 5-14: Adhesives and Gibco™ PBS Formaldehyde Concentrations by Adhesive Mass

It is difficult to distinguish consistent relationships between weight percent adhesive modifier, glycolide amount, and initiator type (linear versus branched) versus formaldehyde release using Figure 5-14 alone. Similar to the mass loss results, however, for all the Cy96s and Cy90s there appears to be a direct relationship between days in hydrolysis and formaldehyde release. This relationship indicates that the adhesive modifier and adhesive degraded when placed in Gibco™ PBS (hydrolyze) at least partially following the unzipping polycyanoacrylate degradation pathway. Increasing the amount of time the polymerized adhesive formulation was in Gibco™ PBS, thus, caused

the adhesive formulation to lose mass more rapidly, and thereby result in an increased amount of formaldehyde released.

As previously discussed, it was hypothesized that the increased amount of adhesive modifier and increased amount of glycolide for several of the adhesive modifiers would decrease the pH of the eluent thereby causing the adhesive primarily to follow the chain scission degradation pathway, thereby decreasing the amount of formaldehyde released (less unzipping degradation). In addition, it was expected that using a branched initiator for several of the adhesive modifiers would cause the polyester to have a decreased crystallinity, and thus more readily hydrolyze. Cy90-G40C60T10 was formulated using 10 weight percent adhesive modifier as included in Table 5-1. The polyester used in particular was synthesized using a branched initiator and 40 molar percent glycolide. Cy90-G40C60T10 would thus be expected to have the greatest mass loss, lowest pH, and potentially lowest amount of formaldehyde released of all the Cy96s and Cy90s formulated. As demonstrated in Figure 5-7 and Figure 5-10, Cy90-G40C60T10 had the greatest mass loss and lowest pH of all the Cy96s and Cy90s. As demonstrated in Figure 5-14, however, this adhesive did not have the lowest formaldehyde release amount. Comparing Cy90-G10C90T10 (polyester with branched initiator and 10 molar percent glycolide, and 10 weight percent in MPC-S) and Cy90-G40C60T10 appears to indicate an inverse relationship between adhesive modifier glycolide molar percent and formaldehyde release rate exists. This formaldehyde release observation provided evidence that the previously stated hypothesis may be true. This same relationship may have not been observed for the linear polyesters because these

adhesive formulations used adhesive modifiers with linear initiators, so they would be at a greater expected crystallinity and thus slower degradation rate than the adhesive formulations containing the triaxial polyesters.

The formaldehyde release results for the polymerized tested adhesives that did not contain adhesive modifiers (MPC-S, EEC, BCA, and 2-OCA) were also summarized in Figure 5-14. The EEC and MPC-S results appear to indicate a direct relationship between alkoxy side chain length and formaldehyde release at the 12-day time point, but not at the 38 days-time point. As previously discussed, EEC had a much lower eluent pH and much greater mass loss than MPC-S. For this reason, the fast degrading EEC would be more likely to have a greater formaldehyde release than the slower degrading MPC-S initially. At later time points, however, MPC-S with its more basic eluent pH would have more unzipping degradation and thus more formaldehyde than the acidic eluent producing EEC degradation with its side chain scission polycyanoacrylate degradation pathway resulting in cyanoacrylic acid and alcohol. Based on the EEC, MPC-S, and BCA results, there also appears to be an inverse relationship between alkane side chain length and formaldehyde release. This result was expected because there is a direct relationship between alkane side chain length and steric hindrance for polymerized adhesives, which in turn causes a decreased degradation (mass loss) rate and thus less formaldehyde released. Lastly, the results demonstrate that stability time did not significantly affect the formaldehyde release except for MPC-S. This result is likely because these four adhesives tested did not contain any adhesive modifier; thus, their degradation path is limited to only the adhesive itself, which will typically more slowly degrade than polyglycolide based polyesters

resulting in less formaldehyde released over time. Although MPC-S contained a methoxy side chain, it also contained a propyl alkane side chain. As previously discussed, the pH for the MPC-S eluent was basic. Increased hydrolysis time thus resulted in more rapid degradation of the adhesive due to the alkoxy side chain group, but basic pH due to the alkane side chain group, thereby producing a large amount of formaldehyde.

The formaldehyde release results were further analyzed using statistics to determine whether there were any significant differences between the means for all the polymerized adhesives tested at each time point. ANOVA with Tukey multiple comparison ($\alpha$=0.05) was performed for the data using Minitab 17 software, with a resulting p-value of 0.000 indicating there was a statistically significant difference between the means. The Tukey multiple comparison demonstrated that the adhesives were split into seven groups; however, the groups had too much overlap across several groups for several of the adhesives, so significant differences between the adhesives in groups could not be distinguished. For this reason, the data was subjected to further statistical analysis through regression analysis to determine whether the factors that describe the adhesives significantly affected the formaldehyde release results for the adhesives. From this data analysis, a p-value was calculated to identify the significance of each factor (see Table 5-1 and Table 5-2) as included in Table 5-7 and Table 5-8. Main effects plots (see Figure 5-15 and Figure 5-16) were also generated to display the relationships between the means from the formaldehyde release for the adhesives and the factors that describe the adhesives.

Table 5-7: Factors Significance for Cy96 and Cy90 Adhesives Formaldehyde Release

| | **Adhesive Modifier Initiator Chemical Structure (Initiator)** | **Adhesive Modifier Weight Percent (Wt. %)** | **Adhesive Modifier Glycolide Molar Percent (Gly Mol %)** | **Hydrolysis Time in Days (Stability Time)** |
|---|---|---|---|---|
| **Significance by p-value** | 0.519 (p-value>α of 0.05, so factor not significant) | 0.002 (p-value<α of 0.05, so factor significant) | 0.202 (p-value>α of 0.05, so factor not significant) | 0.367 (p-value>α of 0.05, so factor not significant) |

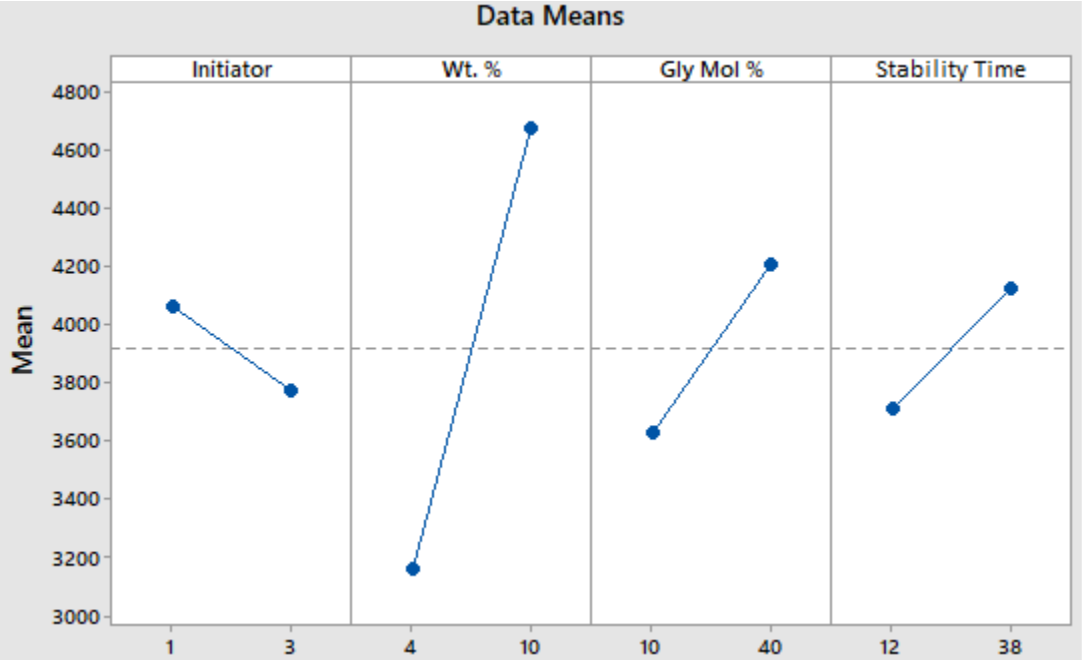

Figure 5-15: Main Effects Plot for Cy96 and Cy90 Adhesives Formaldehyde Release

The results in Table 5-7 and Figure 5-15 demonstrate that the initiator, glycolide content in adhesive modifier, and stability time factors had a minimal effect on the formaldehyde release amount. In comparison, the statistical analysis results indicated that there was a direct relationship between weight percent adhesive modifier and formaldehyde release.

Table 5-8: Factors Significance for EEC, MPC-S, BCA, and 2-OCA Adhesives Formaldehyde Release

| | **Alkane Side Chain Length** | **Alkoxy Chain Length** | **Hydrolysis Time in Days (Stability Time)** |
|---|---|---|---|
| **Significance by p-value** | 0.003 (p-value<α of 0.05, so factor significant) | 0.002 (p-value<α of 0.05, so factor significant) | 0.116 (p-value>α of 0.05, so factor not significant) |

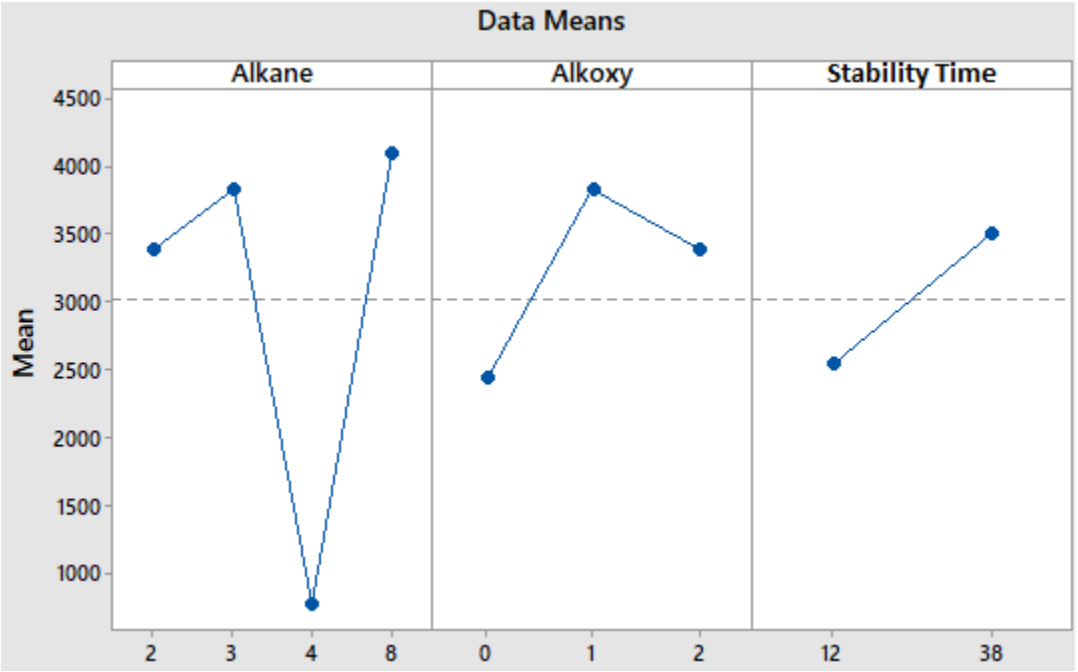


Figure 5-16: Main Effects Plot for EEC, MPC-S, BCA, and 2-OCA Adhesives Formaldehyde Release

The results in Table 5-8 and Figure 5-16 partially demonstrate the relationships observed. Specifically, an inverse relationship is demonstrated between side chain alkane length and formaldehyde release except for 2-OCA for the main effects plot; however, the regression analysis results demonstrate that the alkane side chain length was not a significant factor. This discrepancy is likely because there was only a significant change

in formaldehyde release when the alkane side chain length was decreased from 4 to 3. A direct relationship was also demonstrated between alkoxy side chain length and formaldehyde release. Lastly, both the main effects plot and regression analysis results demonstrate that stability time did not significantly affect the formaldehyde release. This result is likely because these four adhesives tested did not contain any adhesive modifier; thus, their degradation path is limited to only the adhesive itself, which will typically more slowly degrade than polyglycolide based polyesters resulting in the release of less formaldehyde.

### 5.3.6. Potential Effects of Excess DMT on Polymerized 2-OCA

As previously mentioned, the 2-OCA mass loss and formaldehyde release amount was much greater than expected. As mentioned in Chapter 4, all the adhesives except 2-OCA were polymerized with at most 2 µl N,N-dimethyl-p-toluidine (DMT) for every 1 ml adhesive. For 2-OCA, this DMT volume had to be increased to 60 µl per 1 ml adhesive (i.e. 5.6% DMT in 2-OCA) in order for the adhesive to polymerize, which was a 30 fold increase compared to the other adhesives. DMT is a weak base, so at a 30 fold increase compared to the other adhesives, the DMT may have increased the pH for the 2-OCA eluent.[25], [26] As included in Figure 5-10, the eluent for this adhesive was greater than 7 pH (basic) at both time points, which may be a result of the excess DMT. This basic environment may have caused the polycyanoacrylate to not only more readily degrade, but also more readily follow the unzipping pathway resulting in the formation of formaldehyde and cyanoacetate. The DMT may also have diffused out of the

polycyanoacrylate creating an excess of pores in the polymerized cyanoacrylate as compared to the other degraded adhesives, which would increase the degradation rate for the polycyanoacrylate. Other research has demonstrated that there is an inverse relationship between DMT amount and polycyanoacrylate molecular weight.[27] For this reason, the 2-OCA polymerized for this research may have had a lower molecular weight than the 108,000 Da measured in other research.[9] The basic pH and potentially lower molecular weight are potential reasons for the increased mass loss and formaldehyde release amount for 2-OCA for this research.

### 5.3.7. Adhesives Cytocompatibility

The cytocompatibility testing results included a score of 0/4 for all three samples tested for all the polymerized adhesives tested except for Cy90-G40C60T10 and EEC. These latter two polymerized adhesives had a score of 2/4 for all three samples tested for both adhesives. According to ISO 10993, these scores of 2/4 or less indicate all the polymerized adhesives tested were non-cytotoxic. In comparison, a score of 3/4 or 4/4 would indicate a sample was cytotoxic. A representative image of the cytotoxicity results for a score of 0/4 and an image for a score of 2/4 can be found as Figure 5-17 and Figure 5-18 respectively. A 0/4 score was given to Figure 5-17 based on the high cell confluency. In comparison, a 2/4 score was given to Figure 5-18 based on the slightly reduced cell growth as compared to Figure 5-17.

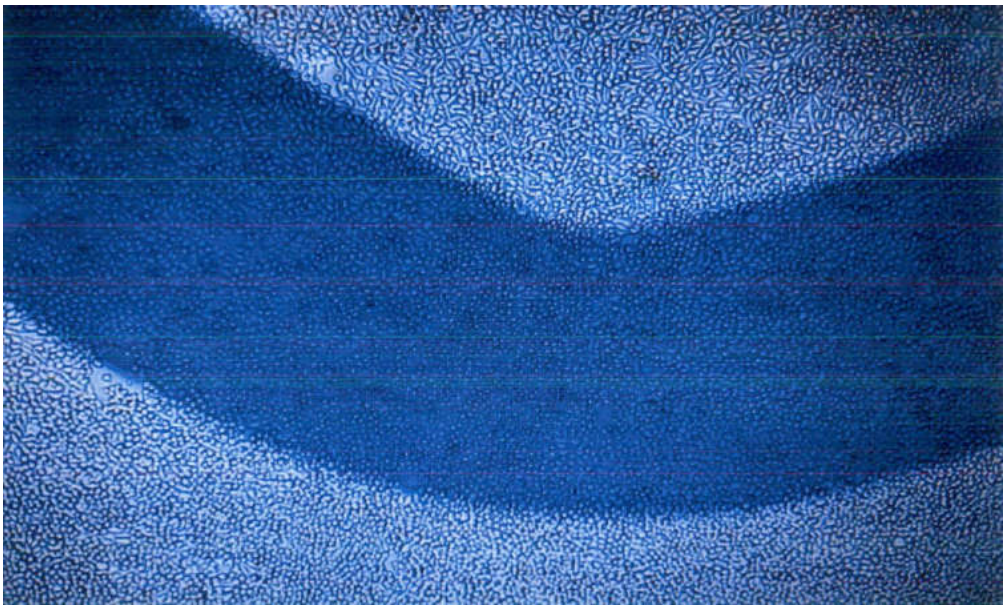

Figure 5-17: Cytocompatibility Results Image at 4x Magnification for Cy90-G40C60L10 with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

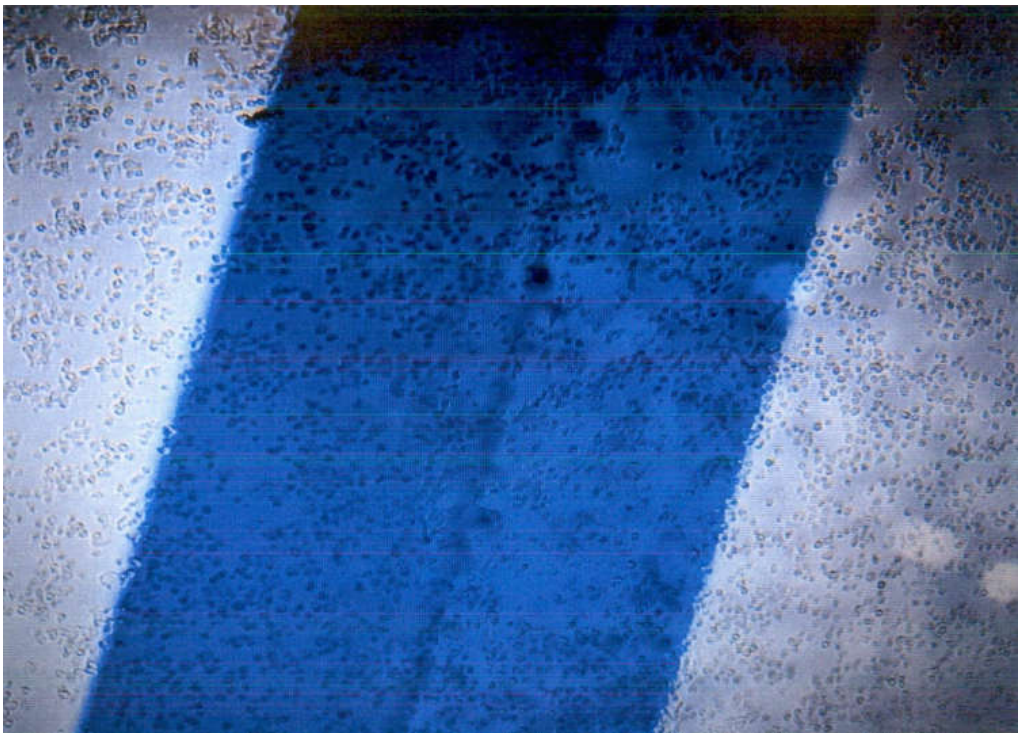

Figure 5-18: Cytocompatibility Results Image at 4x Magnification for Cy90-G40C60T10 with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

The biocompatibility of the adhesives was assessed based on the mass loss, pH, formaldehyde release, and cytocompatibility results as included and previously discussed. Based on the results, for the adhesives and time points tested, all the adhesives would likely be biocompatible if applied internally; however, Cy90-G40C60T10 and EEC may result in cytotoxicity issues. Specifically, these two adhesives produced the lowest eluent pH levels, which may result in tissue necrosis due to cellular activity inhibition.[28] In addition, these two adhesives had the highest cytotoxicity scores for the tested adhesives. Although the scores did not indicate the adhesives were cytotoxic, the scores were greater than the other adhesives, so they may have proved to be cytotoxic if they had been allowed to degrade for a longer amount of time.

### 5.3.8. Adhesives Hydrolysis Testing Broad Analysis

After reviewing the collected mass loss, pH, formaldehyde release, and cytocompatibility data, there were several patterns observed for the adhesives tested. The use of a branched initiator did not result in polymers that were significantly structurally different from their linear initiator analogs. All the polymeric modifiers were synthesized to have a low molecular weight, which resulted in them having an amorphous appearance based on their clear color. If several of the polyesters had an increased crystallinity they would be expected to have a slower diffusion of water into the polymer due to the polymer's packed configuration; thus, the polymer would be expected to have a slower degradation.

The polyester modifiers for the Cy96 and Cy90 adhesives varied depending on their initiator type and glycolide molar percent. Each research adhesive formulation varied depending on its polyester modifier type and weight percent in the adhesive monomer as well as the polycyanoacryalte's hydrolysis time. Due to the differences between the types of research adhesives, they were expected to have different mass loss, eluent pH, and formaldehyde release properties. Reviewing the data collected demonstrated that the adhesives performed as expected for mass loss and eluent pH. Specifically, as the weight percent of polyester blended into them was increased, the mass loss increased thereby releasing more acidic byproducts from the polyester, which was associated with a decreased eluent pH. This relationship between mass loss and pH was also observed for increased glycolide amount in the polyester and increased stability time.

Comparing the results for day 12 and day 38 demonstrates that the research adhesives overall released more formaldehyde than hypothesized. Specifically, although the increased mass loss was associated with a decreased local pH, this lower pH was not consistently associated with a decreased formaldehyde release for the two tested time points. The formaldehyde release was only decreased when the pH was decreased to 5 or lower. Decreased formaldehyde would result in healthier tissue; however, continuous exposure to low pH would likely result in poor cell growth.

The cytotoxicity results included in Figure 5-18 demonstrate less cell growth as compared to Figure 5-17. These results were obtained after 24-26 hours of 37°C incubation for the polycyanoacrylate, agar, and L929 cells in eluent. In comparison, 12

days at 50°C was the minimum incubation time for the degradation study included in this research. If the polycyanoacrylates performed similarly for the cytotoxicity and degradation studies for eluent pH and formaldehyde release, Cy90-G40C60L10 (Figure 5-17) would likely have a higher pH value than Cy90-G40C60T10 (Figure 5-18); however, Cy90-G40C60L10 would likely have a greater formaldehyde release than Cy90-G40C60T10. These specific comparisons provide evidence that the decreased cell growth for Cy90-G40C60T10 may be due to a decreased pH and not an increased formaldehyde release. This decreased cell growth also represented a score of 2/4, which did not indicate Cy90-G40C60T10 was cytotoxic. In comparison, increased formaldehyde levels result in cytotoxicity as previously discussed.

## 5.4. Conclusions

After synthesizing several rheological modifiers, blending them into MPC-S to form adhesive formulations, polymerizing the formulations, and then allowing the adhesives to degrade in PBS, the effect of the rheological modifiers on a polycyanoacrylate's degradation including mass loss, local pH, and formaldehyde release was able to be determined. As expected, a direct relationship between adhesive modifier weight percent versus polymerized adhesive degradation rate (mass loss) was observed. The results also demonstrated that an inverse relationship between adhesive modifier weight percent versus polymerized adhesive eluent pH exists. There was not, however, a consistent inverse relationship between adhesive modifier weight percent versus polymerized adhesive formaldehyde release amount as previously hypothesized. Further analysis of

the data indicated that the hypothesized inverse relationship was only observed for lower local pH environments including the ones produced during degradation of poly(Cy90-G40C60T10) and poly(EEC). This observation provides evidence that low pH local environments can direct a poly(cyanoacrylate) to degrade by side-chain scission rather than unzipping, thereby resulting in less formaldehyde released as the polymerized adhesive degrades. These lower pH environments may decrease the amount of formaldehyde released, while not rendering the adhesive material cytotoxic. These research outcomes may thus provide a method to reduce formaldehyde released from degrading polycyanoacrylates by reducing their local pH, thereby increasing their biocompatibility, and thus likely improving cyanoacrylates for internal clinical use.

## 5.5. References


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# CONCLUSIONS

## 6. Conclusions

The goal of this research was to determine methods to potentially improve polycyanoacrylate biocompatibility to encourage its future clinical internal usage. This goal was accomplished by synthesizing novel chemically active rheological modifiers, blending them into anionically stabilized methoxypropyl cyanoacrylate (MPC-S), polymerizing the adhesive formulations, and then evaluating the ability of the modifiers to reduce the amount of formaldehyde released from the polymerized adhesives as it hydrolyzed. Methoxypropyl cyanoacrylate was selected as the base adhesive for this research due to the work completed in other research that proved the adhesive has an acceptable biocompatibility, mechanical strength, and bioresorption ability. It was postulated that by synthesizing poly(glycolide-co-caprolactone) bioresorbable modifiers, the modifiers would act as a heat sink to decrease the heat generation during cyanoacrylate polymerization. It was also postulated that the acidic byproducts produced through the modifiers' bioresorption would decrease the pH of the local environment at the polycyanoacrylate thereby driving the polymerized adhesive to degrade by side chain scission to produce cyanoacrylic acid and alcohol instead of degrading by unzipping to produce cyanoacetate and formaldehyde.

As an additional component of this research, the need was identified for a method to measure the heat of polymerization, reaction rate, and peak temperature change, and it was developed. This method was necessary in order to measure the success of the

cyanoacrylate blends in decreasing the thermal properties observed during the polymerization of cyanoacrylates. Using this novel method, an inverse relationship between adhesive modifier amount and measured peak temperature change, reaction rate, and HOP was determined. In addition, the test results demonstrated that the research adhesive formulations are predicted to have acceptable biocompatibility in terms of the tissue response to the exothermic reaction from the polymerizing adhesive.

Through the completion of this research, test results were also successfully collected to determine the effect of the adhesive modifiers on the degradation of the polycyanoacrylate research formulations. A direct relationship between adhesive modifier weight percent versus polymerized adhesive degradation rate was determined. An inverse relationship between adhesive modifier weight percent versus polymerized adhesive eluent pH was also determined. An inverse relationship between adhesive modifier weight percent versus polymerized adhesive formaldehyde release amount was also demonstrated for decreased local pH environments. These low pH states were generated by degrading adhesive modifiers with a high molar percent of glycolide. A method to reduce the formaldehyde amount released as a polymerized adhesive degrades was thus determined. When these polymerized adhesives were tested for their cytocompatibility, it was demonstrated that these polymerized adhesives were non-cytotoxic. As a final conclusion, the research was thus able to indicate the potential ability of chemically active rheological modifiers to improve many aspects of medical cyanoacrylates for both internal and external clinical use.

## 7. Recommendations for Future Work

After completing the methods included in this research, there were additional studies noted that could be completed potentially to further the conclusions from this research:

- Synthesize adhesive modifiers with a continuous hydrolysis rate by increasing the amount of glycolide in the polymer and the amount of adhesive modifier in the cyanoacrylate
    - The formaldehyde release amount could be further inhibited through a maintained decrease in the local pH to a value between 5-7 for the entire wound healing cycle and polymerized adhesive degradation
- Blend the adhesive modifiers into cyanoacrylates other than methoxypropyl cyanoacrylate such as ethoxyethyl cyanoacrylate
    - Based on knowledge gained in this research, additional cyanoacrylates may be improved for clinical internal use through the addition of the novel rheological modifiers

- Evaluate the polymerized adhesive formulations long-term to determine their effect on local pH and their formaldehyde amounts released at long-term time points
    - Evaluation of the polycyanoacrylates long-term may indicate their biocompatibility throughout the entire degradation process for the materials
- Reduce the variability between polymerized adhesive test specimen sizes
    - Reduce the tray size (mold) to produce one individual test specimen per tray rather than breaking larger samples into small pieces
- Test the adhesive formulations using ASTM standard test methods
    - ASTM methods would allow accurate comparisons to be made between this research and other completed studies
- Evaluate the polymerized adhesives *in vivo* using a model such as a porcine small intestine
    - Although the aim of this research was not to produce an adhesive product, an *in vivo* study would allow one to determine the ability for the adhesive formulations to function for wound approximation or sealant applications as potential cyanoacrylate products
- Further evaluate the repeatability and reproducibility for the polymerized adhesives thermal properties test method (PATP) created through this research
    - Perform Gauge R&R analysis for the PATP method

# APPENDIX

## A: Supplemental Results from Chapter 5

Figure A-1: Image at 4x Magnification for Cellular Response to Polymerized Cy96-G10C90L4 with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

Figure A-2: Image at 4x Magnification for Cellular Response to Polymerized Cy90-G10C90L10 with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

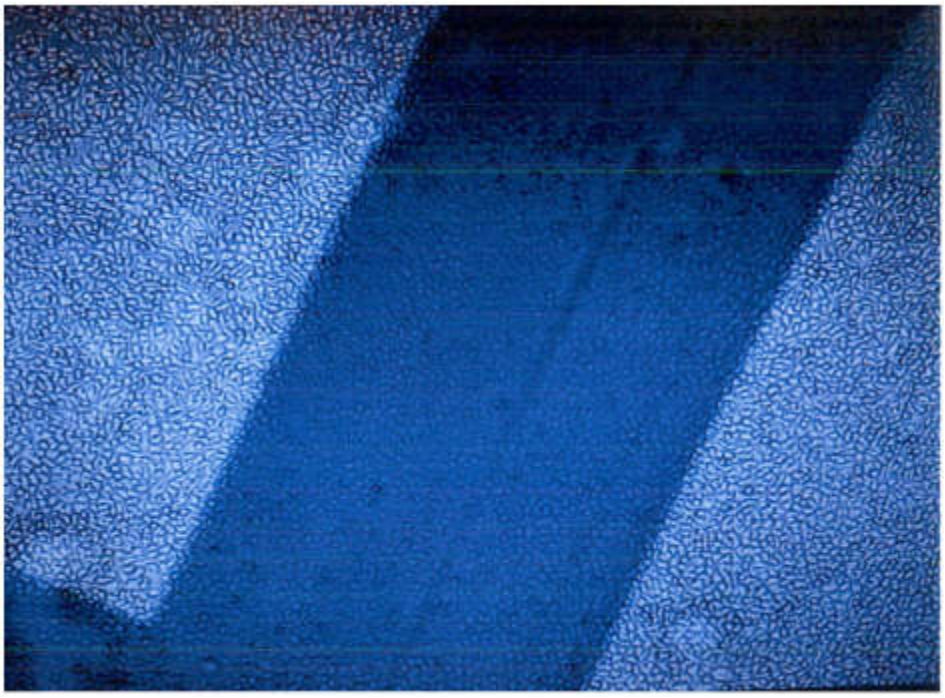

Figure A-3: Image at 4x Magnification for Cellular Response to Polymerized Cy96-G40C60L4 with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

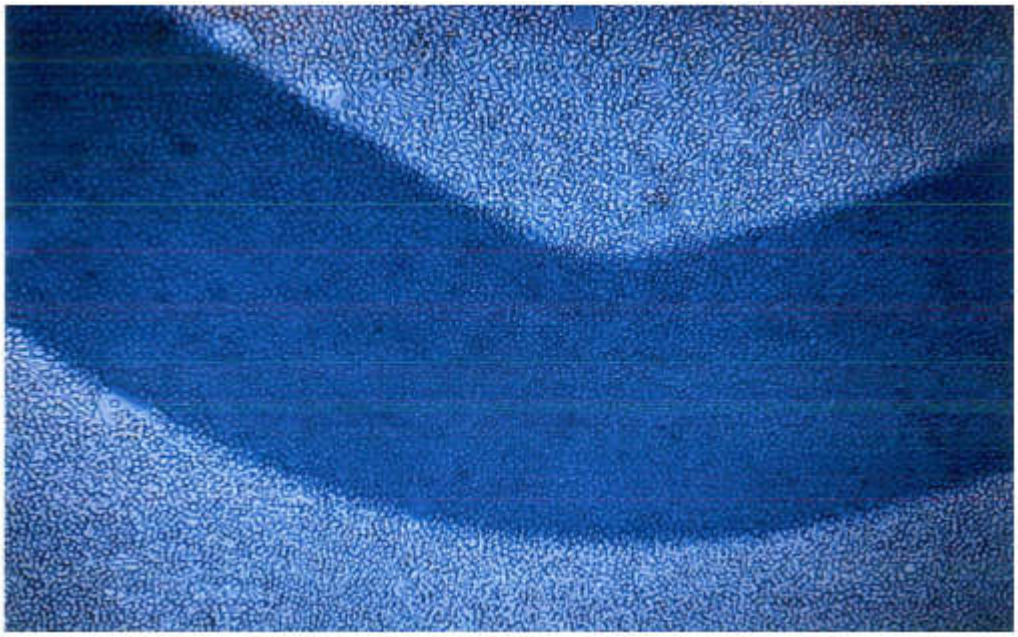

Figure A-4: Image at 4x Magnification for Cellular Response to Polymerized Cy90-G40C60L10 with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

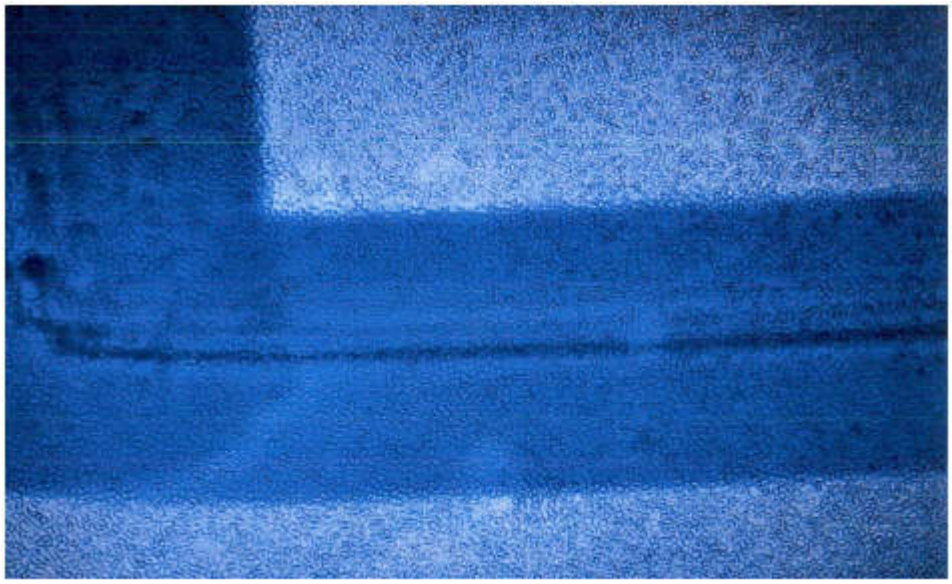

Figure A-5: Image at 4x Magnification for Cellular Response to Polymerized Cy96-G10C90T4 with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

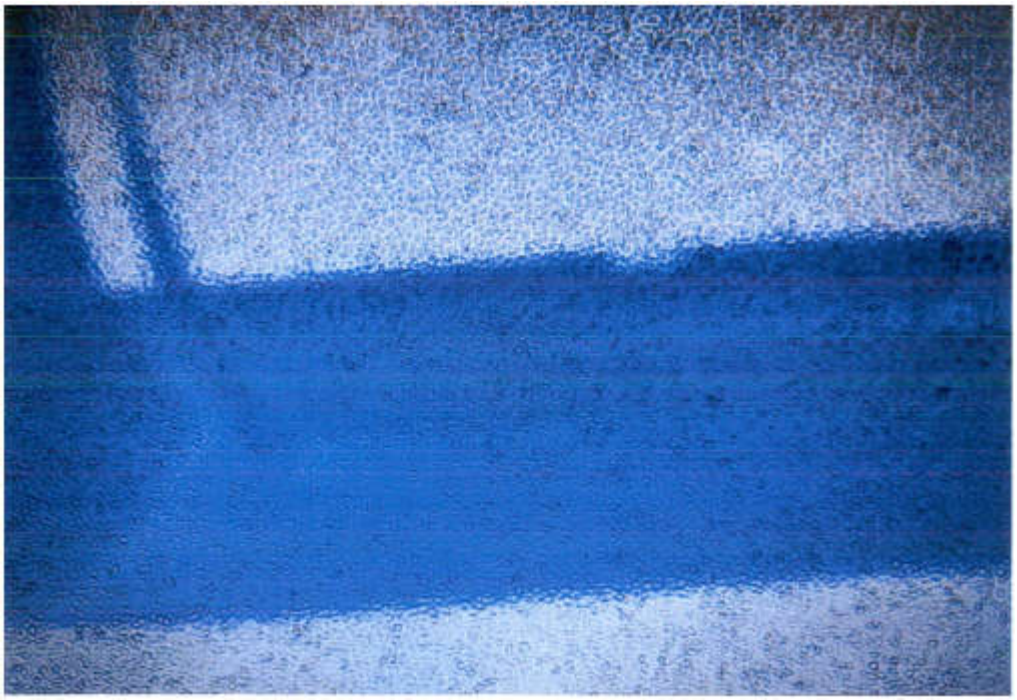

Figure A-6: Image at 4x Magnification for Cellular Response to Polymerized Cy90-G10C90T10 with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

Figure A-7: Image at 4x Magnification for Cellular Response to Polymerized Cy96-G40C60T4 with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

Figure A-8: Image at 4x Magnification for Cellular Response to Polymerized Cy90-G40C60T10 with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

Figure A-9: Image at 4x Magnification for Cellular Response to Polymerized EEC with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

Figure A-10: Image at 4x Magnification for Cellular Response to Polymerized MPC-S with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

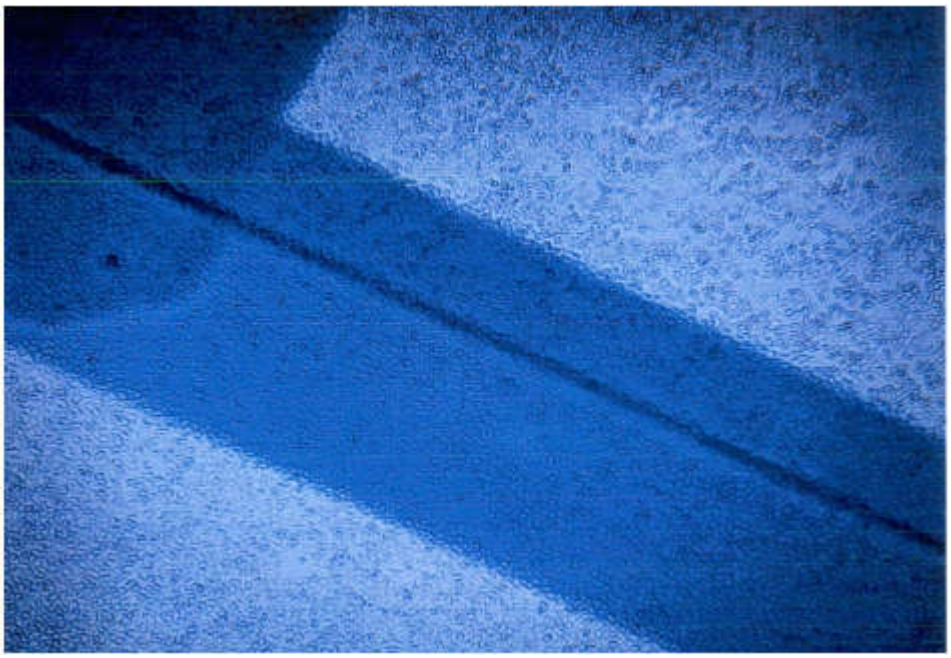

Figure A-11: Image at 4x Magnification for Cellular Response to Polymerized BCA with L929 Cells (dots) and Sample Placement Area (dark outline) Visible

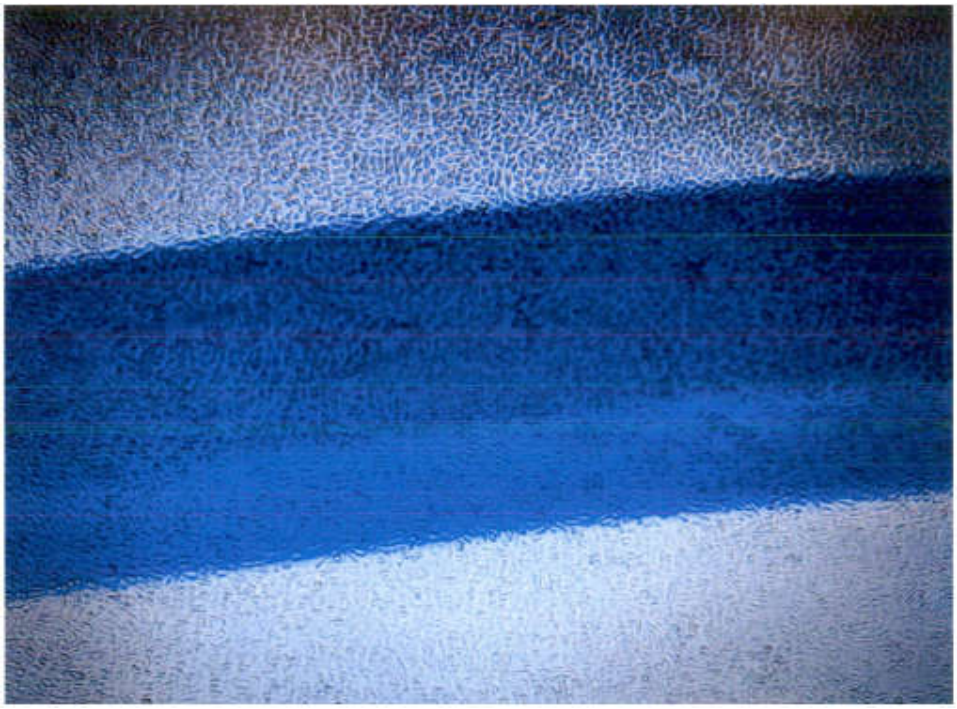

Figure A-12: Image at 4x Magnification for Cellular Response to Polymerized 2-OCA with L929 Cells (dots) and Sample Placement Area (dark outline) Visible