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Quantification of Volatile Residuals in Polydioxanone by Gas Chromatography: Method Development, Validation and Implementation

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QUANTIFICATION OF VOLATILE RESIDUALS IN POLYDIOXANONE BY GAS CHROMATOGRAPHY: METHOD DEVELOPMENT, VALIDATION AND IMPLEMENTATION

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by
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ABSTRACT

Bioresorbable polymers are increasingly utilized for implantable medical devices to avoid complications related to explant and/or long term responses to foreign materials. As with all polymeric materials, residual levels of unreacted monomer, and other volatiles, drastically affect the mechanical and chemical properties as well as the parameters required to process the material into a device. The 1,4-dioxane-2-one (PDO) monomer is a six membered ring that forms an ether-ester polymer via ring-opening polymerization (ROP) with intermediate absorption rate properties that are ideal for numerous implant situations. Developing devices using poly(1,4-dioxane-2-one) (PPDO, PDO, PDX, PPDX, or PDS) is complicated by residual levels of the unreacted, ring-form of the monomer causing the material to be toxic to biologic tissue in addition to the typical negative effects of excessive residual monomer in polymers, especially bioresorbable polymers. It is required to quantify the residual monomer content of PDO polymer throughout the manufacturing and storage of polymer or devices to ensure proper and repeatable processing in addition to the safety aspects related to the end use of the material. This work describes the application and comparison of known gas chromatography techniques, multiple headspace extraction and classical vaporization injection, to separate the residual monomer from the polymer matrix and allow for quantification. Accuracy and repeatability evaluations were used to determine the ideal testing methods to be used in industry as regulated by governing agencies such as the Food and Drug Administration (FDA).

DEDICATION

I could never thank enough the One who taught me love. How to love myself, my actions and those around me will always be the greatest lesson to be learned. This manuscript is dedicated to my loving family. My wife, Lindsey, knows me better than anyone ever has or will. She knows when I need to be pushed and when I need room to grow. I would not have been able to find and achieve the success I know today without her support. We share our most precious blessings in this life, my son Carson and daughter Ella. They have proven to be the truest personifications of love, pride, admiration, humility, and hope. My continued interest in my family's success will always enhance the difficulties and triumphs associated with my efforts. My family is the reason that I want to be successful and the reason I continue my determinations in the face of disappointment. I will spend the rest of my life sharing my success and the weight of my struggles with my family, whom I love more than words will ever accurately express.

ACKNOWLEDGMENTS

I would like to thank Poly-Med, Inc. (PMI) for the continued support, a cutting edge laboratory for research, and an overwhelming belief in my potential. I left a job in a restaurant to work at PMI in 2009. Dr. Shalaby W. Shalaby saw a future in Materials Science within me that I never knew existed. The Shalaby family welcomed me into their lives and business by offering me the opportunity to learn the science of bioresorbable polymers. This changed the course of my life towards an unimaginably exciting, challenging and rewarding career that I can spend the rest of my life pursuing.

I must specifically thank Dr. Joel Corbett for his gracious understanding of my learning style and my unique perspectives on science and the world. The lengthy conversations we had during the past several years have molded my understanding of lab work, bioresorbable polymers, academic research, and industrial scientific advancement. I know that I owe my continued opportunities at PMI to Dr. Corbett's support of my abilities and his continued willingness to aid in my personal, professional, and scientific development into valued assets.

I would like to thank Dr. Gary Lickfield for his initial faith in my future success. I came to Dr. Lickfield in pursuit of graduate studies at Clemson University. His advice and guidance allowed my academic career to flourish. I could never thank him enough for his contributions to the continued satisfaction of my thirst for knowledge.

Also acknowledged are Dr. Scott Taylor, Dr. O. Thompson Mefford, Chris Hawkins, Matt Benton and David Shalaby for all being instrumental to my success as a student, researcher, engineer, man, father, and husband. This manuscript required a great deal from so many, thank you.

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CHAPTER ONE

INTRODUCTION AND REVIEW OF LITERATURE

Industrial Implementation of Polymers

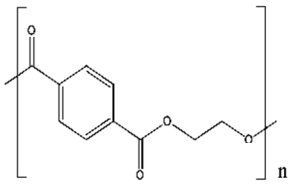
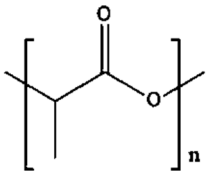
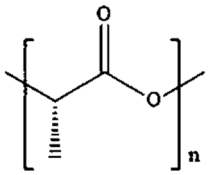
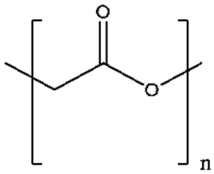
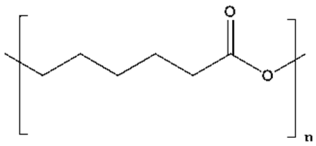
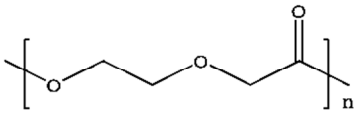
In the broadest chemical definition, polymers are molecular chains of relatively high molecular weight. These high molecular weight molecules are formed from smaller molecules known as monomers. Polymeric materials are seemingly present in every aspect of modern life. Their use and application is widespread and only limited in the field of material science by imagination. Anne and Henry Emblem specifically noted in *Packaging Technology* that:

> "Compared with other packaging materials, plastics are generally lighter in weight, more easily formed into different shapes, and extremely versatile. This versatility is largely responsible for their growth in usage: the ability to carefully tailor the performance of a plastic container to the needs of the product, the market and the demands of the supply chain means that there is a 'plastic' solution to almost all packaging problems [1]."

The utility of plastics is not limited to packaging. Nearly every industry can and has benefited from the modular nature of polymeric material integration into research and business. The well-known polyester, poly(ethylene terephthalate), or PET, that is used to make disposable drink containers, is "the common thermoplastic polyester used in packaging [1]." PET is differentiated from the other polyester materials in Table 1 by the

synthesis of the bioresorbable polyesters. Ring opening polymerization (ROP) is utilized to form high molecular weight bioresorbable materials from six membered ring monomers [2]. Poly(D,L-lactide) (PDLA), poly(L-lactide) (PLA or PLLA), polyglycolide (PGA), polycaprolactone (PCL), and polydioxanone (PDO or PDS) are the most common polyesters used to create bioresorbable medical devices due to their ability to degrade in the body after the intended purpose is complete. The most common example of these polymers use in the medical field is historically in bioresorbable sutures. The future of bioresorbable implants has been the source of speculation for decades [3-5]. In addition to sutures, bioresorbable materials have been considered for uses nearly anywhere non-degrading plastics are currently used. This includes food packaging, composting bags, disposable dishware, and more elaborate biomedical uses [3]. Bone fixation, drug delivery and tissue scaffolding are currently developing markets that are already proving as excellent opportunities for utilization of polyester based bioresorbables [6].

Table 1: Common Polyester Materials and Respective Melting Properties [2, 7]

Polymer	Structure	Melting Temperature (°C)	Glass Transition Temperature (°C)
PET		270	61
PDLA		N/A	57
PLLA		173	60 - 65
PGA		225	35 - 40
PCL		64	-36
PDO		107	-10 - 10

Tensile and tear strength, along with the elongation, are the most relevant properties of a packaging material so that the material can be processed, implemented and utilized [1][2]. These properties are just the beginning of the material requirements for polymeric medical devices. There are dozens more properties that dictate the effectiveness of a plastic for each intended use such as surface friction, thermal resistance, optical properties and cyclic stress endurance. The modern material properties that need to be met or exceeded by biodegradable materials are also widely varied due to the requirements that are dictated by the array of biological properties sought to be mimicked. Thus, a large collection of tailored materials must be developed to meet the many criteria of the shift to bioresorbable or degradable polymers [3].

Bioresorbable Polymers for Medical Applications

The use of polymers in the medical industry is known to be ubiquitous, but bioresorbable polymers are defined by the ability to degrade in the body and remove the need for explant surgeries as well as the risk of long term negative responses [7, 8]. Editor Castia Bastioli noted in a 2014 publication:

> "Today the bioplastics available in the market at different levels of development are mainly carbohydrate-based materials. Starch can be either be physically modified and used alone or in combination with other polymers, or it can be used as a substrate for fermentation for the production of polyhydroxyalkanoates or lactic acid, transformed into polylactic acid (PLA) through standard polymerization processes. Also vegetable oil based polymers are under development."[3]

When discussing the implementation of bioresorbable materials, formally referred to as absorbable polymeric materials, into medical devices, the criteria are known to be extensive and complicated. The requirements of implementing bioresorbable polymeric materials into medical devices originate with matching the existing mechanical and chemical properties of currently used materials or even better, the biological environment of the intended use. The benefit of this engineering and optimization process is a device that does not require explantation and avoids complications related to the presence of the materials beyond their useful life. The current transition to polymeric medical devices was made possible by advances in polymer processing technologies that allowed sufficient strength when compared to metallic and ceramic materials being used, but with

lighter weight and greater flexibility [7, 9-11]. These polymer processing techniques include fiber processing science that was necessary for the success of further transformation of the medical device market with bioresorbable sutures. The rate at which the bioresorbable materials degraded and were removed from the patient through biological pathways such as exhaled carbon dioxide and excreted small molecules was the next characteristic of bioresorbable polymers that required control and modulation for each individual end use [4, 7]. Shalaby and Burg noted in their 2004 book *Absorbable and Biodegradable Polymers*:

> "Interest in synthetic absorbable polymers has grown considerably over the past three decades, principally because of their transient nature when used as biomedical implants or drug carriers. The genesis of absorbable polymers was driven by the need to replace the highly tissue-reactive, absorbable, collagen-based sutures with synthetic polymers, which elicit milder tissue response. This led to the early development of polyglycolide as an absorbable polyester suture. In spite of the many polymeric systems investigated as candidates for absorbable implants and drug carriers, ester-based polymers maintain an almost absolute dominance among clinically used systems and others that are under investigation [4]."

In medical devices benefit to patient health dictates industrial acceptance more than the cost of materials. Gajjar and King claimed the forces that are advancing degradable materials for bioengineering include:

> "(i) improved long-term biocompatibility compared to many of the existing permanent implantable materials; (ii) the advantage of not having to remove the bioresorbable implant once it is no longer needed, thereby avoiding a second surgical operation; and (iii) the growth of emerging biomedical technologies, such as tissue engineering, regenerative medicine, gene therapy, controlled drug delivery, and bionanotechnology, all of which require bioresorbable materials [6]."

The biocompatibility, resilience, potential strength and well defined degradation mechanism initiated by the moisture in the body are the sources of confidence in the family of bioresorbable polyesters [12]. The advantages related to avoiding explants, follow up surgeries, and long-term implant complications should not be underestimated, however, each of the benefits of bioresorbable polymeric materials is affected by the presence of residual level contaminants [13-17]. These contaminants can include residual monomer, catalyst, solvent, initiator, processing aids, side reaction products, as well as any contaminates originally present in the raw materials. Synthesis of polymers by ROP is more susceptible to exhibiting equilibrium reaction kinetics than that of a typical chain growth reaction [2].

$$[Monomer] \overset{CAT}{\longleftrightarrow} [Polymer]$$

ROPs do parallel many reaction tendencies of chain growth polymerizations, but generally proceed at the slower reaction rates known to step growth, condensation polymer syntheses. Chain growth and ROP reactions that undergo reversible polymerization / de-polymerization are observed to transition from primarily polymerization to de-polymerization at a maximum temperature known as the ceiling temperature according to the equation below where $[M]_c$ is the equilibrium concentration of monomer in the polymer at the ceiling temperature, T_c [2].

$$ln[M]_c = \frac{\Delta H}{RT_c} - \frac{\Delta S}{R}$$

When discussing ROP above the ceiling temperature, the stability of the ring form of the monomer drives the reaction backwards to generate monomer from the polymer. A consequence of the application of this formula to ROP with six membered rings, generally the most stable ring structure, indicates that the bioresorbable polyester family must be uniquely prone to non-trivial residual monomer concentrations. For example, the equilibrium residual monomer content of PLA is 3% at approximately 180°C, but can be reduced to 0.3% or less by standard methodologies [18]. Thermal generation of lactide and other monomers during processing is also documented as a common source of residual monomer in many of the most common bioresorbable materials and devices [13].

Residual level components in a material function as defects in the material matrix in addition to potentially serving as catalysts and plasticizers. These actions are often quantified in relation to degradation kinetics. The onset temperature of a bioresorbable, aliphatic polyester's thermal degradation was reduced as levels of residual catalyst, or other transition metals, were increased [14]. In addition to metallic contaminants, residual

levels of volatile organic compounds (VOCs) are also of concern within materials utilized for medical devices as well as other industries due to the potentially toxic effects. Sterilization of bioresorbable polymers is often performed via ethylene oxide gas exposure [4, 7]. The process unavoidably introduces a toxic, volatile residual into the material. Residual levels of this toxic residual monomer are also of concern when ethylene oxide (EO) gas is used to form polyethylene glycol (PEG) via ROP [2]. Any residual level contaminant in a material utilized for a medical device implant can become a leachable in the patient after implantation that results in local tissue damage [4, 19].

In addition to the toxicity concerns related to residual level contaminates, other critical aspects of bioresorbable polyesters are affected. The effects caused by residual monomer on the initial mechanical properties, rate of mechanical strength loss, and the rate of molecular weight reduction (bioresorption) of bioresorbable materials, especially high lactide and glycolide polymers, further support the need for residual monomer quantification [6]. *In vitro* degradation is often quantified by inherent or intrinsic viscosity (IV) analysis and mechanical testing of the polymeric material at specified time points of material exposure to the *in vitro* conditions. IV testing is used to monitor changes in the molecular weight of the polymer while standard tensile testing methods are typically used to quantify the strength loss. *In vitro* degradation of polylactide (PLA) by Ella *et al.* correlated strength loss over time with residual monomer content that was generated via extrusion settings that varied resonance time in the heated extruder and quantified by gas chromatography (GC). The study concluded that monomer content was more statistically relevant to the degradation rate than the diameter of the fibers tested.

Thus, the surface area and diffusion of moisture into the material were not as relevant as the relationship of monomer content to degradation of mechanical properties [15]. A summary of this data is shown in Figure 1 below. The retention of strength in a modelled environment was dramatically affected by relatively small changes in monomer content. This is most evident by observation of the decreasing slope of each trend as monomer content increases.

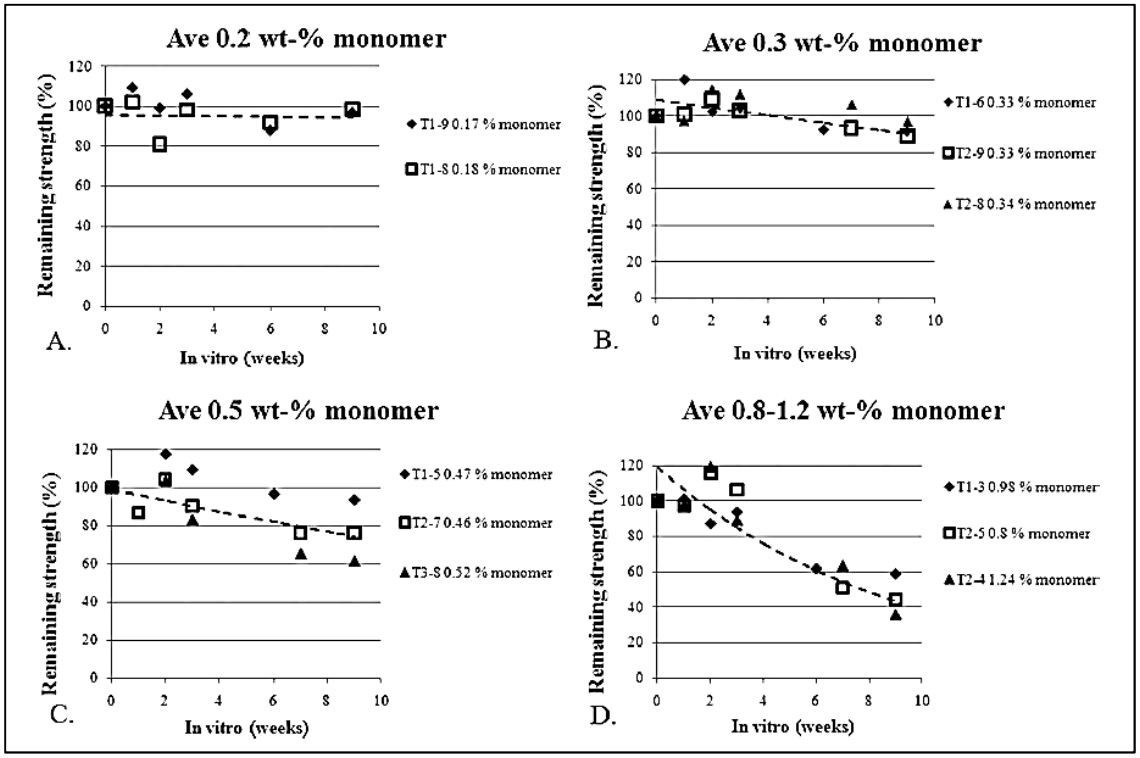


Figure 1: "Mean values of cumulative strength retention of fibers during 9-week hydrolysis. At the start of hydrolysis, fiber monomer content was for (A) 0.2%, (B) 0.3%, (C) 0.5%, and (D) 0.8–1.2%; results are shown as mean values (n = 5) [15]."

The molecular weight decrease *in vitro* is presented as a summary of Paakinaho's study in Figure 2. Paakinaho added L-lactide monomer to an 85% lactide / 15% glycolide random copolymer (PLGA 85/15) via melt blending. Lactide monomer was also

generated thermally during the blending. Total lactide monomer content was determined by gas chromatography with a flame ionization detector (GC-FID) [16].

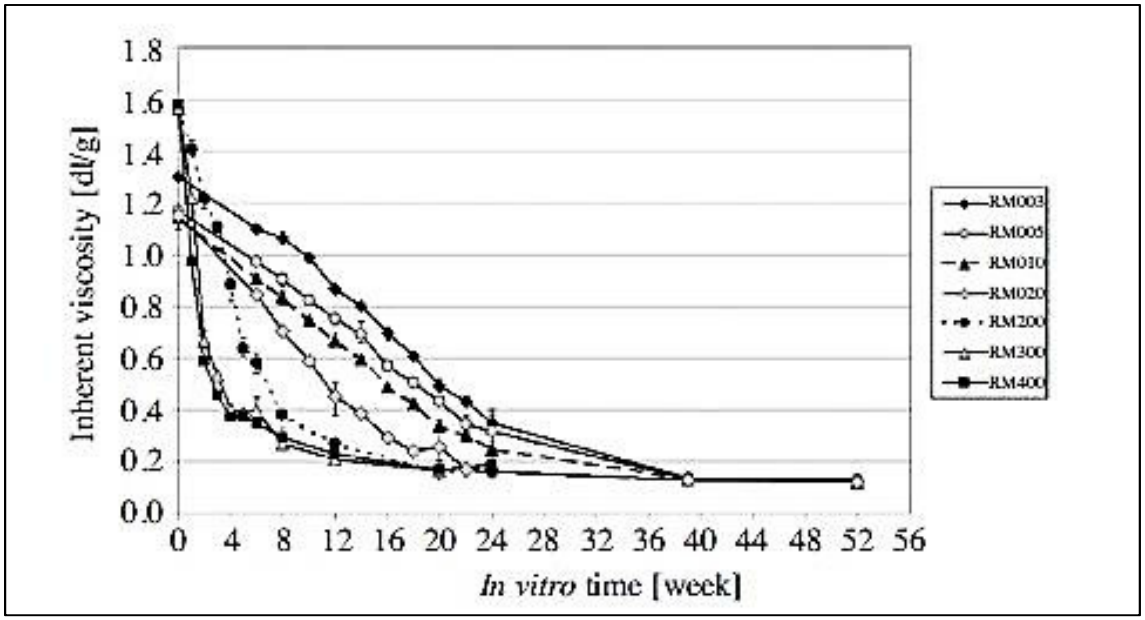


Figure 2: Paakinaho Data Summarized the Effect of Monomer Content on *In Vitro* Degradation, as Shown by Inherent Viscosity Decrease, in PLGA 85/15 (n=3). Monomer Concentration Ranged from 0.03% to 4.0% [16].

Most PLA and PGA based bioresorbable, polymeric devices with high strength characteristics are also too stiff to meet the ideal modulus and mechanical compliance characteristics for materials within many areas of the body. Most PDLLA materials that satisfy the low modulus and high mechanical compliance requirements cannot form strong devices. These conclusions have had an obvious effect on the rising interest related to 1,4-dioxane-2-one monomer based materials, but polydioxanone materials has proven costly and very difficult to process into an ideal device for other reasons [6].

A Review of Poly(1,4-dioxane-2-one)

Degradation rates of bioresorbable homopolymers vary from days to years, but achieving the ideal degradation for most biological implants of several weeks to a few months came at the sacrifice of strength and nearly all other material properties. The most common acceptance of bioresorbable polymers has been previously limited to use in sutures [7]. Polydioxanone (PDO) is an aliphatic polyether-ester that has been shown to degrade in the six to ten month range while providing impressive strength and flexibility when optimally processed [20-23]. This new polymer that appeared suitable when considering the common requirements of medical device manufactures was not truly new. Ethicon US, LLC, a Johnson and Johnson company, previously introduced the PDS® suture and an improved version is known as PDS® II. The PDS® and PDS® II sutures are comprised of polydioxanone homopolymer [7]. Current technology has decreased the cost of monomer and provided processing techniques and specialties that are now making PDO stronger and more available for medical device development [20, 24-26]. Several U.S. patents filed by Dr. Bezwada, *et al.* in the 1990s proposed polydioxanone as an ideal candidate for medical devices [21]. U.S. patent number 6,113,624, titled "Absorbable Elastomeric Polymer", claimed that introduction of dioxanone polymer segments into polymer compositions produced a "…bioabsorbable elastomer [that] has a high percent elongation, a low modulus, and outstanding tensile strength [22]." Decades of investigation into using bioresorbable materials have revealed several shortcomings related to previously commercialized bioresorbable polymers that relate to materials not being both strong and flexible. Despite achieving the necessary strength properties to

meet end use requirements, the shortcomings are summarized as degradation that is too fast or too slow as well as a lack of *in vivo* mechanical compliance. PDO can provide both properties [6].

The difficulties processing PDO are related to the polymer's inclination to degrade at relatively low temperatures by zero-order, unzipping de-polymerization that reduces molecular weight and generates increased amounts of residual monomer. The generation of monomer in PDO begins at approximately 80°C and reaches an ideal rate of degradation for recycling of the polymer into pure monomer by distillation between 200°C and 250°C [27]. This generation of monomer renders the polymer overly degraded or susceptible to autocatalytic degradation. Thus, no desired material properties may remain after non-optimized processing is attempted. Succinctly, PDO polymerization displays a fully reversible equilibrium reaction at temperatures between 80°C and approximately 300°C [27-29]. When exposed to 350°C, only 2% of the degradation products were identified as not PDO monomer by GCMS [29]. Because of the thermal complications, batch reactions of PDO have relatively low yields with as high as 50% residual monomer after polymerization. The desire for higher molecular weight polymers to achieve higher strength materials is contested by the thermodynamics and chemistry that dictates the low yields. Small amounts of inorganic additives are shown to increase tensile strength by promoting crystallization, but the higher crystallinity and residual amounts of inorganic reduced elongation of fibers from 400% to less than 30% [20].

The previously discussed complications with PDO relate to the PDO monomer content. The processing difficulty precipitated the need for a reliable methodology to

both remove volatile residuals and quantify the removal to ensure consistency. The objective was separation of the reacted polymer from the unreacted monomer without generation of a degraded polymer and/or additional monomer. The necessity of monomer removal introduced a need for an accurate, reliable testing method to determine the PDO monomer content of PDO throughout the processing to an ideal device content of 0.1% or less from a minimum starting monomer content of approximately 20%. Testing before, during and after the entire process is necessary to ensure optimization of resources and effectiveness of the process. Quantification of unreacted monomer in PDO, the specific homopolymer of 1,4-dioxane-2-one monomer, was the focus of the current study. Another way to quantify degradation in addition to IV is mass loss or weight retention. By tracking the mass of the material during the specified time points *in vitro*, the amount of low molecular weight material that can diffuse out of the material into the buffer solution can be quantified and indicate the rate of material degradation. This method of quantifying degradation is the slowest. The time required for detectable strength or molecular weight change is much less than the time required for the polymeric degradation products to solubilize in water and elute away from the bulk material. Li *et. al.* made observations of PDO polymer (PPDO) samples that had varying amounts of PDO added. The PDO polymer was heated under reduced pressure to remove monomer to a constant weight, and then PDO monomer was blended into the melted polymer at 0%, 3%, 6%, 9% and 12% by weight in a heated reactor / mixer. The initial mechanical data for each monomer concentration is tabulated in Table 2. Li *et al.*'s data from *in vitro*

degradation of PDO polymer (PPDO) with added PDO monomer can be found in Figure 3 and Figure 4.

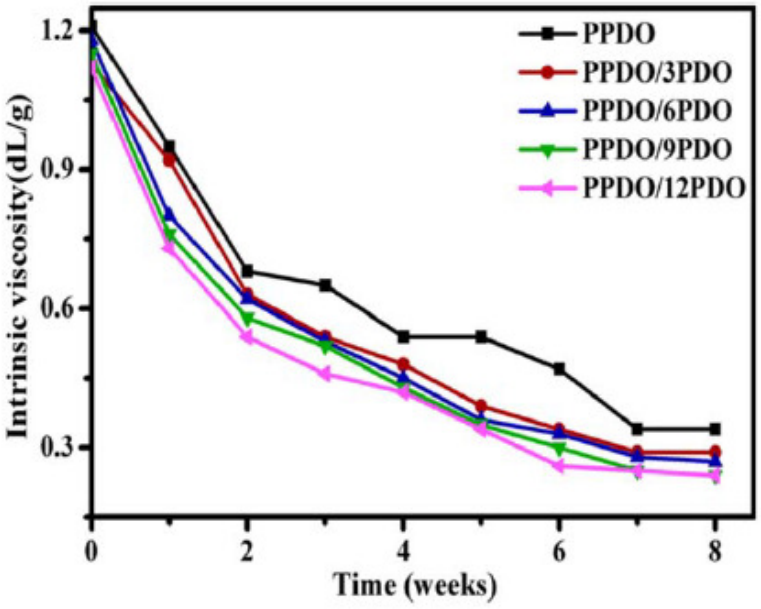


Figure 3: Changes of the Intrinsic Viscosity of PDO Polymer / PDO Monomer Samples during *In Vitro* Degradation. Note: The material descriptions indicate weight percent monomer added [30].

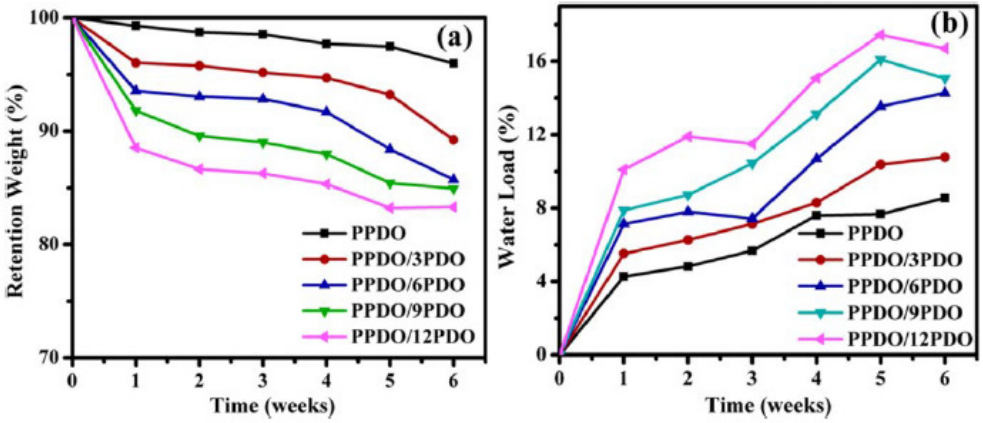


Figure 4: Changes of (a) the Weight Retention and (b) Water Absorption of PDO Polymer / PDO Monomer Mixtures during *In Vitro* Degradation. Note: The material descriptions indicate weight percent monomer added [30].

Table 2: Mechanical Properties of PDO/PDO Monomer Added. Note: The material descriptions indicate weight percent monomer added [30].

Samples	Tensile strength (MPa)	Elongation at break (%)
PPDO	53.2 ± 2.5	713 ± 48
PPDO/3PDO	53.5 ± 2.7	724 ± 65
PPDO/6PDO	48.0 ± 3.2	580 ± 49
PPDO/9PDO	43.0 ± 2.4	573 ± 29
PPDO/12PDO	34.9 ± 1.4	489 ± 30

The mass loss and moisture gain correlated well with the initially added monomer content levels. The 0% added material did experience mass loss and moisture gain as if it contained an appreciable amount of monomer. Nishida reported that heating PDO caused generation of PDO monomer as the polymer unzipped [27]. The extensiveness of the research, literature, and requirements related to quantification, control, identification, and causes of residual contaminants were seen as evidence to the significance of effects caused by residual levels of an impurity. The existence of residual monomers in polymers synthesized by ring opening polymerization is more likely due to polymerization-de-polymerization equilibrium [2]. Sufficient justification for the quantification of residual monomer in PDO is apparent prior to regulation, but the *FDA Guidance Document for Testing Biodegradable Polymer Implant Devices* specifically recommends that:

> "The composition and material structure of the product to be implanted should be characterized quantitatively. These analyses may include the following: low molecular weight components (separate

> components which have and have not chemically reacted with the polymer, e.g., contaminants, curing agents, crosslinking agents, dyes, monomer/dimer content, plasticizers, residual solvents)…" [31].

The guidance document goes on to advise that the volatile contaminants should be quantified using a gas chromatography (GC), or equivalent, method. The monomer and solvent components of medical devices are generally volatile and are also recommended by ASTM International material standards to be quantified by gas chromatography (GC) [32-35]. Polylactide and polyglycolide materials are specifically regulated to less than 2% residual monomer content (RMC). There is not currently a specific ASTM International standard for high PDO materials, nor is there a specific GC method for determination of PDO monomer.

Separation Science / Chromatography

Miller noted, “In chromatography, one phase is held immobile or stationary and the other mobile phase is passed over it [36].” Chromatography is one of the most common methodologies discussed in separation science. Liquid chromatography (LC) includes the most simplified versions such as gravity column separations that employ manual collections of each component rather than automated detection, as well as high performance liquid chromatography (HPLC). HPLC dominates the discussions in separation science as it is not limited to volatile analytes and is commonly used throughout academia and industry for isolation, purification and quantification of a wide array of compounds and molecules. An additional justification for the scientific focus on LC is that the concepts directly apply to GC by considering a gas mobile phase a very low density liquid that exhibits excellent diffusion characteristics.

A common descriptor for separation science to quantify the efficiency of a stationary phase is theoretical plates, a measure of chromatographic separation ability. The plate nomenclature is in reference to distillation columns that take samples or flow of distillate by the insertion of literal plates within the distilled vapor column. The location of the plate in the distillation column controls the vapor concentration that is collected from the distillation process. An increase in the number of plates utilized increases the amount of unique separations made available. The increasing amount of plates is limited by the height of the distillation column and the height between each plate. This concept was adopted to refer to the number of peaks that could be individually resolved by a chromatographic methodology. The number of theoretical plates is an inverse of the plate

height based on the length allowed for the separation to occur. It is this historical origin that defines why isocratic and isothermal separations must be used when determining theoretical plate heights and numbers [36, 37].

Theoretical plate numbers and heights are important to separation science. They allow quantification of not only the column parameters, but the entire instrument's ability to separate and thus the required information for comparing pieces of separation equipment as well as the historical status of the equipment to its current status. With all aspects of the instrument unchanged, the theoretical plate values can be attributed to the column's efficiency and be used to determine the suitability of a replacement column and when a column is due for replacement. Without this quantification the method development and maintenance processes could not definitively determine when and what changes are needed. In an effort to isolate the potential areas for changes in hardware and methodology, chromatographers have attempted to relate plate height to each component mathematically to potentially aid in predictive method development and optimization. The most noteworthy and successful chromatographer in this respect was J.J. Van Deemter. The below equation summarizes the 1956 Van Deemter theory that described the empirical observations of mobile and stationary phase effects on chromatography.

$$H = A + \frac{B}{v} + C$$

The height of each plate, H, was equal to the sum of three components. As H was minimized, more plates could provide higher efficiency separation. The first component, A, related to the phenomenon of eddy type diffusion of molecules in the mobile phase axially that allowed for interaction with the stationary phase. The second component, B,

was the longitudinal diffusion of molecules in the mobile phase that resulted in peak broadening divided by the linear velocity, ν, of the mobile phase. The linear velocity and the volumetric flow rate of the mobile phase are interrelated by the cross-sectional area of the mobile phase in the column. This area is much easier to determine for open tube type columns versus packed type columns. The third component, C, was the mass transfer component that relates to the diffusion, absorption, and/or absorption of molecules into and out of the stationary phase. The mass transfer component has a direct relationship with the speed of the molecules through the chromatographic bed, and thus increases with increased linear velocity. For GC with helium or hydrogen mobile phases and an open tube column with a relatively thin stationary phase, H decreases with increased flow rate until it virtually levels because diffusion and transfer are very fast for gases. Many GC methods are seemingly effective but report much higher flow rates than are necessary for separation [36].

Peak or band broadening describes the spreading of the analyte in time or space as it was transferred to the column. The analyte forms a band as the injection passes through a needle and forms a row of drops. This row of drops forms a volume of vapor that begins and ends at different points in the chromatographic flow. A signal relating the process of the vaporized material then starts with some actual peak width, or breadth. An infinitely small delta function is often used to theoretically describe the peak width at the time of injection despite the inherent inaccuracy. As the material interacts with the stationary phase, a Gaussian shape is formed. The use of a delta function simplifies the mathematics required to predict and represent this phenomenon. The broadening of peaks

is of great interest to chromatographers. Peak width and broadening affect the resolution of peaks when the width of each peak adds additional separation requirements to avoid overlapping peaks. Resolution of two peaks is determined by the distance, or time, between two peak apexes divided by the average of the two peak widths. A resolution value of two is the generally acceptable, minimum resolution of two chromatographic peaks.[36] Resolution is required for reliable and accurate chromatography. Significant resolution of two peaks can be confirmed visually when inspecting a chromatogram. A visual separation of two Gaussian shaped peaks with a flat baseline observed between the peaks yields resolution values much greater than two. A normal distribution of the analyte is indicated by a symmetrical, Gaussian shaped distribution. This peak shape is considered a necessity for the accuracy of the methodology. It indicates that sufficient adsorptions onto the stationary phase have occurred [36]. Column bleed describes the material observed by the detector that originated as stationary phase material. For GC this detector response forms the baseline and its associated noise to which the analyte signal is compared. The maximum amount of analyte that could be analyzed without inducing peak asymmetry was referred to as the column capacity by Miller in the text titled *Chromatography: Concepts and Contrasts* [36].

Generalities of Gas Chromatography (GC)

Gas chromatography (GC) is at a minimum defined by a gas mobile phase, an injector, a column that contains a solid or liquid stationary phase, and a detector. The injector is used to transport a sample to the column from the sampling apparatus. A general schematic of a GC and headspace (HS) autosampler with the primary components labelled is below in Figure 5.

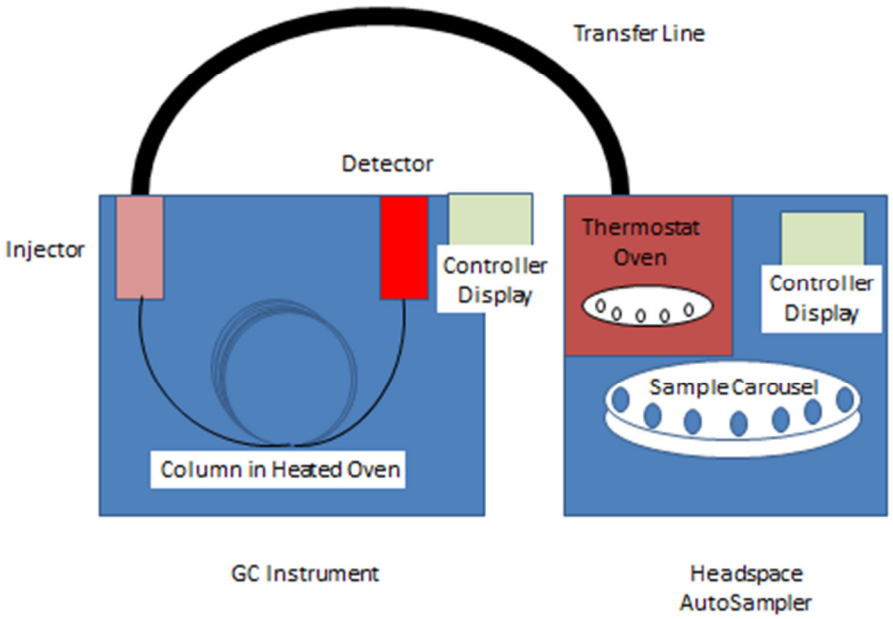


Figure 5: Annotated Schematic of General Gas Chromatography Instrument with Headspace Autosampler

The mobile phase flows into the injector where it typically mixes with the injected material and first interacts with the vaporized sample. The mobile phase in relation to GC is the inert gas that carries the volatile components of the injection across the stationary phase in the column. Aside from ancillary instruments that can be added before the

injector to help prepare a sample for injection, the injector is seemingly the simplest and least modular aspect of the GC instrument. In its most basic form, a GC injector is a heater with access for an injection and the entry point of the column. A schematic of a basic injector is below in Figure 6.

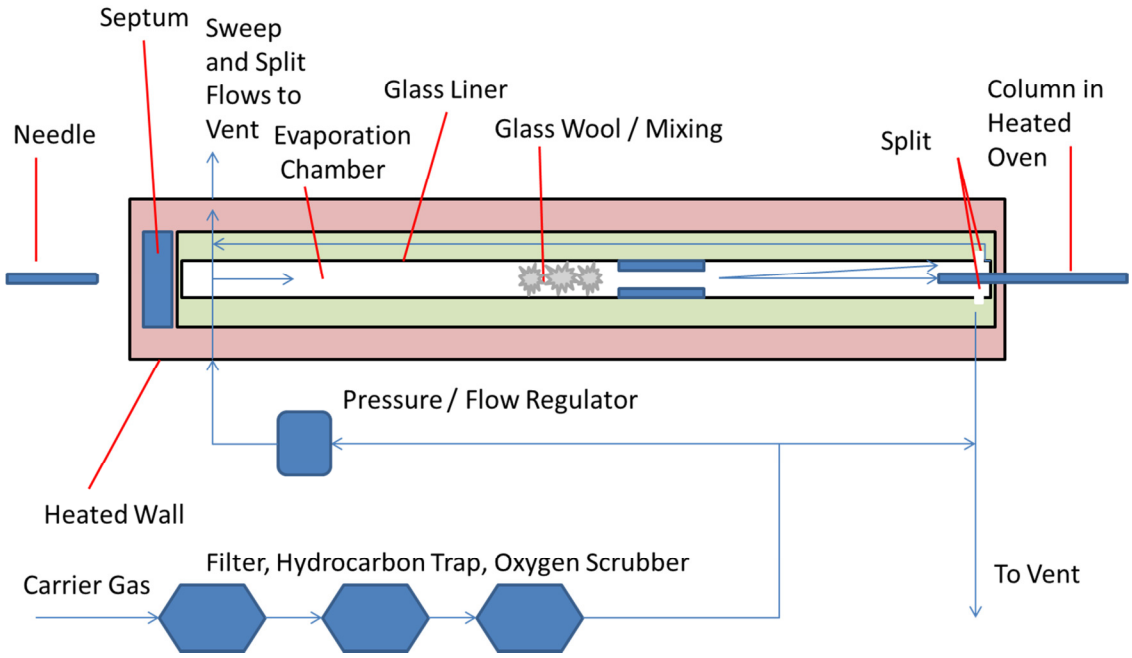


Figure 6: Annotated Schematic of a Split/Splitless Injector

The injector typically contains a deactivated glass liner with a volume available for the sample to expand. The length of the injector is fixed, so the inner diameter of the liner is the critical aspect that dictates the allowable volume of the vaporized sample [36]. The extent of expansion due to a phase change in the injector is otherwise determined by the injector temperature, the pressure as regulated by mobile phase flow rate, and the thermal properties of the sample. These conditions within the injector are of particular importance in GC.

The modern GC column is comprised of inert, fused silica. When capillary columns were made available, packed GC columns were soon found to be less common. Capillary, open tube columns are tubes comprised of silica with the inner surface coated with the polymer matrix that can provide separations with lower temperatures and pressure drops due to the use of less stationary phase. The fused silica capillary's inner surface is uniformly coated with the desired stationary phase and then the two are crosslinked together to provide maximum stability [36]. The thickness and polarity of the stationary phase applied (column selection) depends on the analytes planned for analysis. More polar stationary phases are more selective for polar compounds as the compounds are retained longer, and thicker coatings allow for larger samples of more volatile samples by increasing the column's capacity [38]. The guard column, or pre-column, is an uncoated and deactivated fused silica capillary to protect the column from deterioration and contamination that is commonly due to nonvolatile material within liquid solution injections such as polymer [36]. The stationary phase is within the column, and the column is housed within the GC oven. The oven controlled temperature of the phases controls the separation and is observed by the detector.

The chromatographic detector is used to identify and quantify each component in the volatile portion of the sample as it exits the column. The stationary phase and column ensure the components reach the detector separately, but there are many detectors available for the many different GC techniques. The most common detectors are thermal conductivity (TCD) and flame ionization (FID) because both were historically proven as universal and sensitive. TCD was more universal and non-destructive while FID

combusts the material as it passes through. The destructive combustion provides a very large linear range for nearly any organic material. Non-organics such as carbon dioxide and water cannot be detected by FID, but water often interferes with quantification and resolution as peak tails. Avoidance of this and any other distortion to analyte peaks is appreciated when possible [36]. While the actual ionization efficiency in the FID is technically low, the sensitivity is one of the highest available for GC. See Figure 7 below for a general schematic of an FID. The FID flame is lit by an external igniter and is kept burning by a continuous flow of hydrogen and air. Both gases must be filtered to ensure the highest purity.

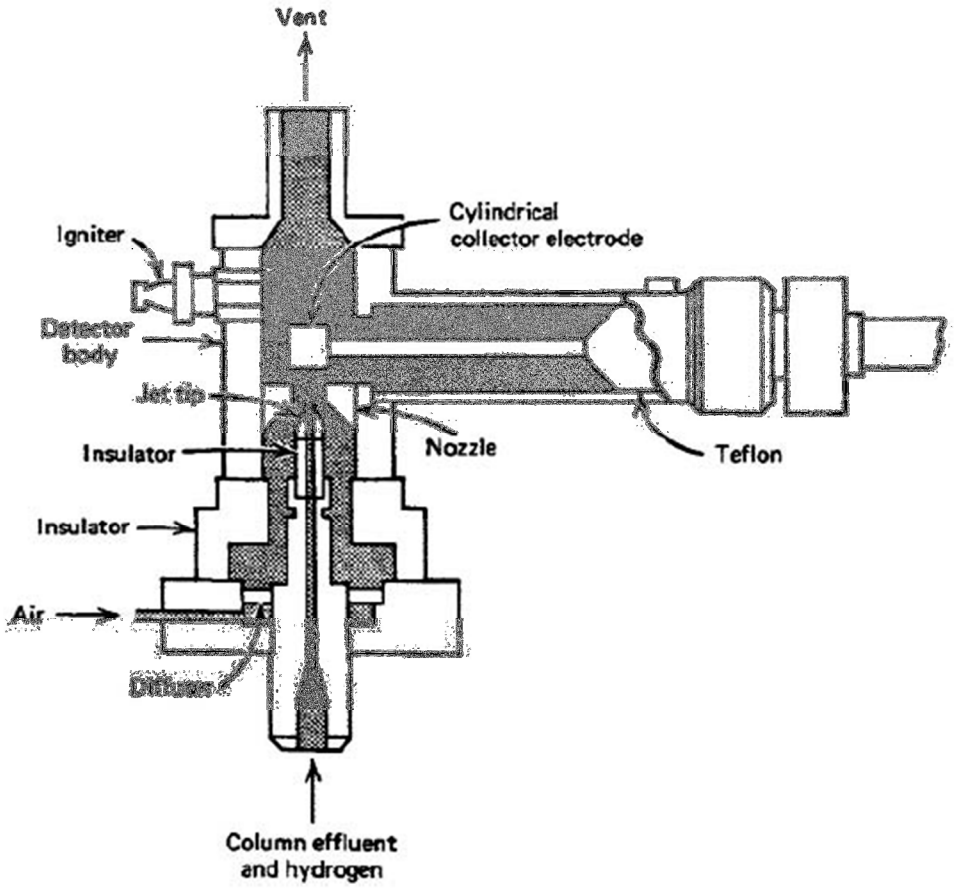


Figure 7: Schematic of FID (courtesy of Perkin Elmer) [36].

The TCD does not require additional gases but is subjected to selectivity based on the mobile phase. The analyte must have a different thermal conductivity than the carrier gas to be detected. The peak areas yielded by TCD are also flow dependent because TCD is a concentration detector versus the mass flow detection of FID. A small leak in a system with a TCD allows air to corrode and destroy the detection filaments [36]. An electron capture detector (ECD) was invented by Lovelock in 1961 as a selective option to specifically detect materials that capture electrons such as halogens, nitrogen containing compounds and unsaturated aromatics. This is especially useful for analysis of pesticides and other harmful chemicals of environmental importance. The ECD requires an ion source to ionize the mobile phase and has a smaller linear range when compared to the previously mentioned popular detectors [36].

The injector however has non-obvious and sometimes counterintuitive adjustments that greatly affect the success or failure of a testing method. Gas phased fluids are inherently very affected by changes in temperature, pressure and / or volume. Special care must be given to the selection of mobile phase flow rate, injection volume, injector temperature, and injector liner to account for this property of gasses. Often the sample is diluted in a solvent to facilitate the injection. The flow rate can be further adjusted when using split/splitless injection by applying a split flow. The split allows for a specified volume, or ratio of the flow rate, to pass out the split line at the base of the vaporizing chamber and bypass the column [38]. The use of a split is especially useful when smaller injections are required, but not otherwise feasible. By splitting the injection, less material enters the column than was injected. This is useful when a smaller sample is desired, but the injection is already only a fraction of a microliter. Another effect of the split includes the concentration of less volatile species in the sample relative to species within the sample that vaporize more quickly at the injector temperature. The more volatile components expand faster and to a larger volume than the less volatile, lower concentration components and thus the analyte(s) of interest occupy a higher concentration of the flow onto the column than without the use of a split. This results in relatively more analyte per solvent going onto the column in the case of classical liquid injection where volatile solvents are used to dissolve relatively less volatile analytes. Split injections were recommended by Grob for concentrated solutions and headspace analysis [38]. In the injector, special liners are available that contain glass wool to trap

non-volatiles dissolved in the solvent portion of a sample and thus prevent contamination of the stationary phase. The use of split flows and glass wool packed liners allow the analysis of trace volatiles within polymers injected via a solution of the polymer. The nonvolatile polymer collects on the glass wool where the sample vaporizes and mixes. The solvent preferentially expands and leaves the injector through the split flow while the relatively less volatile monomer is concentrated in the portion of the vaporized sample that enters the column. Splitless injection is recommended for dilute samples and trace analysis due to improved sensitivity because twenty to fifty times more material is typically carried onto the column, but splitless injections requires a thermal gradient that begins after the injection and well below the elution temperature of the analytes to ensure resolution [36, 38]. A dilute sample in the context of splitless injection is not necessarily referring to the sample that was dissolved into the solution for analysis. The sample's contaminates can have varying volatilities and other chromatographic properties that dictate not using a split for reliable analysis. The ability of the split to change the ratio of a sample based on volatility and concentration dictates that less splitting is required when relative quantifications within the sample are intended.

The stationary phase, as well as vapor pressure differences, induces a separation of the sample into its components relative to the volume of the mobile phase. This relative difference is then indicated by the detector as the mobile phase carrying analytes reaches the detector. The use of oven a temperature below the boiling points of the solutes is recommended for all analysis to ensure opportunity for molecules to interact with the stationary phase in the column. Vapors existing below boiling points seem odd,

but just as moisture in the air exists as humidity despite being below the boiling temperature of water, solvent and monomer vapors are carried down the column. The analyte interaction with the column is akin to how humidity can adsorb to, or wet, a surface if the surface is relatively cool [36, 39, 40]. This concept of vapor below boiling temperatures, even at high pressures, is further evidence for the GC requirement of small dilute concentrations of analyte for reliable analysis. The analyte is essentially dissolved in the carrier gas, just as solid samples can be dissolved in liquid samples for liquid chromatography analysis. The relatively high diffusivity of vapor ensures sufficient sample interaction with the stationary phase despite the low mass per column length of the phase versus packed columns and liquid chromatography columns. The stability of the stationary phase is ensured by adhering to maximum temperature recommendations and adding a guard column between the main column and the injector.

The separation volume is represented as a time relative to the mobile phase flow rate and inner column dimensions [36, 38, 39]. The time required for the maximum concentration of an analyte presence within the mobile phase to reach the detector from the injector is referred to as the retention time, with time zero being the moment of injection and the specific retention factor for an analyte designated a lowercase “k”. The apex of the peak is used because it is relatively easy to identify and it is the truest representation of equilibrium on the column [36]. The times discussed in relation to GC are in fact chromatographic volumes converted to time relative to the cross-sectional area and rate of flow. There is a minimum retention time that relates to the column volume and mobile phase flow rate known as the holdup time, or void volume. The maximum

retention time depends on nearly all other aspects of the methodology. The optimum retention time(s) is defined as the minimum amount of time required to provide an efficient separation of the analyte(s) from the rest of the sample while still retaining sufficient confidence for the signal to noise ratio and peak(s) resolution from all other peaks.

The most common choice of carrier gas in the United States is helium, especially when flame ionization detectors (FID) are used. Hydrogen and nitrogen are other common gasses utilized for GC analysis. Hydrogen was reported as especially preferred mobile phase when electron capture detectors (ECD) are needed [36]. Hydrogen is an increasingly common carrier gas for use with FID. The number of required gasses is reduced, and the risk of contamination via the gasses is thus reduced. It is of note though that use of hydrogen as a carrier gas will significantly increase the rate of hydrogen use. This increases the risks associated with compressed hydrogen tanks and the connection processes. Connecting and disconnecting hydrogen tanks improperly can lead to fires and explosions. Hydrogen generators are often employed when hydrogen is utilized as a carrier gas to reduce the costs and risks associated with high pressure tanks. The generator removes the need for continuous tank exchange. The ideal carrier gas for a GC mobile phase was described as inert to not affect the analysis, as well as thermally diffusive enough to ensure that the oven temperature is quickly and evenly imparted to the sample components being carried. This typically removes argon from the discussion due to its thermal diffusivity being an order of magnitude lower than helium or hydrogen. Nitrogen has thermal properties similar to argon but is significantly the cheapest gas to

obtain in very high purity levels. While nitrogen is not ideal, the cost effectiveness does lead to its use as a GC mobile phase. The thermal properties of the mobile phase and oven temperature are critical to efficient separations.

Variations of Gas Chromatography Methodologies

According to Miller, “Sample introduction is most often accomplished with a microsyringe through a self-healing rubber septum [36].” Miller was referring to a classical injection technique. The liquid sample is flash vaporized by the heat of the injector and the mobile phase carries the vapor onto the column leaving non-volatiles behind in the deactivated glass liner of the injector. Gas chromatography analysis methods in literature vary as much as the analytes analyzed and quantified. Methods include headspace analysis and classical direct injection. Other variations, especially column technologies, are continuously developed to increase the productivity, accuracy, sensitivity, and versatility of GC analytical methods. Miller also described several variations of injection via a syringe and needle. The most general was known as classical vaporization injection (CVI). CVI was defined as injection into a permanently hot split/splitless injector. Cool needle injection was a variation of CVI that specifically referred to when a sample was drawn into an ambient temperature syringe, the needle was inserted into the hot injector, and the plunger was depressed without delay. Conversely, hot needle injection allowed a delay of three to five seconds between the insertion of the needle and the depression of the plunger. Direct injection described the non-splitting vaporization of an injection into a liner directly connected to the column entrance [36].

In the case of headspace analysis, the solvent for extraction of volatiles is the air in the vial diluting the vapors formed during the thermostat step. The headspace is defined as the volume above a sample, specifically in a sealed container such as a GC

headspace vial. By using an inert vial of fixed volume to contain a specified mass or volume of sample, the sample is heated to generate a vapor concentration of the volatiles from the sample in the headspace. The vaporized portion of the sample is then sampled from the headspace in the sample vial. The vapor sample travels to the GC injector and then the chromatography is completed as previously described. Straight tube liners can be utilized in the injector to minimize dead volume and opportunities for adhesion. The concentration present at equilibrium with the vial volume and thermostat temperature is understood to be relative to the concentration of all volatiles in the sample. This understanding is supported by Henry's Law discussed below. The sample is often a mixture of the analyte(s) being quantified in a matrix that dilutes the analytes and allows them to be sampled. GC analysis by headspace extraction is performed by inserting a needle into the vial to remove a volume of the headspace and then transferring this volume into a GC injector. This transfer is performed manually by syringe, but the use of an autosampler to pierce the vial, take the sample volume and transfer the volume to the GC injector greatly increases the repeatability and usefulness of headspace analysis.

Inappropriate application of headspace techniques can relate to the thermodynamics of the sample system or the hardware used for sampling and transfer of the sample to the GC. The matrix of the sample contains the analytes and is typically much less, or much more, volatile than the analytes to prevent interference with the chromatography separation. The sample is already in the vapor phase when it reaches the injector so excessive expansion is no longer of concern for the injector liner selection. Dead volumes and adhesions reduce repeatability, reduce accuracy, and cause analyte

distribution in the mobile phase to abnormally expand and deviate from Gaussian distribution. Unnecessary expansion and non-Gaussian peaks are indicative of poor chromatography that is less quantitative and less repeatable. Large concentrations of less volatile matrix material with respect to the analyte(s) elute before the analytes and often negatively affect the quantification of the analyte(s) without excessive resolution [36-39]. In some cases the matrix is a nonvolatile solid requiring analytes to slowly diffuse toward equilibrium with the headspace.

Multiple headspace extraction (MHE) takes advantage of Henry's Law that predicted the linear relationship of vapor concentration with the analyte in the sample when the analyte is at low concentrations in the sample matrix. Henry's Law is an adaptation of phenomenon observed for ideal mixtures as described by Dalton's Law and Raoult's Law but only applicable to a finite range of low concentrations. Further, Henry's Law only applies when the condensed phase and vapor phase are in equilibrium. The detection of vapor concentrations that can be related to condensed phase component ratios requires this equilibrium between the two phases to remain constant during the entirety of the observation and measurement. This is especially true when MHE is applied and multiple iterations of the equilibrium between the phases are required. Dalton's Law described the concentration of the analyte in the headspace as proportional to the partial pressure of the analyte. Raoult's Law described the vapor pressure of the analyte in the headspace as proportional to the mole fraction in the solution below. Henry's Law reduces to Raoult's Law in an ideal case where Henry's Constant is equal to a value of one [39, 41].

During MHE and after the sample volume is removed from the headspace of the vial, the vial is allowed to purge to remove the previously vaporized material. The thermostat and sampling process is then repeated to generate multiple injections for GC analysis. The volatile material is removed from the system, causing a lower concentration to be generated upon each repetition of the extraction process. Using the GC to quantify this reduction until no material can be further vaporized, allows a total amount of analyte to be quantified without recreating the sample matrix when preparing standards for calibration. MHE curves relate the peak areas observed, or the natural log of the peak areas observed, on the y- axis versus each extraction number on the x-axis. The follow equations are applied to the constants determined from the best fit regression curve generated by the observations.

$$A_i = A_0 \times e^{-q \times i}$$

and

$$\sum_{i=1}^{i \to \infty} A_i = \frac{A_1}{1 - e^{-q}}$$

When each subsequent extraction determines a peak area that fits an exponential decay, equilibrium between the headspace and condensed phase prior to each sampling of the headspace (HS) was established. This exponential decay forms a linear curve when the natural log of the area counts are plotted versus the extraction numbers. A representative MHE calculator is shown in Figure 8 from Perkin Elmer's Application Note on MHE to determine monomer content in a polymer [42].

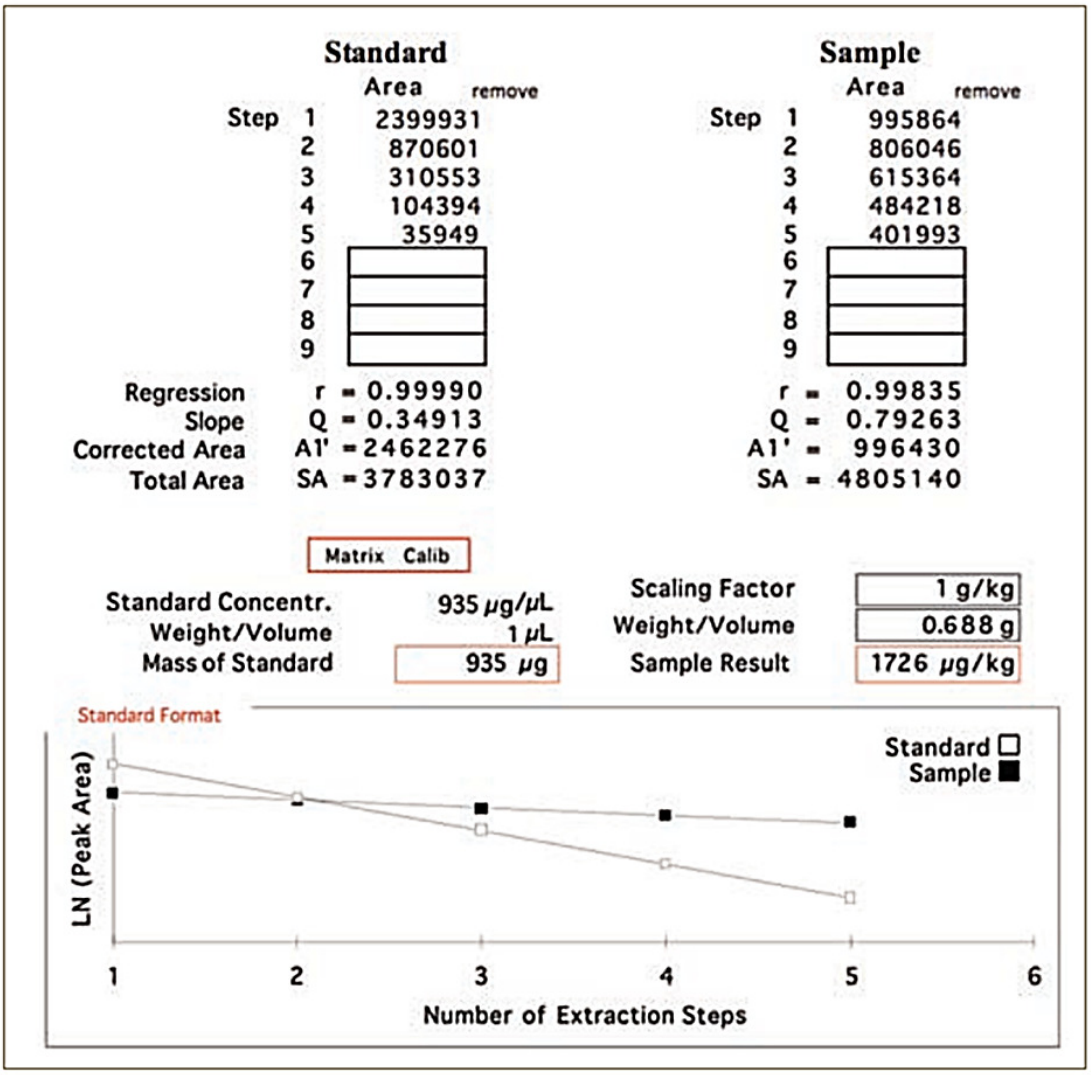


Figure 8: Excerpt of Excel Type Macro from Perkin Elmer's Application Note for MHE determination of Residual Monomer in a Polymer (MHE Calculator) [42].

The sample's MHE curve was less sloped due to slower extraction of monomer from the polymer than the total evaporation of the neat monomer. MHE determined there was 27% more monomer in the sample vial than in the standard vial based on the total area counts in the MHE calculator. The monomer standard was fully vaporized and removed from the HS vial (5^{th} extraction area count is 1.5% of first extraction, 0.95% of total area

calculated, sum of 5 extractions is 98.4% of total area calculated). The monomer in polymer response decreases slightly with each extraction (sum of 5 extractions is 68.75% of total area calculated. Single extractions would have generated a response factor of 2566.77 area counts / ug and determined the monomer in polymer concentration to be under reported as 563.93 ug/kg vs 1726 ug/kg. Reduced response per mass of monomer in HS vial is matrix effect (polymeric material is the matrix and must diffuse out and equilibrate material still in the polymer). Each GC run can be very brief with MHE, the extraction in HS vial separates monomer from most of the sample mass and limits the injection to just the volatiles. This pre-separation prior to injection reduces the rate of maintenance required by GC. There is no nonvolatile material to build up in the instrument.

Conversely to MHE, coordinating the matrix of standards with known amounts of analyte for use in calibration to a specific sample matrix is known as matrix matching to avoid inaccurate quantification related to matrix effects. Matrix effects describe the influence of the sample matrix, the non-analyte components, on the quantitation. For each matrix/analyte system exists an equilibrium described as the partition coefficient, designated a capital “K”, determined by the ratio of the concentration of the analyte in the matrix divided by the concentration in the headspace. This is important because Henry’s Law predicts the relationship between the vapor phase and the condensed phase to be specific to each condensed phase matrix and each volatile material it contains. Henry’s Law also only pertains to the linear range of the relationship that occurs at low concentrations. The maximum concentration for which Henry’s Law will apply was also

specific to the matrix and the volatile material it contained. The multiple headspace extraction method was especially useful for novel materials such as polymers that contain volatiles because the matrix was not only difficult but often impossible to replicate exactly. MHE methods are highly dependent on matrix and analyte stability so that multiple equilibrium states at elevated temperature can be achieved without heat induced generation of analyte from the sample matrix itself. The ability of the analyte to form a partition system via diffusion from the condensed phase is also a critical requirement of the methodology. Both requirements relate to the ability of the analyte to reach an equilibrium concentration between the headspace and the matrix phases. If the matrix generates the analyte through thermal degradation, or if the analyte tends to adsorb onto a surface in the vial, either the vial wall or the surface area of the matrix, equilibrium that would be predicted by Henry's Law cannot be obtained. Troubleshooting the development and implementation of MHE attempts to diagnose the reason for lack of equilibrium by inspection of the changing analyte peak areas over the course of at least three extractions. For example, if the peak areas related to the analyte(s) increased with each extraction, sufficient time for equilibrium was not allowed prior to the headspace sampling [39]. Hence, analysis of a material that is unstable in both the monomer and polymeric form, as in this case, is troublesome.

The equilibrium state of an unstable material could potentially not represent the sample intended for analysis. To test for equilibrium in a HS vial, a progressive experiment can be performed. This involved preparing multiple sealed vials of the same sample and sample size in each vial. Special care must be taken to ensure that the sample

in each vial was of similar mass and size. Each vial is then subjected to an increasing thermostat time at a set thermostat temperature before a HS volume is sampled. The results of a progressive experiment should show the analyte peak area increase to a constant peak area when plotted versus thermostat time. The thermostat time required to reach the constant peak area value is then the thermostat time required for equilibrium and thus repeatable and quantitative HS analysis.

The United States Pharmacopieal Convention (USP) standard testing method for residual solvents provides a detailed GC testing method and tables for solvent concentration regulations that lists Class I, II and III solvents [43]. Standard methods, such as *ASTM D4526 – 12 Standard Practice for Determination of Volatiles in Polymers by Static Headspace Gas Chromatography*, recommended using gas chromatography to determine trace quantities of low molecular weight components in polymers [44]. Comparing a headspace GC (HSGC) method versus a classical injection method, the ASTM D4526 standard specifically states that use of a HS method is intended to avoid the contamination of a GC column with dissolved polymer in a sample or to avoid the added complexity of a precipitation step in sample preparation. Headspace methods via GC are described extensively for identification and quantification of residual solvents in *USP <467> Residual Solvents* [43]. Measuring monomer content by headspace methodology is standardized via ASTM for some polymers such as poly(vinyl chloride) (PVC) in *ASTM D3749-13 Residual Vinyl Chloride Monomer in Poly(Vinyl Chloride) by Gas Chromatographic Headspace Technique*, but classical vaporization injection methods are still utilized in industry due to the relatively simple nature of the approach

and the lack of additional instrumentation or equipment required [18, 45]. ASTM D3749 recommends the use of multiple headspace extraction (MHE) when prepared monomeric standards are not available and replaced the solution injection standard method *ASTM D3749-13 Residual Vinyl Chloride Monomer in Poly(Vinyl Chloride) by Gas Chromatographic Headspace Technique* [46]. MHE determination of monomers in polymers is also recommended by the GC equipment manufacturer, Perkin Elmer, in the form of an application note that specifically describes the procedure and associated calculations,

> "Multiple headspace extraction is used because it is a technique to quantify samples in a solid or difficult matrix without matrix matching the calibration standards. The standard is analyzed, without matrix, in a total evaporation headspace with MHE, determining a response factor…" [42].

The utilization of an MHE-GC method versus a CVI method has the benefits of aligning with currently suggested standard methodology for other non-bioresorbable polymeric materials and mitigating:

- the need for frequent maintenance of the GC instrument,
- the need for additional sample preparation steps such as solvent dissolution of the polymer, and
- the need for commercially unavailable calibration standards that account for matrix effects related to the extraction and vaporization of the analyte(s).

With any GC method utilized, one of the key steps in quantifying an analyte is the development of a standard curve and the corresponding response factor for the analyte. By injecting known amounts of analyte across a desired range, an external standard curve is generated. The response factor for an analyte is the number of area counts generated by the detector for a given mass of analyte that is detected. A useful and relative version of the response factor value is determined by external calibration for each analyte to be quantified. Use of this calibration curve is referred to as the external standard method. In contrast, the internal standard method is the determination of analyte concentration by comparison of the analyte peak with another peak within the chromatogram that relates to a known amount of another substance added to the sample. A combination of the two standard methods yields the standard addition method. Standard addition describes the adding of a known amount of analyte to a sample previously containing an unknown amount of analyte. By comparison of the sample with and without the addition, quantitation is permitted [36-39].

Related to analysis of polymers, the monomers and solvents used to make and process the polymers must be analyzed for purity as well. The impurities in the raw materials are transferred to the product material as residual contaminates and contribute to the negative effects previously mentioned. Additional detection techniques used in conjunction with GC such as mass spectrometry are also used to aid in identification of the contaminants found via GC analysis. Standard methods such as *ASTM D3465 – 00 (2007) Standard Test Method for Purity of Monomeric Plasticizers by Gas Chromatography* provide clear directions for performing this type of analysis accurately

and repeatably via solution injection GC techniques. The described method is a very general CVI method applicable to nearly any volatile organic compound of 99% or greater purity with additional instructions for analysis of materials of lower purities. Volatile materials are defined by this standard as having definitive boiling points and molecular weights less than 1000 Daltons [47].

Gage R&R Statistics

Statistical validation of a testing method is necessary because it is unknown if and to what extent the operator could affect the results of the analytical method [48]. Repeatability and reproducibility are quantified by performing Gage R&R statistical analysis on a range of data generated by multiple operators analyzing the same set of samples. Each operator also performs replicate analysis of each sample. This data allows statistical examination of the method's, the operators' and the instrument's ability to perform the analysis. The purpose of this type of study is to understand the sources of variation that could affect the results produced by the whole of the methodology [48]. For a given level of confidence, the Gage R&R study determines a percentage value, the percent study variation (%SV), to quantify the repeatability and reproducibility of the testing method. The level of confidence increases as the sample size increases up to a sample size of approximately twenty. Above twenty samples the slope of the increase in confidence with increased samples reduces significantly. Thus, an ideal Gage R&R study would include six operators and analysis of at least twenty parts [49, 50]. The typical study design includes three operators and ten parts due to non-ideal constraints present in the industrial setting. Further decreasing the number of operators is allowed in special circumstances where only two operators are to be trained to a testing method. Burdick noted the "quality of a measurement system can be defined by how well it discriminates between good and bad parts [49]." The better the repeatability and reproducibility of the testing method, the lower the percentage value determined by the study, and the less likely the test could have called a bad part good or a good part bad.

The repeatability and reproducibility are subdivisions of the precision of the measurements that respectively referred to analysis of the same part with the same operator and instrument and the analysis with a different operator [50]. A value for number of distinct categories (NDC) is also determined by the Gage R&R study. This value corresponds to the range of samples input into the study and the number of distinct samples that could be discerned within the range. Specifically the number of distinct categories is defined as the "number of non-overlapping 97% confidence intervals for the true value of the measured characteristic that will span the expected product variation [51]." Based on this definition, the range input into the study should have reflected the expected range of the product being tested. A large range combined with a very repeatable and reproducible test returns a higher value for distinct categories. The automotive industry developed the Gage R&R testing method to determine if multiple gauges could return the same measurement when the same parts were measured with each gauge [52]. The total Gage R&R value combines the repeatability and reproducibility percentages. The determined percentages are ratios of the standard deviations, not the variances, of the repetitions [51]. A %SV of 10% to 30% was related to the noise level in the measurement respectively accounting for 1% to 9% of the total variation [53]. The %SV and NCD are directly related [54]. It is important to note that the reported NDC value is a truncated value because a fraction of a category is not non-overlapping.

$$NDC = \frac{\sqrt{2}}{\%SV}\sqrt{10000 - (\%SV)^2}$$

Acceptable total Gage R&R study values are separated into three potentially acceptable categories. Less than 30%, or greater than 4.497 NDC, may be acceptable depending on the time and cost requirements of the analysis. Less than 20% but greater than 10%, six to thirteen NDC, is considered acceptable and confirms confidence in the use of the testing method. A value less than 10%, fourteen or greater NDC, was returned for the most acceptable and confident testing methods [48, 51-54].

CHAPTER TWO

OBJECTIVES

The literature revealed that gas chromatography (GC) is the preferred instrumentation for the quantification of volatile residuals, such as unreacted monomer, in polymeric materials. The application of GC methods specifically engineered for the quantification of residual monomer, and other volatile residuals, is not defined for the polyester family of bioresorbable polymers and copolymers, especially poly(1,4-dioxane-2-one) (PDO). The prevalence and reported benefits of multiple headspace (MHE) analysis for quantification of residual monomer in other polymer systems suggests that successful application of MHE with a headspace (HS) autosampler would provide the most reliable and efficient use of the GC instrument for frequent implementation of the test method. Moreover, the literature revealed that unsuccessful application of the MHE technique would indicate that a classical vaporization injection of a polymer solution directly into the GC injector could provide an alternate testing method to investigate. A review of the Gage R&R techniques revealed that a properly designed study could determine the level of variation when performing the test method within a relevant range of samples intended for analysis. Therefore the objectives of this study were to:

1. Develop an analytical GC method to repeatably and reproducibly quantify residual monomer in polymeric materials;
2. Apply the GC method to PDO polymeric materials to quantify PDO monomer for optimization of polymer processing and industrial quality control;

3. Evaluate MHE-GC and its limitations as applied to the quantification of volatile residuals in bioresorbable polyester materials;
4. Investigate the development of a classical vaporization injection (CVI) GC method for quantification of residual monomer in bioresorbable polyester materials;
5. Compare the benefits and limitations of each method developed; and
6. Utilize Gage R&R to determine the precision of the testing method within the intended range of analysis.

CHAPTER THREE

MATERIALS EXPERIMENTAL AND METHODS

Materials

Materials sourced to develop the GC testing methods were provided by Poly-Med, Inc. including those purchased from outside sources. Table 3 lists solvents, monomers, polymers and analytical gases that were used in this work. Table 4 lists the analytical equipment utilized to develop the testing methods in addition to the consumables required for GC analysis.

Two distinct PDO polymers were utilized to develop the GC methodologies. Both were received in granular form. The two materials were predominately differentiated by the fact that one was representative of PDO polymer that had been successfully processed to a relatively low PDO monomer content (low monomer PDO polymer) while the other PDO polymer was representative of PDO polymer that was not processed to minimize monomer content and was thus considered to be a relatively high PDO monomer content material (high monomer PDO polymer). A polycaprolactone (PCL) polymer was analyzed via the same general GC methodologies developed for the PDO polymer.

Table 3: Materials and Respective Suppliers

Material	Supplier
Monomers	
ε-Caprolactone, 99% (Caprolactone)	Acros Organics
Supomer-PDO®: para-dioxan-2-one, 99.99% (PDO)	MediChem
Solvents	
Dioxane	Sigma-Aldrich®
Hexafluoroisopropanol (HFIP)	DuPont
Polymers	
Dioxaprene™: Polydioxanone	Poly-Med, Inc.
Capa™ 6500 (PCL)	Perstorp
Resomer® X 206 S: Polydioxanone	Evonik Industries
Gases	
Helium, UHP grade	AirGas®
Air, Ultra grade	AirGas®
Hydrogen, UHP grade	AirGas®
Nitrogen, Flashed from High Pressure Reservior of Liquid Nitrogen	AirGas®

Table 4: Equipment and Respective Suppliers

Equipment	Supplier
Gas Chromatograph (GC)	
Model: Clarus® 580	PerkinElmer, Inc.
TotalChrom™ Software	PerkinElmer, Inc.
Columns	
Guard Column (Polyimide Uncoated Fused Silica Capillary Tubing) 5 meter, 0.25 mmID	PerkinElmer, Inc.
Elite – 624 (6% cyanopropyl phenyl – 94% dimethyl polysiloxane) 30 meter, 0.25 mm ID, 1.4 um df CAT# N9316201, Max temp 240°C	PerkinElmer, Inc.
GC Syringes	
1 - 10 µL ±2%	PerkinElmer, Inc.
0.1 - 0.5 µL ±2%	PerkinElmer, Inc.
Volumetric Flasks	
10 ±0.02 mL	VWR
25 ±0.05 mL	VWR
Autopipettes	
500 - 5000 ±1.0% µL	VWR
101 - 1000 ±1.0% µL	VWR
10 - 100 ±1.0% µL	VWR
Headspace Autosampler (HS)	
TurboMatirx™ 40	PerkinElmer, Inc.
22 mL Sample Vials	
Teflon-lined Silicone Crimp Top	PerkinElmer, Inc.
Headspace Vial Crimper	PerkinElmer, Inc.
Sample Vials, 20mL, Teflon lined screw top	
Amber	VWR
Differential Scanning Calorimeter (DSC)	
Model: DSC6	PerkinElmer, Inc.
Balance (0.00001 - 220 ±0.00005 grams)	Sartorious®

Experimental Development: General

Review of the literature related to 1,4-dioxane-2-one (PDO) and gas chromatography (GC) revealed the need for a quantification method for residual PDO monomer, the prevalence of GC methodologies for similar work, and the benefits of multiple headspace extraction (MHE) for analysis of residual volatiles in polymers. Classical Vaporization Injection (CVI) techniques related to GC analysis of polymers were also noted. A GC method is defined as an injection technique and all temperature, flow, pressure, etc. settings required to perform an analytical run. A GC run is defined as the process of data collection that begins when the sample enters the GC injector and concludes when the temperature profile of the GC oven is completed.

The study was designed to initially evaluate the application of multiple headspace extraction (MHE) GC to quantify residual PDO monomer in PDO polymer. A second method was also developed for quantification of residual PDO monomer in PDO polymer using classical vaporization injection GC technique. The design of the study was such that the two methods could be compared for benefits and limitations specific to bioresorbable polyester materials. Additionally the study was designed to determine the Gage R&R statistical level of precision for at least one of the methods that would relate to the analysis of PDO monomer in PDO polymeric material ranging from approximately 0.1% to 5% by weight monomer.

The Clarus®580 gas chromatograph (GC) and TurboMatrix™ 40 headspace (HS) autosampler instruments in Figure 9 were utilized for all GC analysis. Use of the mid-

polarity Elite-624 column chemistry was based on the column recommendation made in the USP standard method for analysis of residual solvents [43].

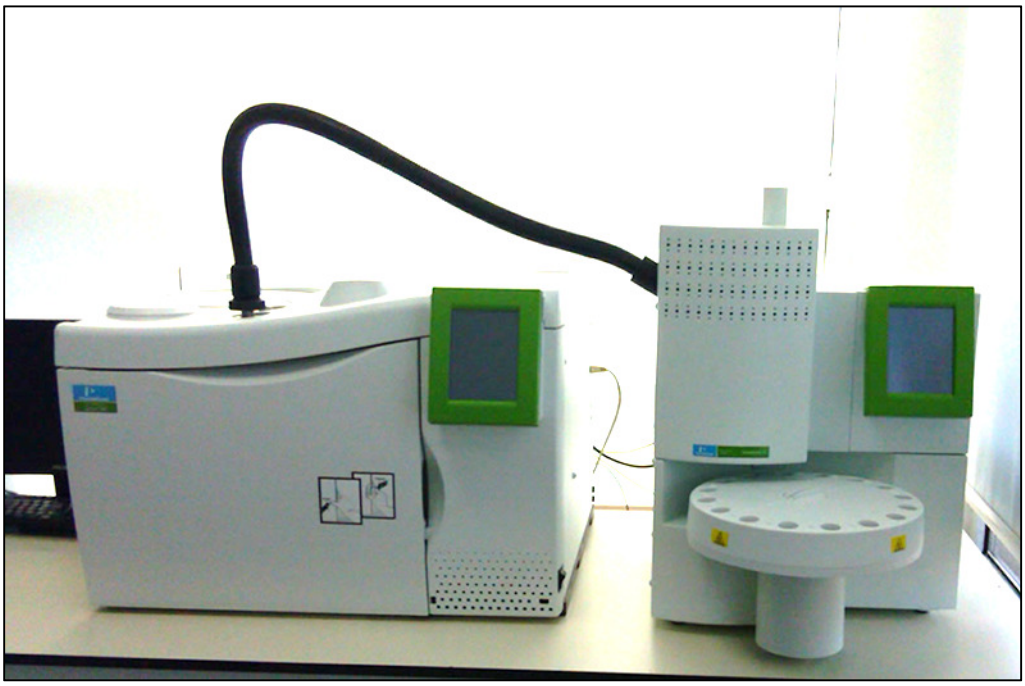


Figure 9: Clarus® 580 GC with Attached TurboMatrix™ 40 HS Autosampler (Image Courtesy of Perkin Elmer)

The GC is equipped with dual capillary column injectors and dual flame ionization detectors (FID). Both of the injectors and detectors were fitted with programmable pneumatic control (PPC) modules to regulate and monitor the flow rates / pressures of the three gases utilized for operation of the instrument. The pressures, flow rates, and temperatures for the GC method are controlled by the TotalChrom™ software on a separate personal computer. Within this software the GC carrier gas flow rates could be varied from 0.5 to 4 mL/minute; split ratio settings for the injector could be varied from 0 to 100; injector temperatures could be varied from room temperature to 350°C; initial oven temperatures could be varied from room temperature to the maximum column

temperature; gradient temperature profiles could be varied from 2 to 40 C°/minute; up to four ramp rates per method could be utilized; hold times at each temperature could be programed for up to 999 minutes; detector sensitivity could be varied via attenuation from 0 (least sensitive) to -6 (most sensitive). Timed events within the method could also change any of the method parameters during the data collection. Between each run the GC was programed to return to the start conditions and equilibrate for two minutes prior to indicating readiness for the next injection / run.

The general GC method utilized a helium carrier gas flow rate of 2 mL / minute, an injector temperature of 200°C, an injector split ratio of 15:1, an initial oven temperature of 40°C, a detector temperature of 240°C and a detector attenuation of -6. Detector gases were programed based on manufacturer recommendations of 45 mL/minute hydrogen and 450 mL/minute air. The detector flame was lit prior to the first run after the detector was above 100°C via the touch screen control “ignite” button for each detector. An initial one minute hold at the starting temperature of 40°C was followed by a 20°C / minute ramp of the oven temperature to 220°C and a final ten minute hold. Cool down time between runs was dependent on lab conditions, but generally required seven minutes prior to the two minute equilibration. Total run time including cool down and equilibration was approximately 30 to 40 minutes. Gases utilized included UHP grade hydrogen, UHP grade helium and Ultra grade Air (20% - 22% oxygen, per supplier specification). The grades of gas specify ≥99.999% purity. The three tanks of gas were secured to the wall next to the GC by strap and chain to ensure a safe operating environment. High pressure regulators were used to maintain all three feed

pressures to 70 psi. Additional precautions in the lab were also made related to the presence of hydrogen gas ("No Smoking" signs in clear view) and the GC system was installed just below a nuisance vapor hood to remove any undesirable fumes vented during analysis.

The general CVI injection was performed using a Perkin Elmer 0.5 µL syringe. The sample solution was used to fill and empty the syringe ten times. The syringe was filled to 0.5 µL, emptied to the 0.3 µL mark, and the needle was wiped to prepare the injection volume. After injection the syringe was cleaned by flushing with solvent a minimum of ten times.

Injections were made within ten seconds of filling the syringe to minimize evaporation of the solvent. The time allowed for the actual injection was between one and two seconds from the initial puncture of the injector septum to when the needle is removed from the injector. This timing is coordinated with the start of the run by a five second countdown started by a button on the GC touchscreen display. Following the injection the needle was cleaned by performing a minimum of ten flushes with clean HFIP solvent. CVI analysis was performed using the Perkin Elmer Elite-624 column with the Perkin Elmer Guard Column installed between the column and the injector.

The HS autosampler is equipped with both an external sample vial carousel and a thermostat oven internal sample vial carousel. It is controlled by the TurboMatrix™ software on the separate personal computer that communicates with TotalChrom™ and the GC for updates related to readiness for injection. Within this software the autosampler can be used to perform Constant (single injection), MHE, and Progressive analyses. The

software controlled thermostat time could vary from at least 5 to 99 minutes; thermostat temperature could be varied from 30°C to 200°C; carrier gas pressure could vary from 30 to 60 psi; vent times could be varied from 0 to 1.0 minute; MHE number of extractions / injections could be varied up to nine; progressive thermostat time could be varied from at least one to ten minutes; pressurization time prior to expansion to the GC injector could be varied from 0.25 to 2 minutes; and sampling time allowed for the expansion could be varied from 0.02 to 0.20 minutes.

The general HS method was MHE with four automated extractions and thus four runs per sample. The needle remained in the vial after the first injection to ensure material was not lost through the septum hole created by the needle. The septum itself minimizes these loses, but by not removing the needle, the loss of material due to leaking is kept to an absolute minimum. The GC run time setting and MHE thermostat times were 45 minutes to allow the general GC method sufficient time to prepare for each run. The thermostat oven temperature was 100°C. Pressurization was performed for 0.5 minutes after the autosampler needle punctured the sample vial septum. Injections were 0.12 minutes and venting was one minute. Following the final run of MHE analysis, the four peak areas were input into the excel macro developed for applying the MHE extrapolation equations from the literature (MHE calculator). The Perkin Elmer Elite-624 column was used for all HS analyses.

A progressive analysis experiment is used to determine the minimum required time at temperature for equilibration of a volatile component between the matrix and the headspace. In this experiment a series of same mass samples are prepared into individual

HS vials. The vials are heated to a specific temperature and held for a specific time before the single HS injection. For each experiment the sample size was not allowed to vary more than ±2% of the nominal weight reported. Thermostat temperatures of 60°C, 80°C or 100°C were used with progressive time increments of 5 or 10 minutes. The general progressive experiment used a thermostat time of 100°C, and the first sample was heated for 10 minutes, the second for 20 minutes, the third for 30 minutes, etc. until no further HS vials were available or the experiment was manually halted.

Sample Preparation: Multiple Headspace Extraction (MHE)

MHE analysis by gas chromatography (GC) entailed sample preparation of monomer or polymer in a headspace (HS) vial followed by the general HS method. Sample mass measurements needed to be both precise and accurate to ensure the GC analysis could also be precise and accurate.

MHE monomer standards were created by addition of liquid monomer to a tared volumetric flask on the balance, and the mass of monomer was recorded. The liquid monomer was transferred dropwise via glass pipette and rubber bulb. The volumetric flask was then filled to the line with hexafluoroisopropanol (HFIP) to create a known weight / volume solution. Autopipettes were then used to transfer known volumes, and thus known masses of monomer to 22 mL glass HS vials. New glass HS vials were used to prevent sample contamination. The HFIP was then removed from the vial during a nitrogen purge step where pressurized gas was allowed to flow into the vial until the solvent was no visible and the precipitated monomer was observed. This technique was utilized to ensure accurate weights of less than 1 mg could be obtained in the vial. The vial was then sealed by crimping the aluminum top onto the vial. The top contained the Teflon -lined silicone septum. Figure 10 below is a schematic of the GCHS vial crimping process performed.

Polymer samples for HS analysis were created by adding polymer to a tared HS vial on a balance. The mass of polymer was recorded, the vial was purged with nitrogen gas and the vial was sealed with a crimp top as shown in Figure 10 below.

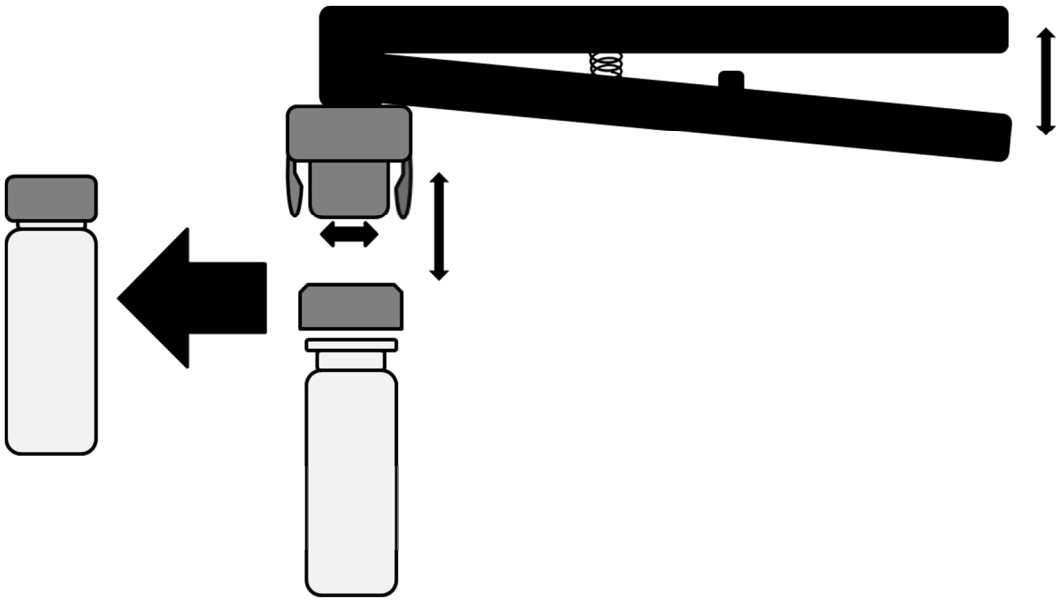


Figure 10: Schematic of HS Vial Crimping

HS vial crimping must be performed very carefully to ensure the septum creates a seal over the top of the vial and the aluminum cap full crimps around the top of the vial. The two handles of the crimper are squeezed together to activate the crimping clasps that reach around the cap and compress the bottom of the cap walls under the lip of the vial top from all sides at once. There is a set screw midway down the handle of the crimper to aid in a repeatable crimping technique.

Sample Preparation: Classical Vaporization Injection (CVI)

The CVI technique uses a small volume needle and syringe to achieve nearly instantaneous vaporization. Total vaporization of low boiling solvent results in a very large volume change which dictates that a very small injection is performed. The very small volume of sample solution, 0.3 µL injected, per run when performing the general CVI method required very specific knowledge of precise and accurate weight / volume solutions of monomer in solvent and polymer in solvent. Accuracy of monomer and polymer masses was initially ensured by starting with greater than 25 mg of monomer or polymer, and greater than 100 mg when feasible. A large mass of monomer required dilution of the solution to generate the desired concentration for use as monomer standards. Larger volumes of solvent for the solution were not feasible due to the cost and availability of the hexafluoroisopropanol (HFIP) solvent. Accurate dilution of monomer solutions dictated that weight to weight concentrations of initial solutions were also needed due to the compounding error associated with continued use of autopipette volumes when transferring volatile organic solvent and solutions. Autopipettes are calibrated to better than ±1% with water. Significant deviation from the density or volatility of water could decrease the accuracy of the volume transferred when using autopipettes with non-water liquids.

CVI monomer standards were created by addition of liquid monomer to a tared volumetric flask (plus stopper) on the balance, and the mass of monomer was recorded. The liquid monomer was transferred dropwise via glass pipette and rubber bulb. The volumetric flask was then filled to the line with HFIP and quickly sealed with the top to

create a known weight / volume solution. The mass was recorded in order to calculate weight / weight solution concentration also. Autopipettes were then used to transfer known volumes of the monomer solution to another tared volumetric flask on the balance and the mass recorded. Filling the flask with HFIP to the line created a dilute solution of confident weight / volume concentration. Varying the volume transferred to the second volumetric flask allowed creation of the desired concentration range of monomer in HFIP standard solutions needed for calibration and test method validation.

The standard addition samples were made to test the accuracy of the CVI method. By adding known amounts of monomer to the polymer solution, a calibration curve could be generated that intercepts the y-axis at 0.0% monomer added to the polymer. The y-intercept would then indicate the original monomer content in the polymer. Comparison of this intercept with actual measurements performed using the same batch polymer would indicate the level of accuracy of the analytical method.

Five separate flasks were prepared with PDO polymer weighed into each (149.9 to 150.2 mg in a 10 mL flask). A volumetric pipette was used to add 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, or 0.5 mL of PDO monomer solution to each flask of polymer. After the additions were made, the flasks were filled to 10 mL with HFIP and mixed for analysis. The CVI injection method and PDO monomer standard curve were utilized to analyze the PDO monomer solution to determine the weight / volume monomer concentration of the addition solution. This concentration and the volumes added to each flask of polymer were used to determine the percent PDO monomer added to each flask of PDO polymer.

CHAPTER FOUR

DEVELOPMENT OF TESTING METHODS AND RESULTS

Multiple Headspace Extraction (MHE)

MHE injection methods for gas chromatography (GC) account for differences in vaporization rates and other matrix effects between standards and samples. With respect to monomer content in polymer analysis, this allows total vaporization samples of monomer to be used for external calibration of the method. External calibration requires plotting multiple analytical results generated by varying the amount of analyte used for each analysis. With an external calibration for PDO monomer established, a solution of unknown concentration could be analyzed to determine the PDO monomer concentration based on the peak area observed at the retention time associated with PDO monomer.

Monomer standards for external calibration of the MHE method were prepared as previously described using 1,4-dioxane-2-one (PDO) monomer and hexafluoroisopropanol (HFIP). The desired minimum limit of quantification was approximately 0.1% PDO monomer in polymer, and the desired maximum limit of quantification was approximately 20% PDO monomer in polymer. The ideal polymer sample size was between 25 mg and 1 gram of PDO polymer. The dilution and volumes added to each vial for the pre-calibration curve samples also served to check the detection ability of the general MHE-GC method and determine the retention times of the PDO monomer analyte. The specific dilution scheme used to create the MHE-GC pre-calibration PDO monomer standards is tabulated in Table 5.

Table 5: PDO Monomer Standards for MHE Pre-calibration Curve

PDO Monomer (g)	**0.05630**		
HFIP Volume (mL)	**10**		
Concentration (g/mL, mg/µL, w/v)	**0.00563**		
Volume Added to GCHS Vial (µL)	**50**	**70**	**100**
Mass of PDO Monomer Added to Vial (mg)	**0.2815**	**0.3941**	**0.563**

These concentrations were prepared to represent 0.3, 0.4 and 0.6% PDO monomer in a 100 mg polymer sample.

Figure 11 is a representative chromatogram of PDO monomer as analyzed by MHE-GC. The chromatogram generated using the general GC method included a small solvent peak eluting relatively early that represented the HFIP followed by a larger peak corresponding to the PDO monomer.

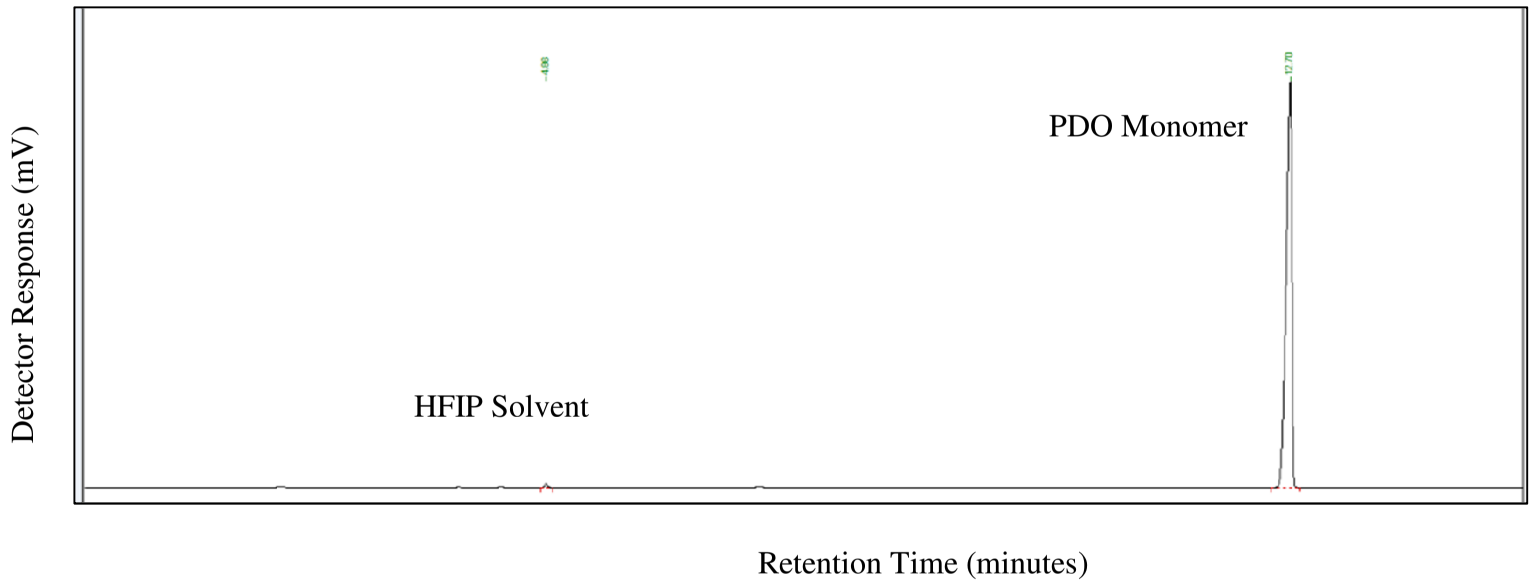


Figure 11: Representative Chromatogram of PDO Monomer Standard as Analyzed by MHE GC

The chromatograms generated by analysis of the PDO monomer standards were exactly as expected. There were no additional peaks observed in the maximum temperature hold portion of the chromatogram that was removed from Figure 11. This suggested that no volatile degradation products were generated during the heating of the PDO monomer. The only peaks present were expected. The chromatography indicated that the general method provided a great deal of resolution power for PDO monomer. The retention time for PDO monomer occurred well into the method at 12.7 minutes. The retention time for the analyte peaks varied less than 0.1 across all injections for all samples.

Each injection of MHE generated an individual chromatogram. All chromatograms thus far in the GC analysis of PDO polymer and PDO monomer appeared the same as Figure 11 but with varying peak sizes. Figure 12 is an overlay of the four chromatograms generated as the smallest of the PDO monomer standards was analyzed.

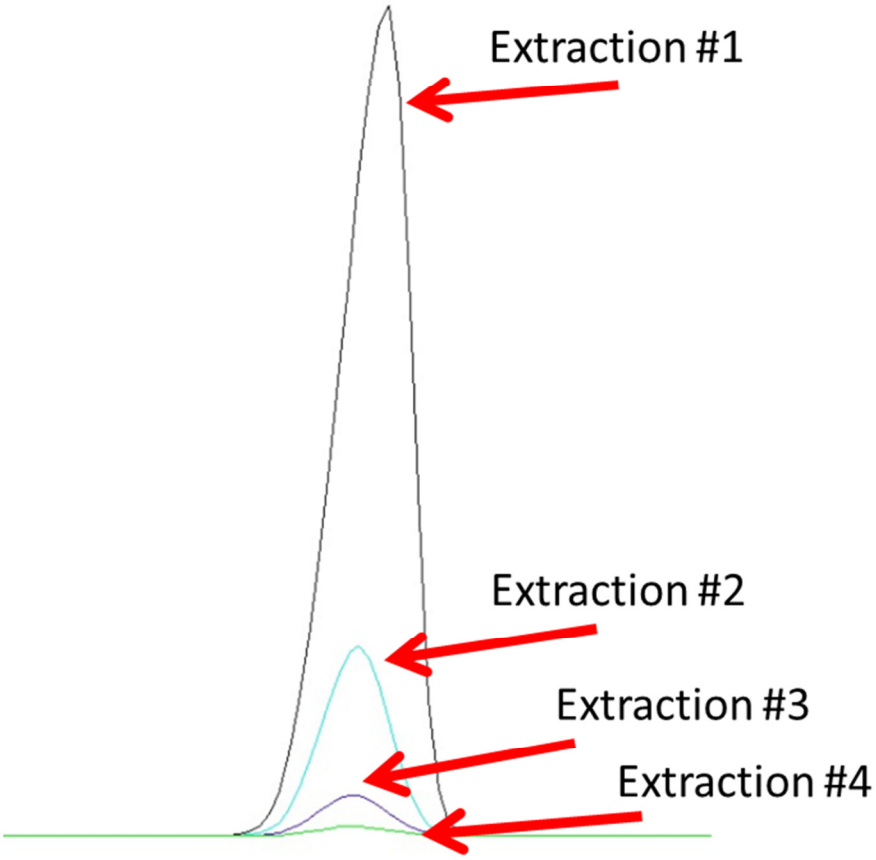


Figure 12: Overlay of MHE Chromatograms for PDO Monomer Standard (0.2815 g)

Figure 12 shows exactly what is expected from MHE analysis based on the literature representations of the total vaporization technique (TVT) that describes MHE analysis of monomer standards. The first three extractions generated for the smallest of the PDO monomer standards were well above the noise threshold of the baseline indicating a great deal of sensitivity for the PDO monomer analyte when utilizing the general MHE-GC method. The reduction of each successive peak appeared as expected for MHE analysis that predicts exponential reduction in analyte concentration in the HS vial.

Figure 13 is a plot of the area counts for PDO monomer versus the number of extractions. Included in the plot is the best fit exponential curve and equation.

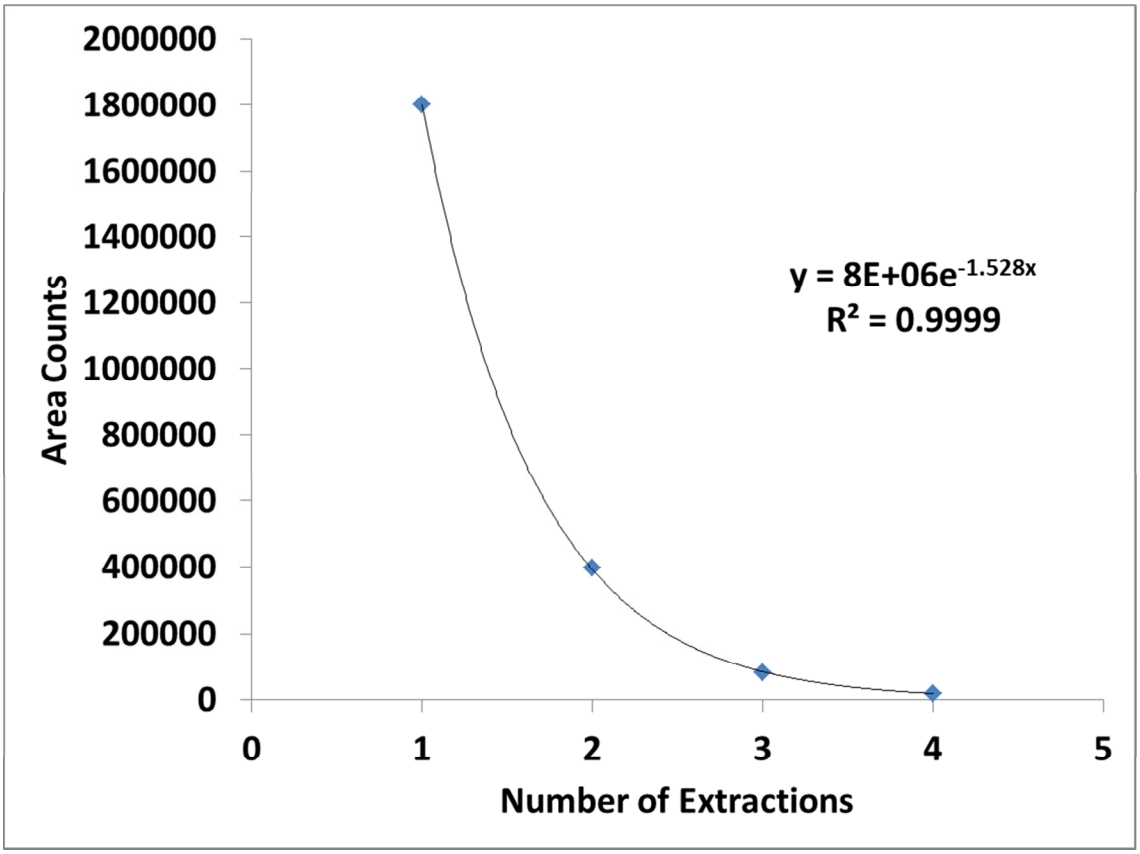


Figure 13: Plot of PDO Monomer Area Counts versus the Number of Extractions Performed on the PDO Monomer Standard (0.2815g) HS Vial (MHE Curve)

The coefficient of determination for the regression curve in Figure 13 is nearly perfect. This indicated the sample was able to achieve equilibrium prior to sampling. The reduction in peak area to nearly zero peak area suggests that total vaporization of the PDO monomer did occur and it was completely removed from the HS vial via the extractions. The best fit curve equation is used with the MHE extrapolation equations to

calculate a total number of area counts that corresponds to the standard mass of monomer in the HS vial. This response factor was used to calibrate the MHEGC method for PDO monomer analysis.

Figure 14 is a plot of total area counts for each of the three PDO monomer standards versus the actual mass of monomer used. A best fit linear curve was applied to quantify the linearity of the data. The equation of this curve is shown on the plot.

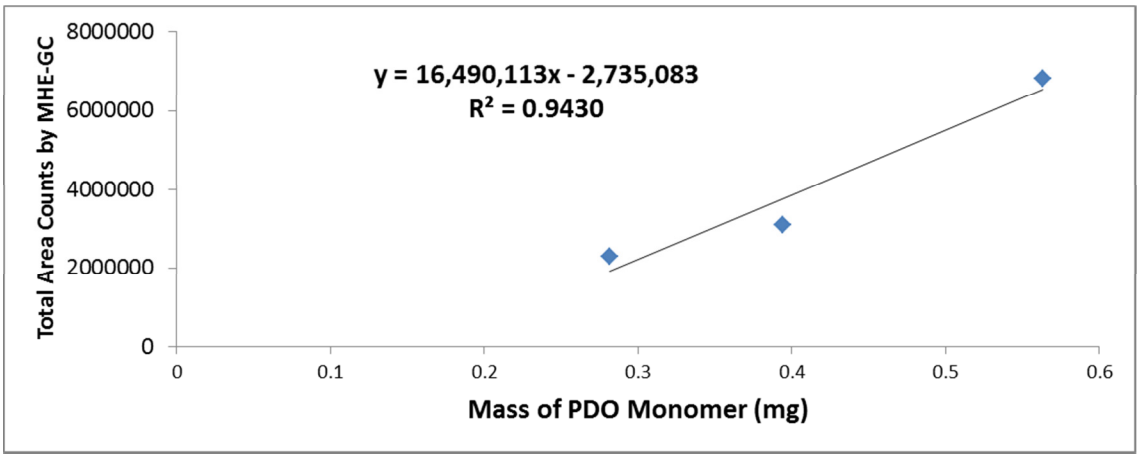


Figure 14: Pre-calibration plot of Total Area Counts of PDO Monomer by MHE versus the Known Mass of PDO Monomer in Each Vial

Even though this curve is only three data points, the linear fit equation and coefficient of regression were not as expected for a calibration curve. The greatest concern was that the y-intercept did not approach the origin. The negative y-intercept is normally interpreted as all of the material weighed into the vial not being quantified. This indicated a problem with the samples. For example the HS vials could have leaked. However, the data in Figure 12 and Figure 13 indicated all of the material was extracted from the vial and quantified via TVT.

The HS vials utilized for the PDO monomer standards were inspected for any evidence to confirm or deny the unintentional loss of PDO monomer from the sealed vials. This inspection was expected to reveal a leak. An opaque solid was observed in the vials. Figure 15 is a photograph of one of the HS vials inspected.

Figure 15: Observation of Material Remaining in HS Vial of MHE-GC Analyzed PDO Monomer Standard

This material in the HS vial appeared to no longer be PDO monomer. The GC results generated via analysis of this vial suggested this material was a nonvolatile degradation product of heating the PDO monomer. The identity of the opaque material in

these HS vials was not further investigated because the conclusion that it was not PDO monomer was the only information relevant to the external calibration of PDO monomer via the MHE-GC method.

It was observed that the vial of PDO monomer used for standard solutions would no longer melt. The material in the PDO monomer container appeared hard and opaque, similar to that which was observed in the bottom of the HS vials. Differential Scanning Calorimetry (DSC) was performed from 0°C to 120°C at 20°C/minute on two separate containers of monomer. One sample was a fresh container of monomer from the freezer. The other sample was the container above that had been melted at 50°C approximately ten times for sampling purposes before it changed appearance and no longer melted at the previously used temperature. Figure 16 and Figure 17 are thermograms collected from both the thermally affected PDO monomer and the fresh PDO monomer, respectively. The previously heated material displayed an endothermal melting event beginning at a temperature greater than 50°C and bifurcating into approximate peak melting temperatures of 84°C and 92°C. The fresh PDO monomer displayed an endothermal melting event at an approximate peak temperature of 30°C.

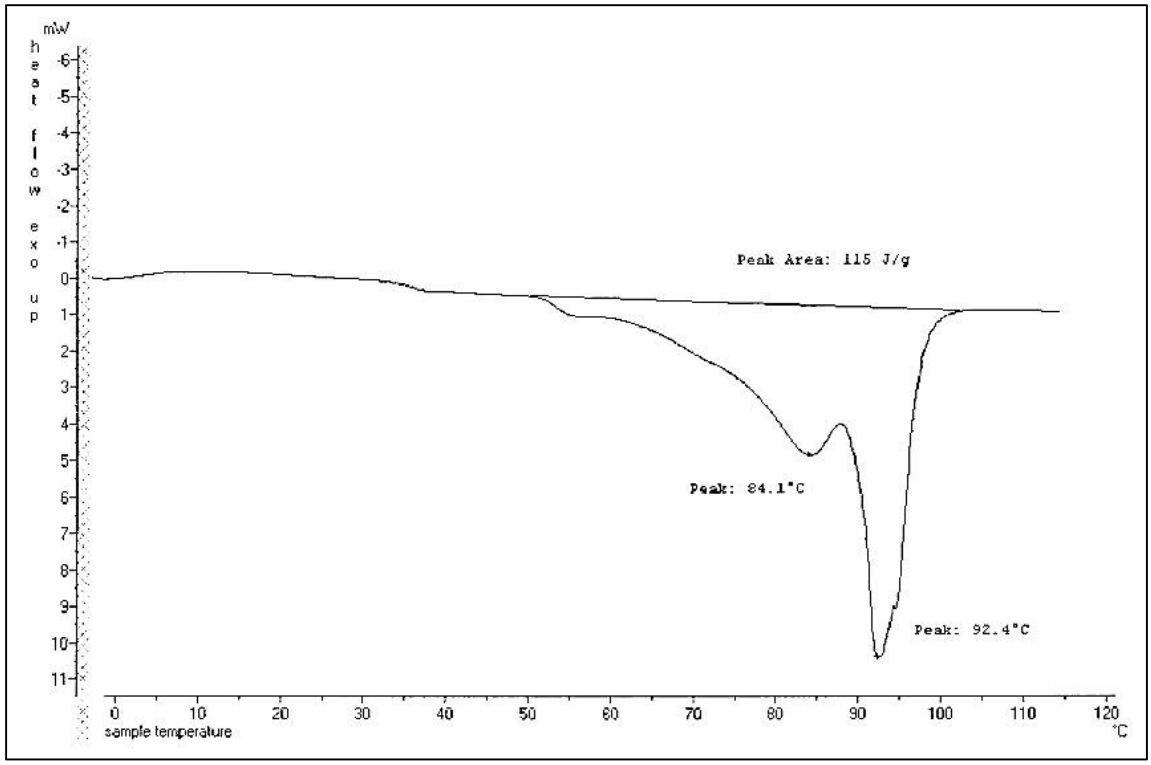


Figure 16: DSC Thermogram of Thermally Affected PDO Monomer

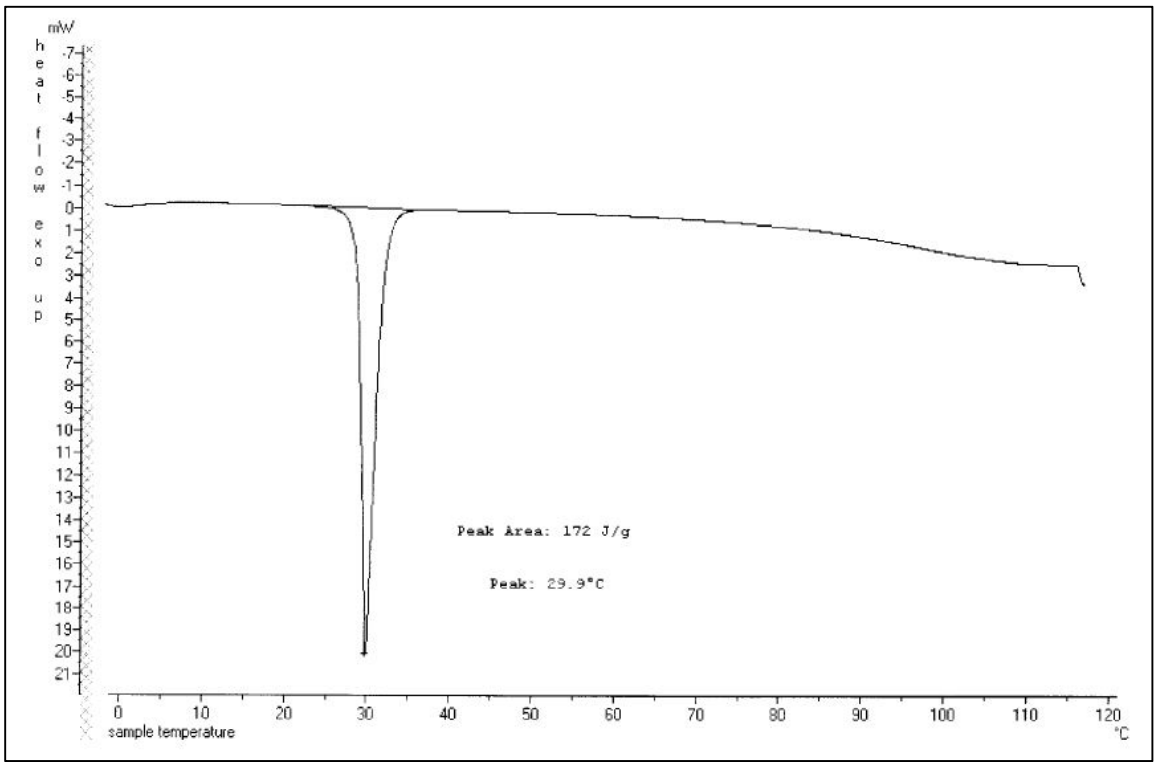


Figure 17: Representative DSC Thermogram of Fresh PDO Monomer

The results confirmed that the previously heated material was no longer monomer. PDO polymer is known to have a melting temperature of approximately 107°C. The thermally affected PDO monomer was not fully polymerized, but was also no longer useful as PDO monomer for use a monomer standard material.

A review of literature provided by the PDO monomer supplier confirmed an exponential relationship between storage temperatures and a measureable effect on the monomer purity by GC of the PDO monomer at the reported number of days. In addition to GC purity analysis, the PDO monomer was monitored using free acid titration analysis. The two quantitative tests were further supplemented by qualitative confirmations of monomer instability via polymerization reactions. Table 6 below lists the temperatures, in both °C and K, which relate to stability observations reported in days. Figure 18 below is a plot of the thermal stability in days versus temperature in Kelvin.

Table 6: Monomer Supplier Recommendations for PDO Monomer Storage

Temperature (°C)	Temperature (K)	Days Until Monomer Stability Issues are Reported
25	298	6
-8	265	120
-17	256	180

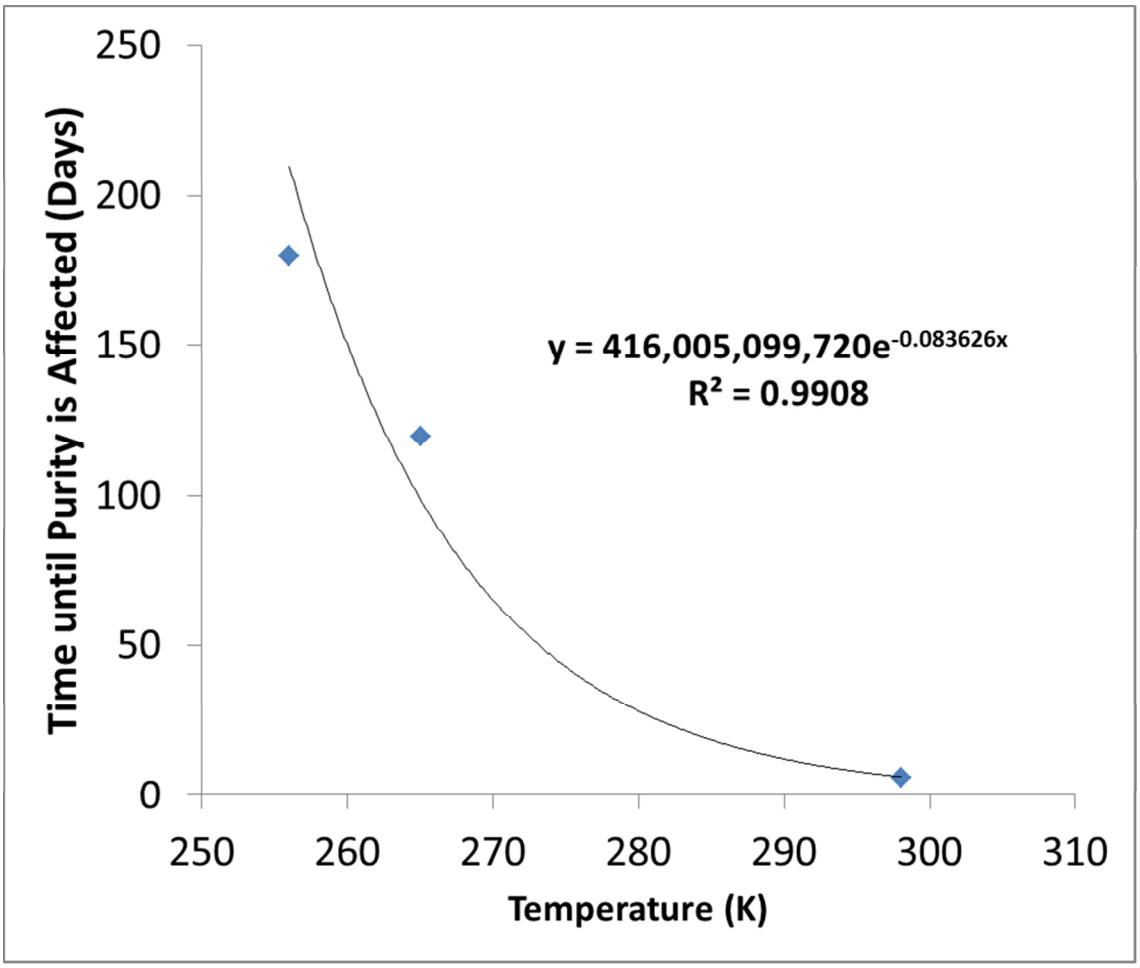


Figure 18: Time Dependence of PDO Monomer Purity with Exponential Trendline

The absolute temperature in Kelvin was utilized so that an exponential trendline could be fit to the plotted data. Extrapolation of this trendline to the 50°C used to melt the monomer for sampling and the 100°C thermostat temperature yielded stability predictions of 19 hours and 17 minutes respectively. The PDO monomer standard samples were concluded to have degraded prior to the first thermostat time of the first HS extraction.

The temperature utilized during the melting step when sampling PDO monomer was reduced to 37°C, and the time allowed for melting was minimized for all future sampling in an attempt to further avoid PDO monomer stability complications.

In spite of the above problems with monomer stability, the general MHE-GC was utilized to analyze PDO polymer for PDO monomer content. Polymer analysis began with the use of the higher monomer content PPDO material available for method development. It was believed to contain 2 to 4 % PDO monomer by weight based on proton nuclear magnetic resonance analysis performed previously. Figure 19 is the plot of PDO monomer area counts versus number of extractions (MHE curve) for a 0.094 gram sample of high monomer PDO polymer.

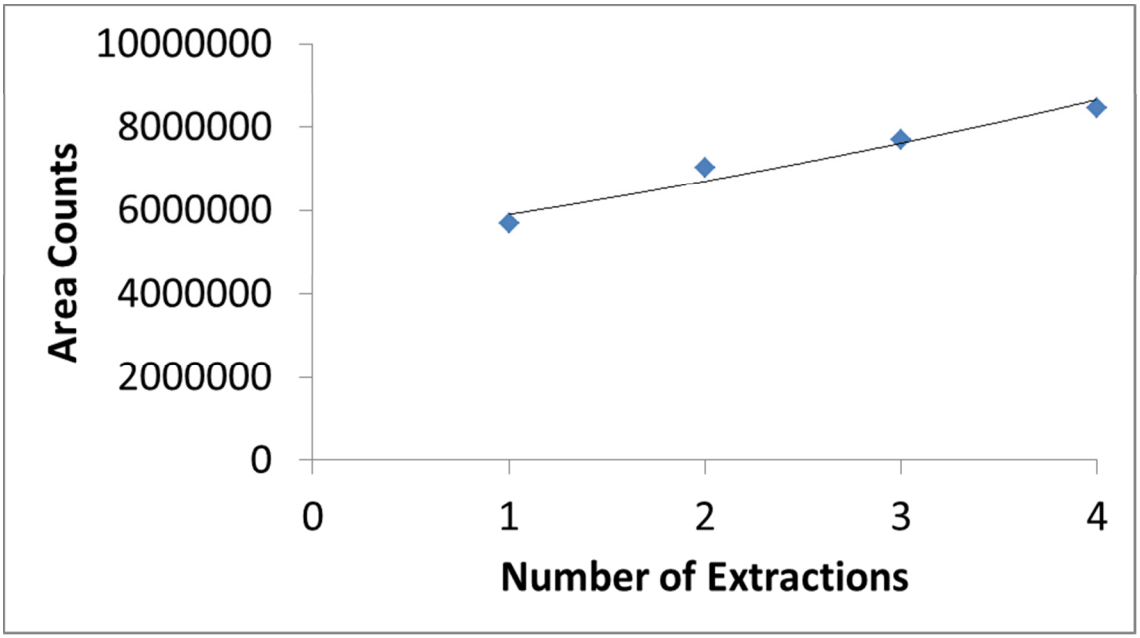


Figure 19: Plot of PDO Monomer Area Counts versus Number of Extractions for High Monomer PDO Polymer using a 0.094 Gram Sample

Surprising, the peak areas for PDO monomer increased with each extraction instead of showing the expected exponential rate of reduction. A larger amount of PDO monomer was detected despite the purge step removing the vaporize PDO monomer from the vial following each sampling for GC. In spite of the increasing slope, the MHE calculation was used and determined a negative monomer content value. It was understood that sample size should not have an effect on PDO monomer content results calculated for a stable system at equilibrium, but the literature suggested that this shaped MHE curve was indicative of insufficient time allowed for the sample to reach equilibrium between the vapor and condensed phases in the HS vial. The literature did suggest that smaller samples should equilibrate the vapor and condensed phases in less time. Other non-ideal MHE curve examples in the literature were due to adhesion tendencies of the analyte. Adhesion of the monomer to either the polymer surface or inner HS vial surface could be overcome by addition of an additional volatile material into the HS vial prior to crimping to preferentially coat the potential adhesion surfaces.

Due to both large sample size and adhesion complications occurring, new samples were prepared with smaller amounts of polymer along with an added solvent. HFIP was added by glass pipette with a rubber bulb such that approximately 20 µL was allowed into the HS vial just before crimping. Figure 20 is the plot of PDO monomer area counts versus number of extractions (MHE curve) for the smaller PDO polymer sample with HFIP added.

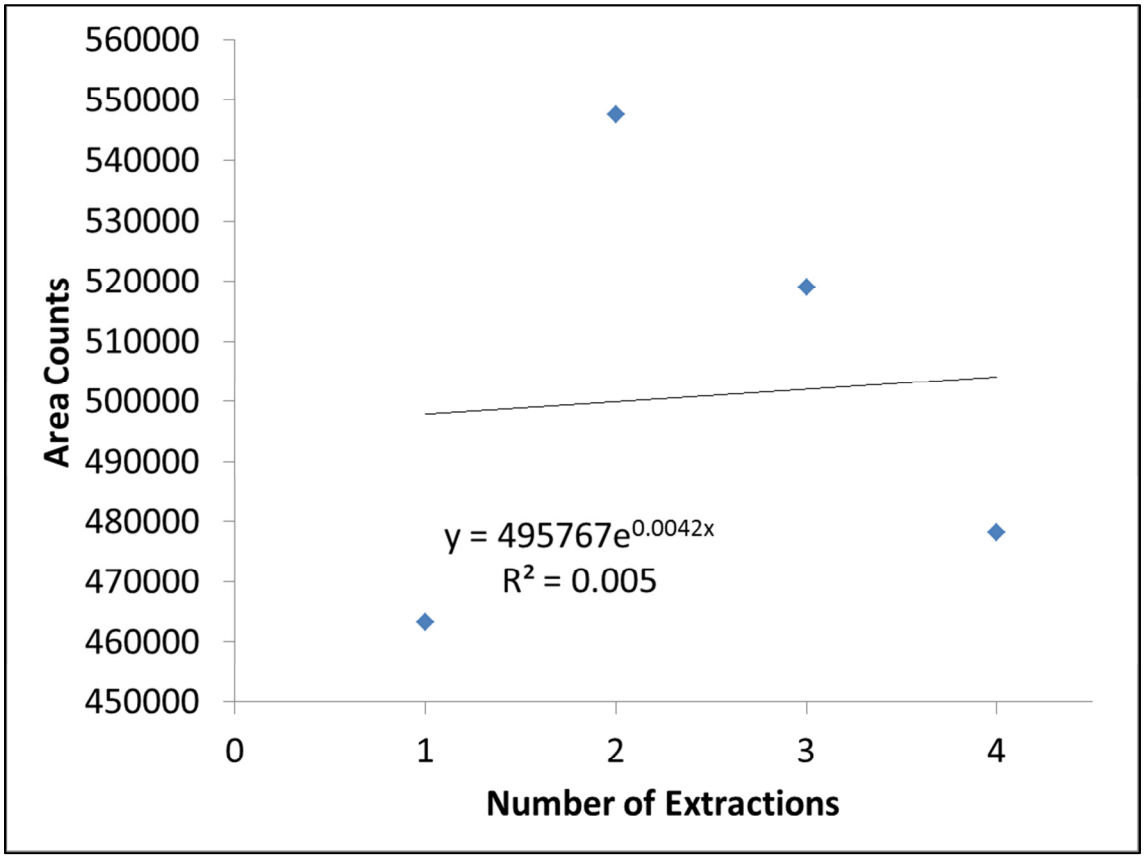


Figure 20: Plot of PDO Monomer Area Counts versus Number of Extractions for High Monomer PDO Polymer using a 0.025 Gram Sample with HFIP added.

Smaller samples with HFIP added in the vial appeared to reach equilibrium in less time as evidenced by the decreasing peak areas from extraction 2 to 3 to 4. The first extraction result did not fit well with the remainder of the MHE curve. Removal of the first extraction data point was commonly referenced in literature. Based on review of literature, the sample was unable to reach equilibrium from ambient conditions during the thermostat time or the first extraction was otherwise differentiated from the remaining extractions. This may be due to a change in the composition of the headspace which is primarily nitrogen for the first extraction. The remaining extractions occur with a

primarily helium filled vial that also contains the HS autosampler. Figure 21 is the plot of PDO monomer area counts versus number of extractions (MHE curve) for the high monomer PDO polymer 0.025 gram sample with HFIP added and first extraction data point removed from the plot and calculation.

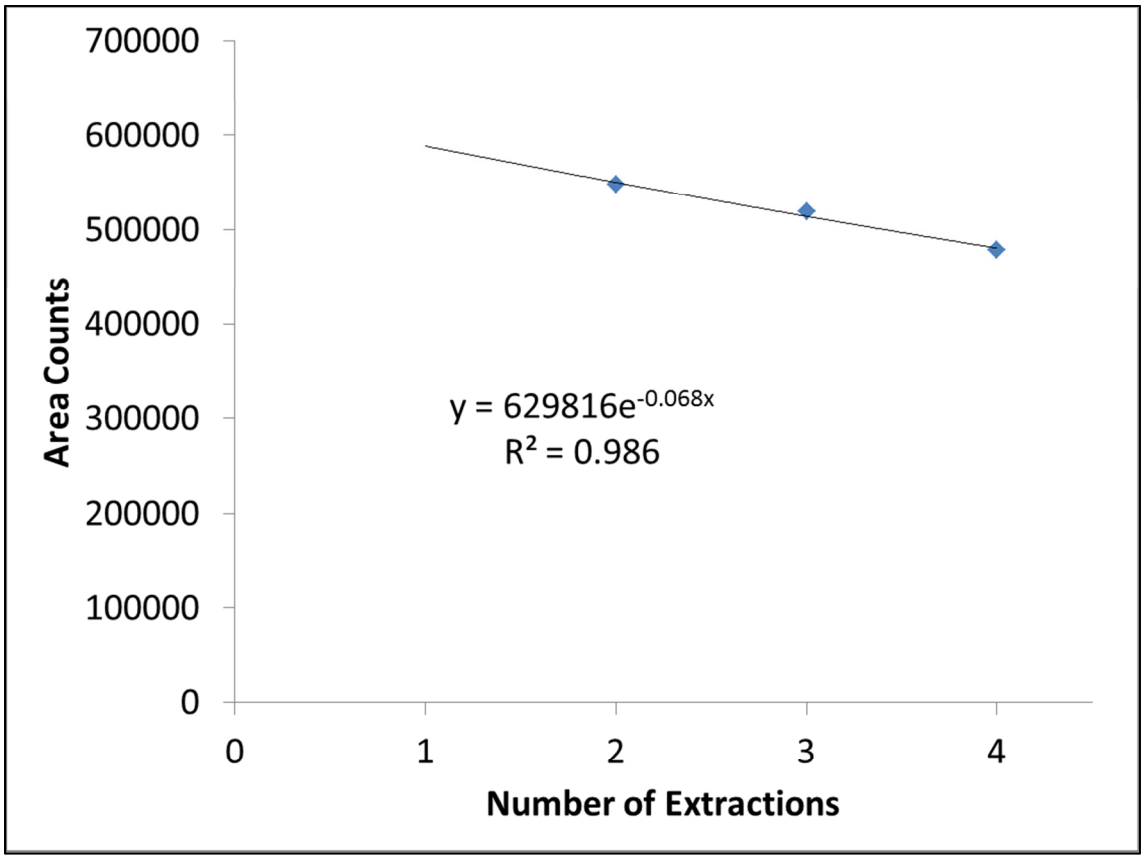


Figure 21: Plot of PDO Monomer Area Counts versus Number of Extractions for High Monomer PDO Polymer using 0.025 Gram Sample with HFIP added (without First Extraction Result).

A non-negative monomer content was calculated from the slope of the MHE curve without the first point. Using the pre-calibration trendline in Figure 14, a monomer content of 2.2% was calculated for the high monomer PDO polymer.

The material added to the vial to prevent adhesions was suggested by the literature to be similar in chemical composition to the analyte to best prevent adhesions from occurring. The low monomer PDO polymer was hypothesized to be a more sensitive sample for analysis due to the smaller analyte signals anticipated. Smaller signals could be more susceptible to errors related to non-idealities and inherent noise in the detector signal. The analysis of low monomer PDO polymer by MHE-GC was thus performed with 10 µL of dioxane solvent added to the vial. An autopipette was utilized to make the addition to the polymer in the HS vial just before crimping. No other change to the general method was made. Dioxane was understood to be much more chemically similar to PDO monomer than HFIP.

Figure 22 is a plot of PDO monomer area counts versus number of extractions (MHE curve) for a 0.025 gram sample of low monomer PDO polymer that had 10 µL of dioxane added just before the HS vial was crimped.

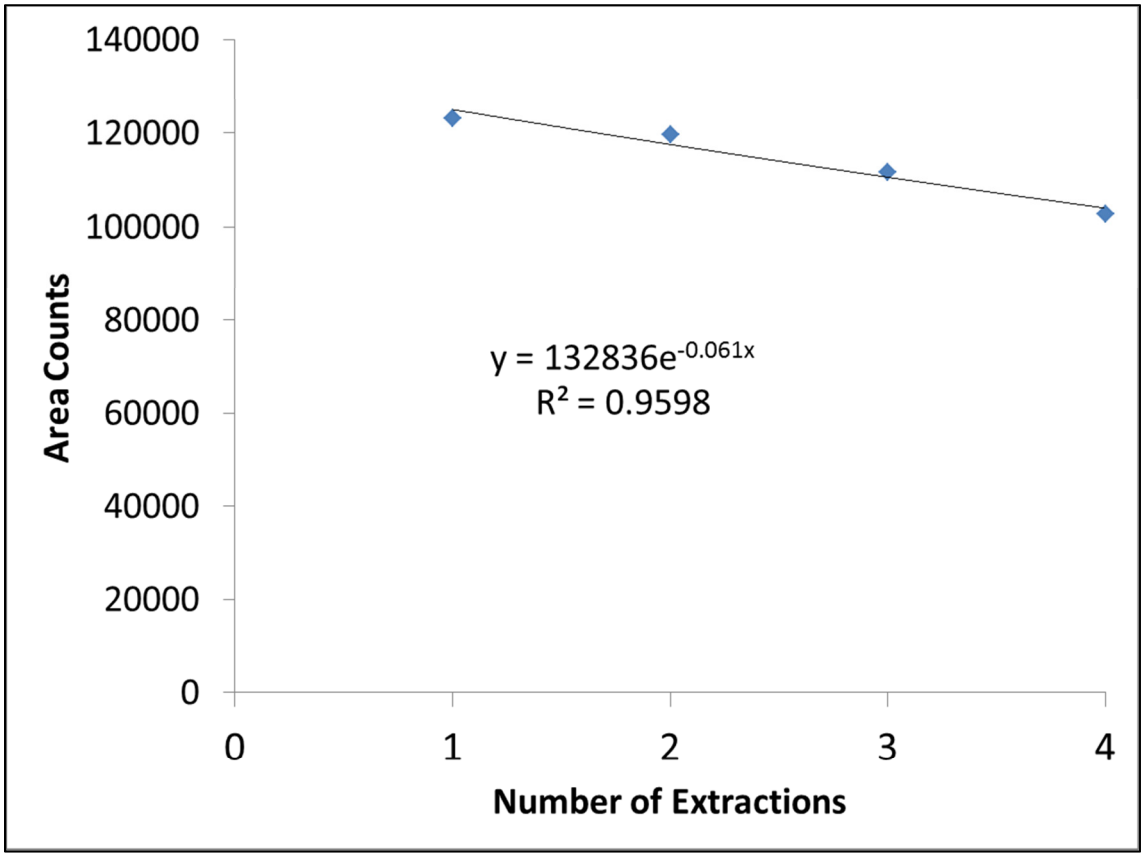


Figure 22: Plot of PDO Monomer Area Counts versus Number of Extractions for Low Monomer PDO Polymer using a 0.025 Gram and 10 µL Dioxane Added to the HS Vial per Sample.

A quantitative result was feasible with or without the first data point. With the first data point included, a monomer content of 0.5% was calculated. Without the first point, the monomer content result was shifted to 0.4%. The trendline coefficient of regression was increased to 0.99 by removing the first data point.

The addition of HFIP, dioxane or no solvent addition did not conclusively improve the repeatability of the PDO monomer in PDO polymer MHE-GC analytical results. An average and standard deviation of 13 MHE-GC results for the high monomer PDO polymer, including all attempts of additions to the vial, was 5.86% ±8.55% residual PDO monomer content (weight to weight). An average and standard deviation of 14

MHE results for the low monomer PDO polymer, including all attempts of additions to the vial, was 0.49% ±0.45% residual PDO monomer content (weight to weight). The highly variable monomer content results compounded the concerns about the stability of the PDO monomer and polymer during the MHE analysis. Addition of solvents in the HS vial was not proven sufficient to ensure adhesion was not affecting the MHE-GC results. Variation in the sample size was not proven sufficient to ensure phase equilibrium was observed via the MHE curves, and this prompted an investigation into the specific requirements for HS equilibrium of PDO polymer and PDO monomer.

Volatile components in a condensed phase are reduced as the temperature of the condensed phase is increased. Consequently, the concentrations of the volatiles in the vapor phase headspace above the condensed phase are increased as the temperature is increased. When the material is exposed to a constant temperature that is greater than the ambient temperature, the temperature of the material increases at a decreasing rate as the temperature of the material approaches that of the constant temperature environment. This thermal equilibrium is also accompanied by phase equilibrium. Quantitative headspace (HS) analysis techniques require equilibrium between the condensed and vaporized phases. A “progressive” experiment was performed that progressively increased the amount of time the sample was allowed to equilibrate at the thermostat temperature with multiple equivalent samples. The results of this experiment should generate a plot of analyte peak areas versus increasing thermostat times that sharply increases until equilibrium is achieved and the analyte peak areas reach a maximum value for all greater equilibration times. This equilibration should be observed as a constant

area count for the analyte with all thermostat times greater than the minimum required thermostat time for quantitative HS analysis. Equilibrium in the headspace vial, as determined in the progressive experiment, has always been critical to the success of any quantitative HS method. Equilibrium must be achieved for Henry's Law to be applied and allow the assumptions required for quantification of volatiles in the condensed phase. Unexpected results were defined as inconsistent changes in analyte peak areas or a non-linear, horizontal curve observed as thermostat time was increased. Another indicator of an unsuccessful progressive experiment is if the analyte peak areas did not increase to a consistent, maximum value as thermostat time was increased. An unsuccessful progressive experiment indicates the sample is not achieving equilibrium as predicted by Henry's Law.

The general progressive analysis method was utilized with the general GC method to analyze for an optimized thermostat time. Figure 23 is a plot of PDO monomer area counts versus progressively increasing thermostat times using 0.1 grams of low monomer PDO polymer per HS vial.

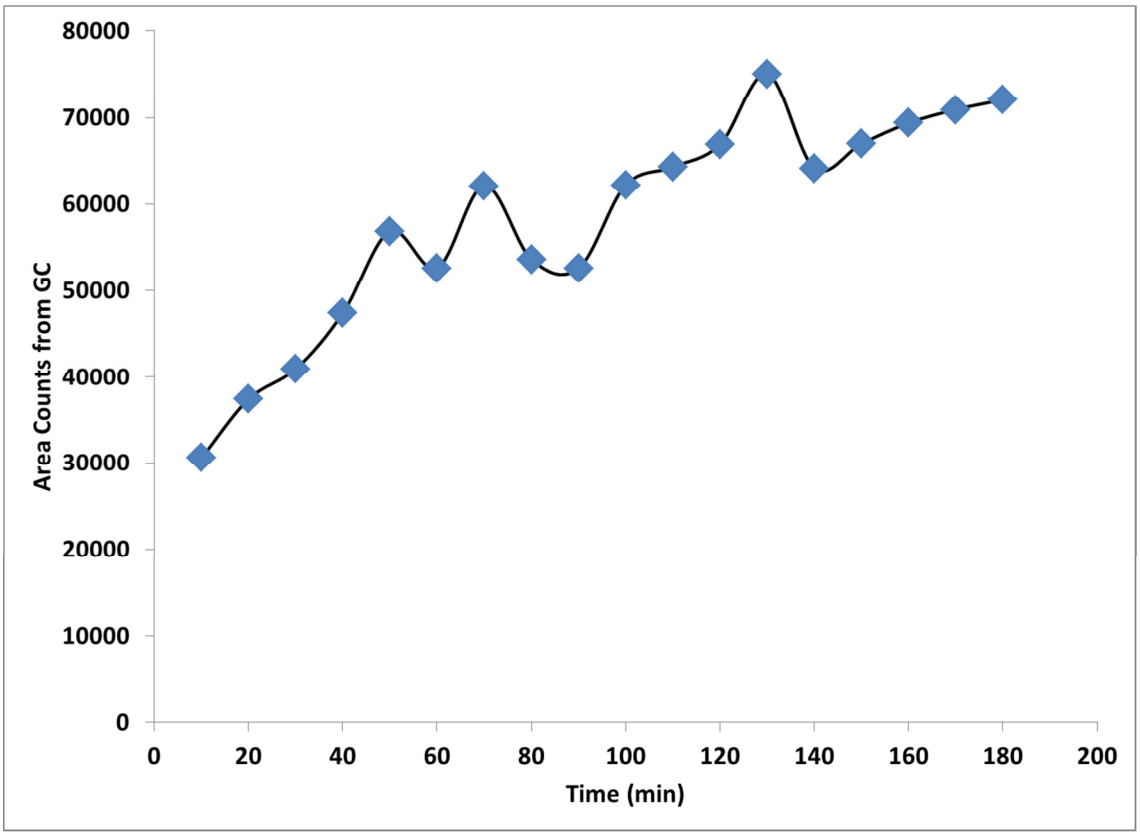


Figure 23: Plot of PDO Monomer Area Counts versus Progressively Increasing Thermostat Times using 0.1 Grams of Low Monomer PDO Polymer per HS Vial.

The PDO monomer area counts initially increase as expected until the 60 minute thermostat time and above. The expected horizontal and consistent curve was not observed. The variation was greater than 40% after the 50 minute thermostat result between maximum and minimum PDO monomer area counts.

Due to this work utilizing smaller sample sizes to reduce the time required for equilibration, it was concluded that the progressive experiment should be repeated using a smaller sample size of low monomer content PDO polymer. Figure 24 is a plot of PDO

monomer area counts versus progressively increasing thermostat times using 0.025 grams of low monomer PDO polymer per sample.

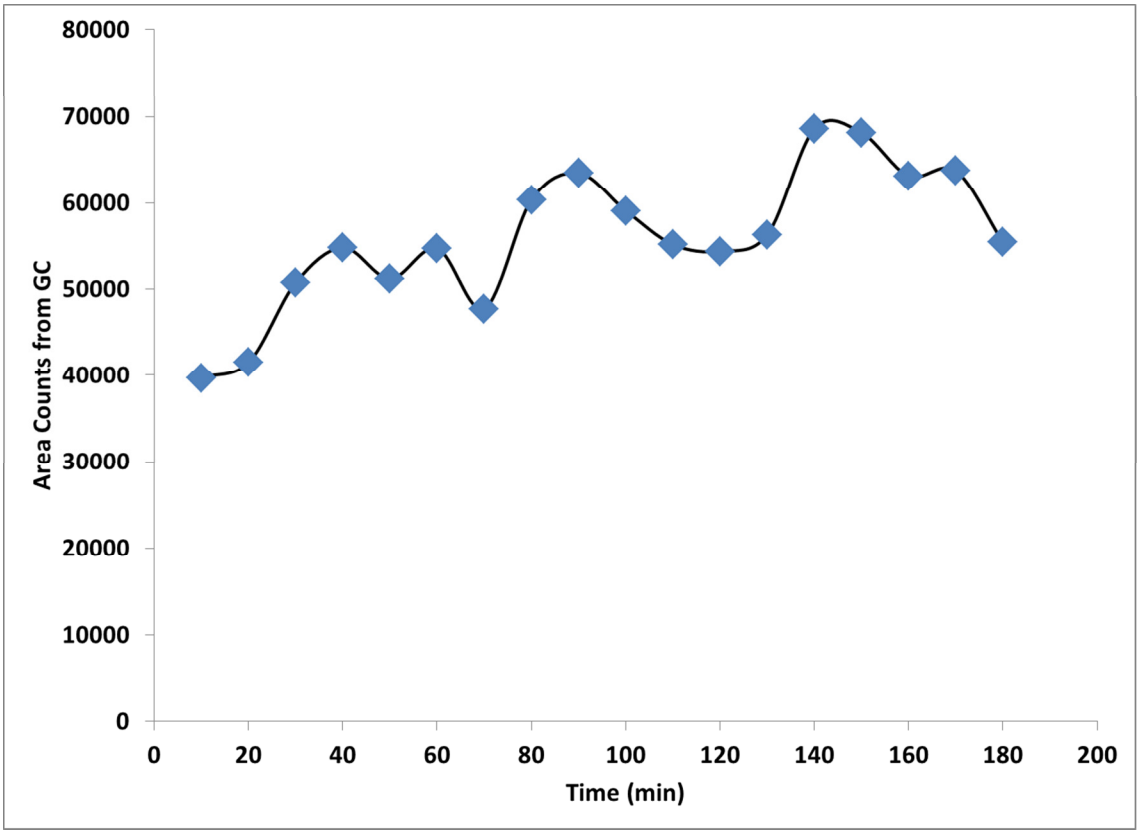


Figure 24: Plot of PDO Monomer Area Counts versus Progressively Increasing Thermostat Times using 0.025 Grams of Low Monomer PDO Polymer per Sample.

The PDO monomer area counts increased until the 40 minute thermostat time was reached. Progressive increases in thermostat time beyond 60 minutes were observed to vary by greater than 40% despite the change in sample size.

The progressive experiment was performed at a temperature of 80°C, but the results of the experiment did not generate results that were closer to equilibrium than the

observations made using a 100°C thermostat temperature. The peak areas were observed to be smaller than those observed when a temperature of 100°C was utilized, but variation beyond the initial increase of PDO monomer peak areas was not improved. The progressive experiment was performed at a temperature of 60°C. Inspection of the chromatograms confirmed that no peaks were present at the PDO monomer retention time. No additional results or analysis were collected for PDO monomer or polymer using thermostat temperatures of 60°C or 80°C.

Previous analysis of PDO polymer investigated the use of dioxane solvent as an adhesion prevention additive for analyzing PDO monomer in PDO polymer samples. Dioxane solvent was added to the vial with the sample using an autopipette just before the HS vials were crimped. Additional progressive experiments were utilized to determine the effects of adding dioxane solvent to the HS vial of PDO polymer. Figure 25 is a plot of PDO monomer peak areas versus progressively increasing thermostat times using 0.025 grams of low monomer PDO polymer and 10 µL of dioxane solvent added to the vial.

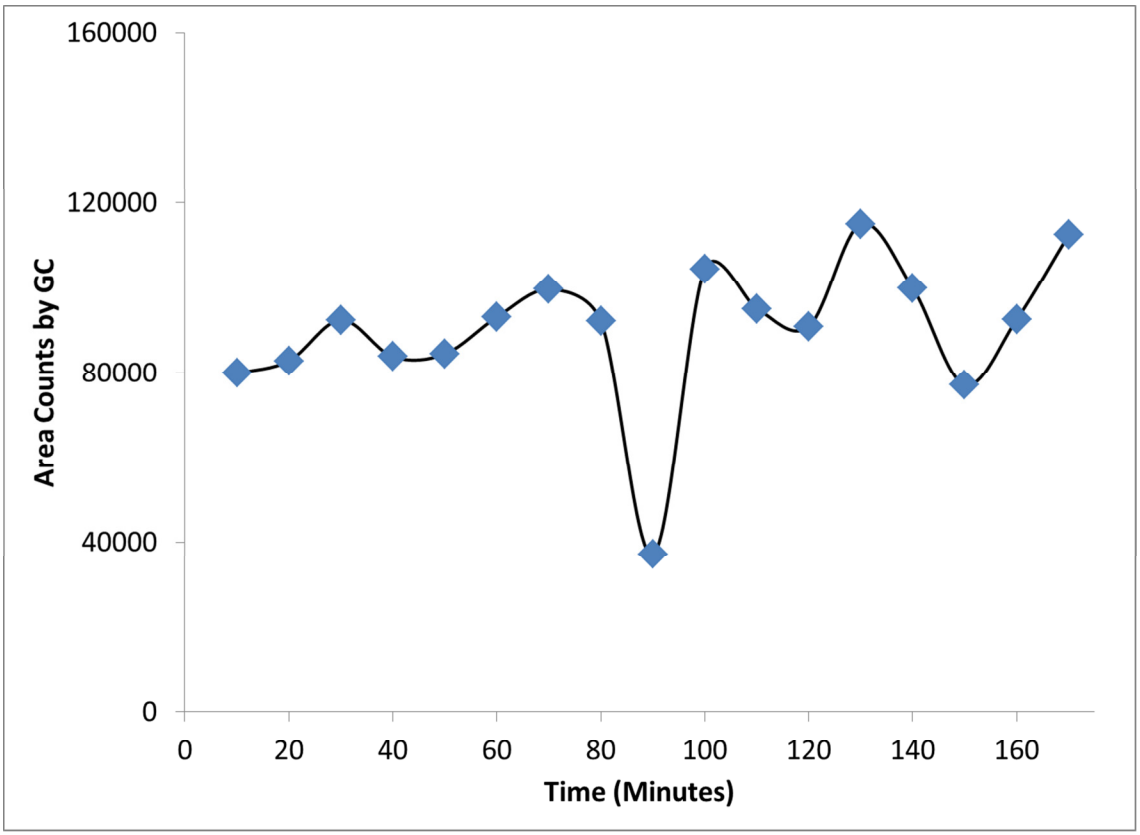


Figure 25: Plot of PDO Monomer Peak Areas by GC versus Progressively Increasing Thermostat Times using 0.025 Grams of Low Monomer PDO Polymer with 10 µL of Dioxane Added to the HS Vial per Sample.

The PDO monomer peak areas were not observed to reach a maximum and consistent result with increasing thermostat time. Greater than 200% variation was observed between peak areas related to thermostat times greater than 70 minutes. Removal of the outlier at 90 minutes reduced the variation of peak areas to approximately 50%. The outlier could have been due to a vial leak.

A second experiment was performed using a greater volume of solvent added to the HS vial. Figure 26 is a plot of PDO monomer peak areas versus progressively

increasing thermostat times using 0.025 grams of low monomer PDO polymer with 100 µL of dioxane solvent added to the HS vial.

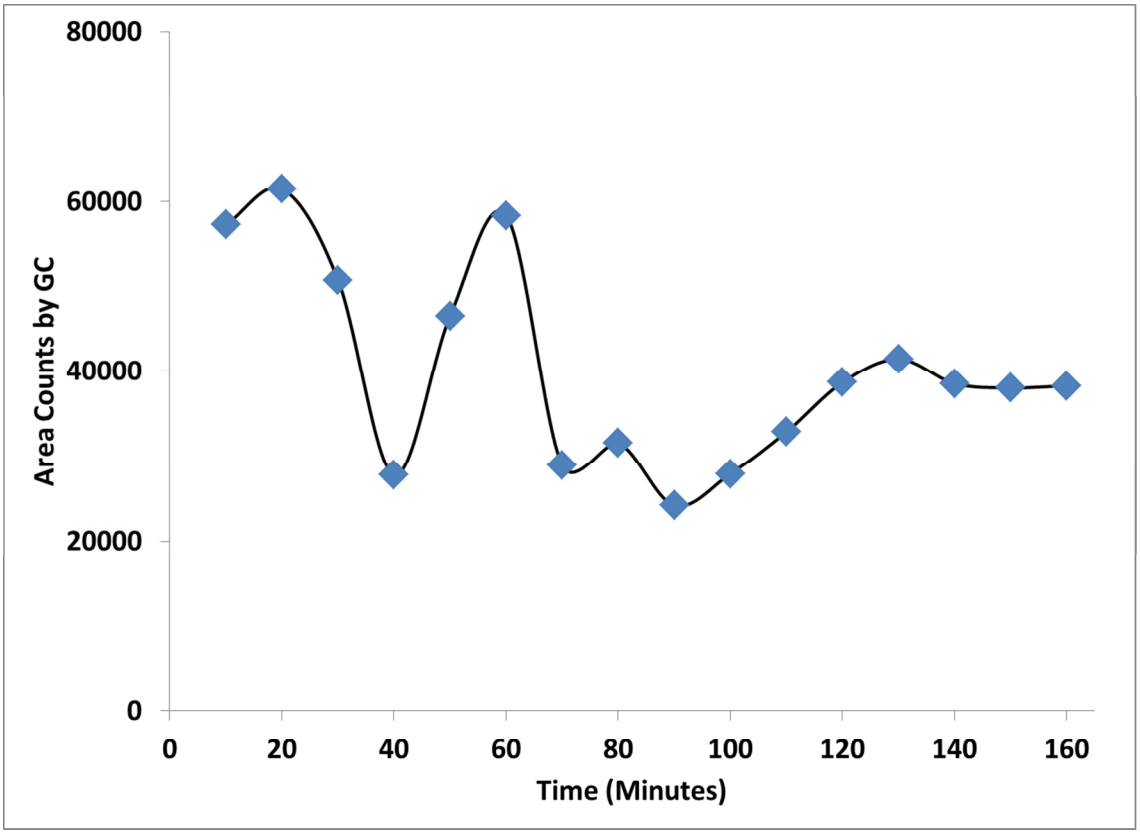


Figure 26: Plot of PDO Monomer Peak Areas versus Progressively Increasing Thermostat Times using 0.025 Grams of Low Monomer PDO Polymer with 100 µL of Dioxane Added to the HS Vial per Sample.

The addition of 100 µL of dioxane into the HS vial prior to analysis was observed to decrease the time required to achieve a maximum peak area for the PDO monomer analyte from 60 minutes to 20 minutes. The consistency indicative of an equilibrium system was again not observed. The variation in peak areas for thermostat times up to 130 minutes exceeded 100%.

Classical Vaporization Injection (CVI)

Classical Vaporization Injection methods of injection for gas chromatography (GC) analysis generally involve the injection of a liquid solution via needle and syringe into a heated injector operating with or without a split flow in use. The injector is generally set to a sufficient temperature to fully vaporize the mass of analyte injected nearly instantaneously and transfer it into the mobile phase gas and onto the column for interaction with the stationary phase for the desired separation to occur. The near instantaneous rate of separation of volatiles from the non-volatiles in the sample solution is the primary reason that CVI was considered a potential alternative to the multiple headspace extraction (MHE) method of GC injection.

Initial investigation into a classical injection technique began with the injector temperature that would be required. Figure 27 is an arrangement of chromatograms to show the effect of injector temperature on PDO monomer peak shape. The four chromatograms in Figure 27 were cropped to highlight the relevant information from the experiment. The top is the GC response when a monomer in solvent standard solution was injected using the general GC method. The second shows the response when a polymer solution was injected using the same general GC method. The third shows the response when a polymer solution was injected using a higher injector temperature of 250°C rather than the 200°C injector temperature utilized in the general GC method. The fourth chromatogram on the bottom is the response when a polymer solution was injected using an injector temperature of 150°C.

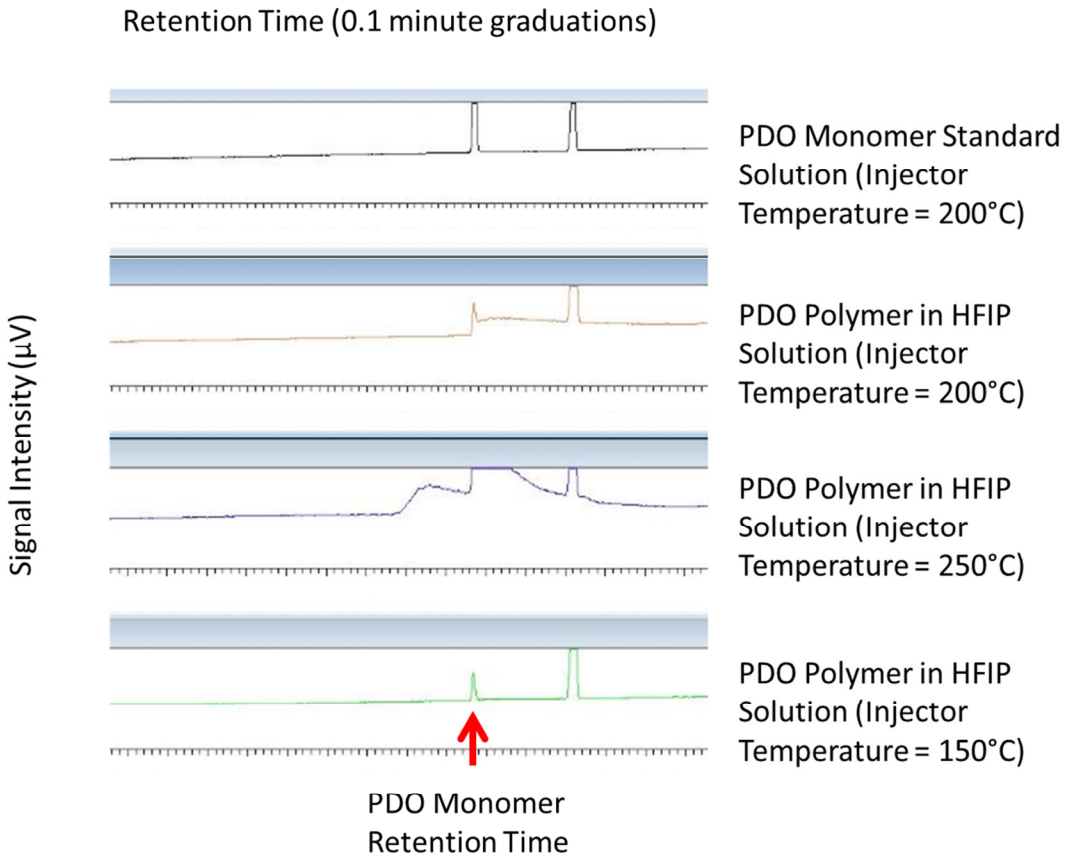


Figure 27: Arrangement of Four Cropped GC Chromatograms that Plot Detector Response versus Retention Time: Top Chromatogram is a PDO Monomer Standard Solution; Second is a PDO Polymer Solution; Third is the same PDO Polymer Solution with the Injector Temperature 250C°; and the Fourth is the same PDO Solution with the Injector Temperature 150C°.

The accepted injector temperature of 150°C had a significant role in the resolution of the PDO monomer peak. The injector vaporized the monomer to form a resolved peak later in the chromatogram. This separation of the monomer from the polymer was the original and most vital intent of the testing method.

Using a CVI method provided an opportunity for method optimization. Without using an internal standard, there was not a mechanism within the method for the GC

operator to mitigate or even identify an improperly performed injection. A bubble that greatly reduces the volume injected could be unknowingly present and negatively affect the analysis. Additionally, quantification of a single monomer allows a much shorter run time.

Figure 28 is an annotated representative chromatogram generated when the optimized CVI-GC method. Optimizations made to the general GC method include a timed event related to the sensitivity of the detector and an increase in the starting temperature of the GC oven. Detector attenuation was adjusted during the method using a timed event that changes the detector attenuation setting from 0 to -6 to account for sensitivity required to integrate the large solvent peak and the small monomer peak. An attenuation value of -6 corresponded to no attenuation applied to allow for maximum sensitivity. Additional timed events were programmed to turn off, and on, the software's automated integration of peaks just before, and after the attenuation change.

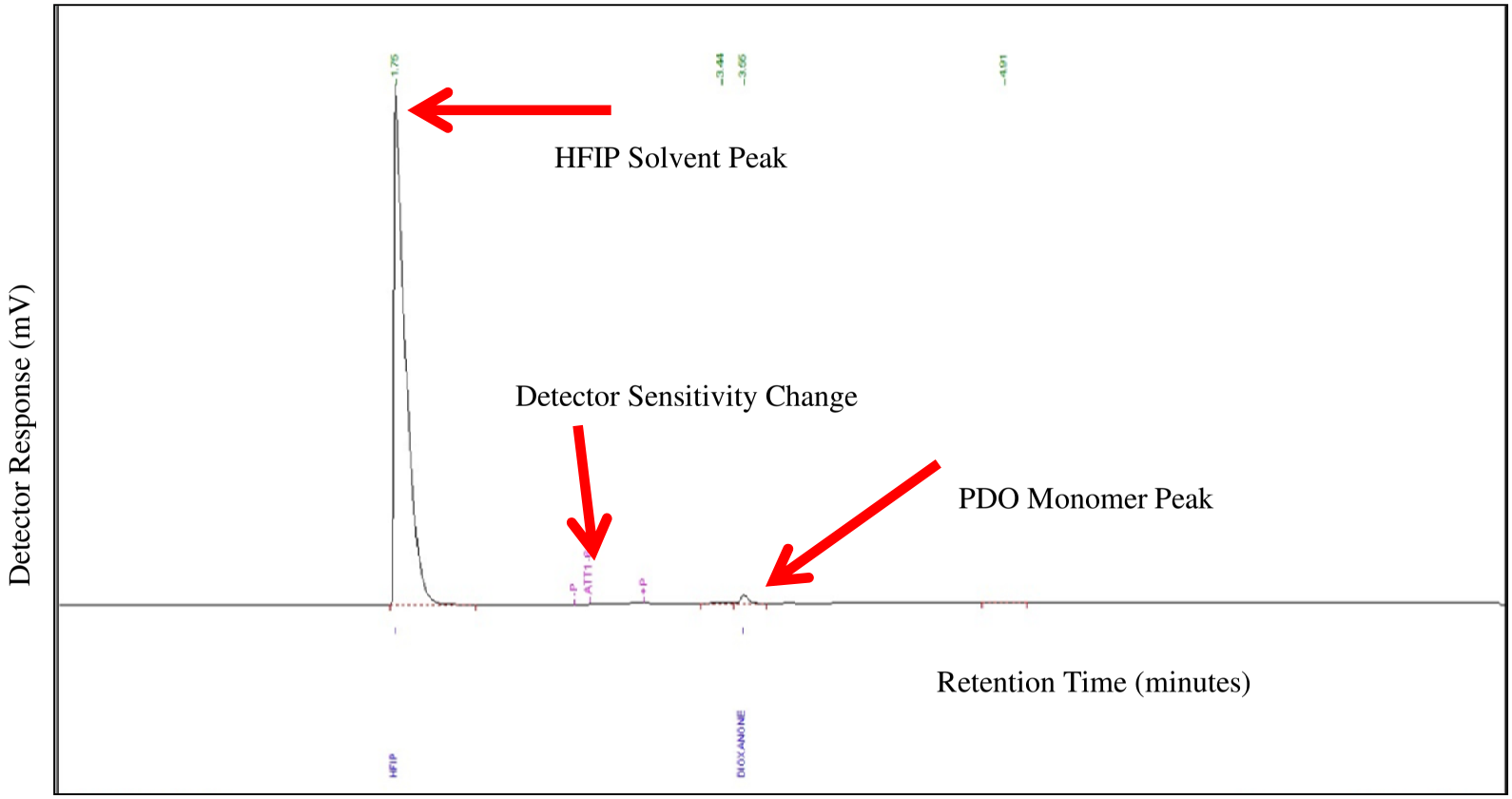


Figure 28: Annotated Version of a Representative Chromatogram of Optimized for PDO Monomer CVI-GC Method

Higher attenuation values allowed greater detector responses to be integrated. In addition to the actual solution volume injected, the HFIP solvent peak area is subject to the injection rate, needle puncture rate into the injector and needle extraction rate from the injector. These relationships make the HFIP peak well suited to monitor the use of manual GC injection. By employing the attenuator so that the solvent peak was integrated, both risks associated with failed equipment or operator error can be mitigated prior to the reporting of inaccurate results. Increases to the initial temperatures were made to minimize run time. The initial GC oven temperature setting of 40°C was optimized to an initial temperature of 150°C. An additional edit to the general GC method was to reduce the hold time at the final GC oven temperature from ten minutes to three minutes. The final run time of the optimized for PDO monomer GC method was 7.5 minutes and the time required between sample injections was reduced from 30 to 40 minutes down to less than 15 minutes. The optimized for PDO monomer CVI-GC method was used to analyze PDO polymer samples of differing residual monomer content values.

A PDO monomer standard solution was created. A stock was made with 7.9 mg of monomer in a volumetric flask brought to 10 mL volume with HFIP and diluted into two additional different concentrations (0.79 mg/mL, 0.079 mg/mL and 0.013 mg/mL) to allow generation of a three point pre-calibration curve. This curve was evaluated for linearity and used to determine if further edits to the method were required. Figure 29 is a plot of the PDO area counts by the optimized for PDO monomer CVI-GC method versus the known masses of PDO monomer injected.

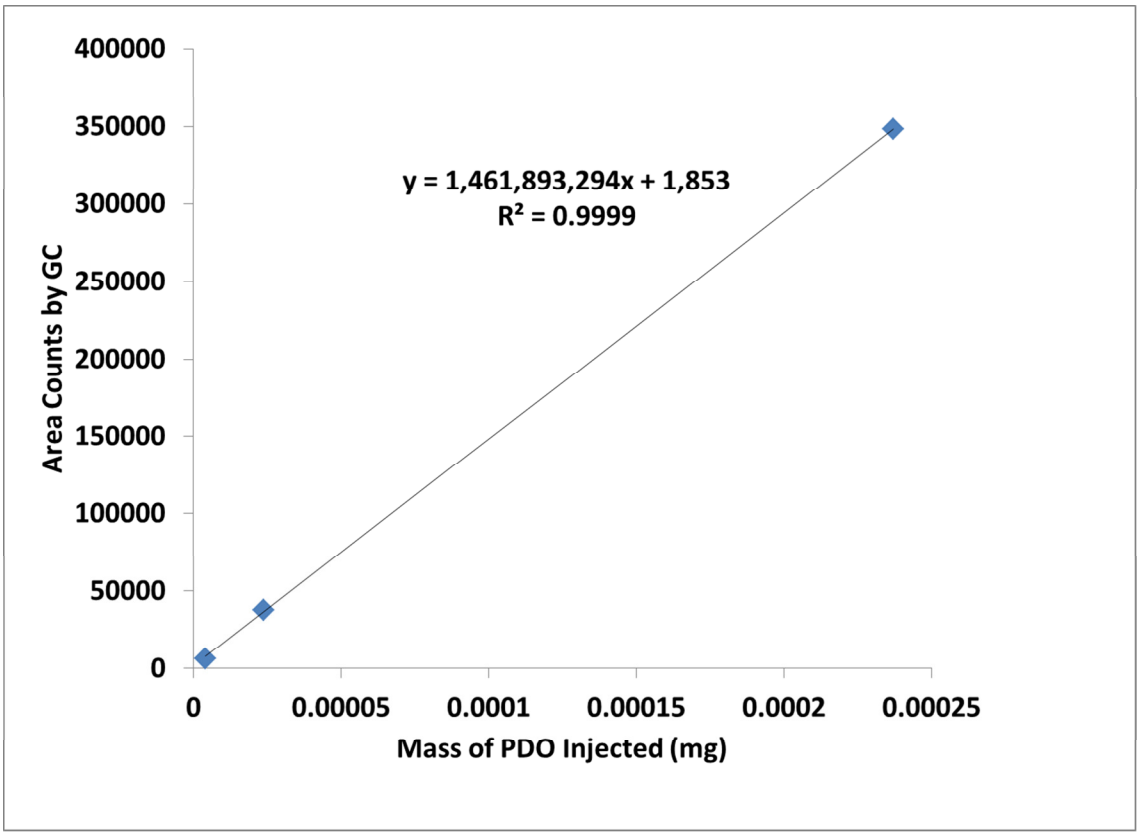


Figure 29: Plot of PDO Monomer Area Counts by CVI-GC versus the Known Mass of PDO Monomer Injected (mg): Pre-Calibration Curve

The graph displayed a linear response that extrapolated well to the origin. The mass injected was calculated using the known amounts of monomer added to the solution and assuming an ideal injection volume of 0.3 microliters. With confirmation of the testing method sufficiently determined to justify additional studies, the linear range, accuracy and repeatability of the method needed to be established. While this curve would not be used for quantifications, it demonstrated that performing an external calibration by injecting a range of concentrations, the PDO monomer response would be linear with respect to the masses determined through weight measurements, dilutions and calculations.

CHAPTER FIVE

VALIDATION OF THE TESTING METHOD

External Calibration

Previous external standard calibration curves only included minimal sampling to speed the process of method development. A confident test method based on the previously discussed criteria warranted a more extensive curve to test the range of the method. Known amounts of PDO monomer in hexafluoroisopropanol (HFIP) were used to create 10 different concentrations from 0.01 mg/mL to 0.8 mg/mL. With the test method's recommended polymer concentration, 0.150 grams PDO polymer in 10 mL (HFIP), the concentrations relate to 0.07% to 5.39% residual PDO monomer. It was recommended that higher polymer concentrations not be used with this method. Each concentration was analyzed with four chromatograms to determine a range of area counts for each amount of monomer injected.

Figure 30 is the dilution scheme and part labelling for the preparation of ten different concentrations of PDO monomer standards. A stock solution was created by adding a mass of 0.24506 grams of PDO monomer to a tared volumetric flask on the balance. The flask was filled to 25 mL and the mass of the total solution was also recorded. This solution was referred to as the "Stock" PDO monomer standard. Ten different volumes of the stock were added to tared 10 mL volumetric flasks. The masses of the different volumes were recorded. The ten flasks were topped off with HFIP and then each solution was analyzed four times.

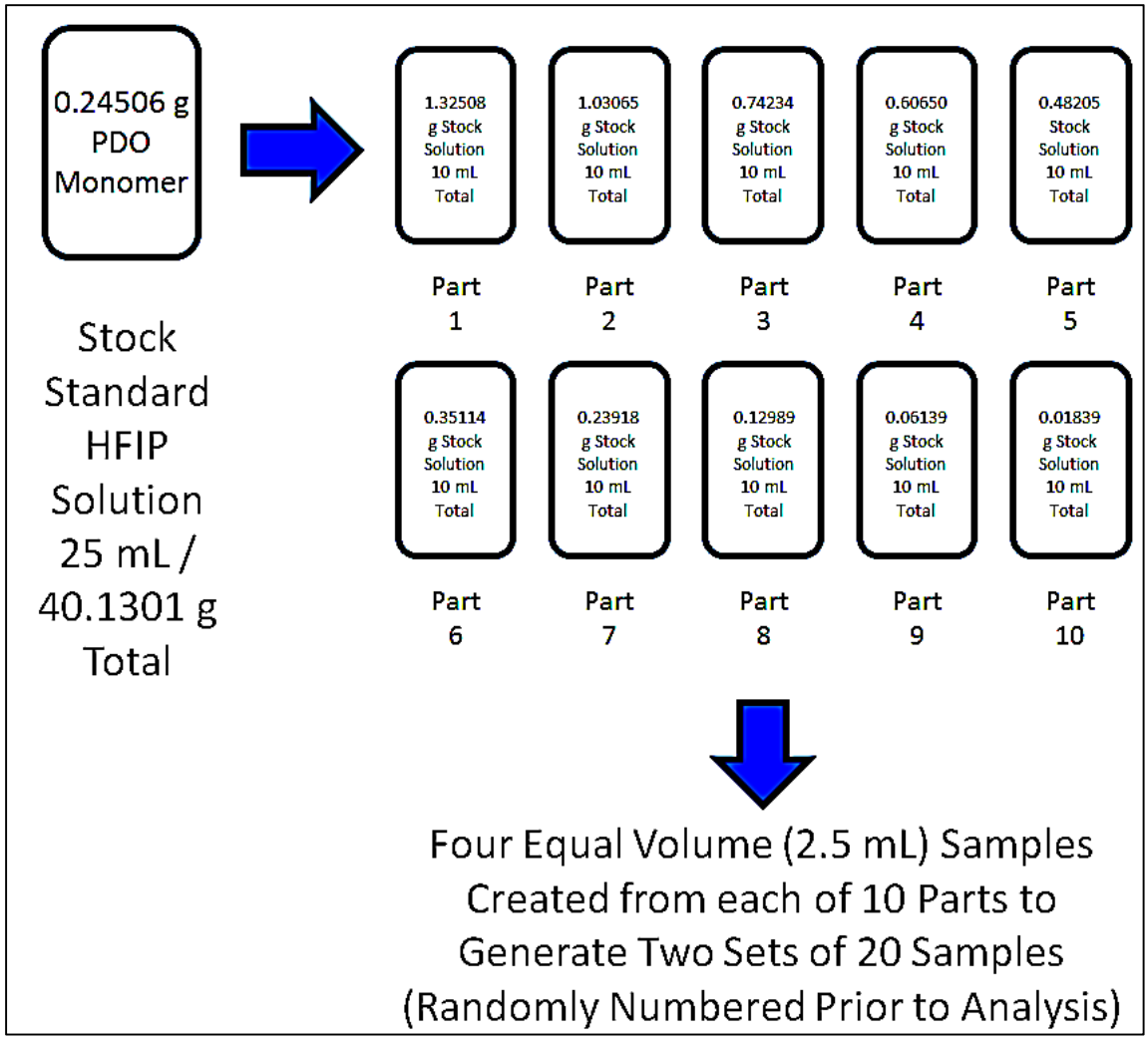


Figure 30: Illustration of Dilution Scheme for Creation of Validation Standard Parts and Replicate Solutions

The dilution in Figure 30 specifically describes the successful preparation of monomer standard samples used for both the external calibration curve and the Gage R&R validation. The resulting external calibration curve is used to relate area counts of a PDO monomer peak that is generated when an unknown concentration solution is injected via the CVI-GC method to the mass of PDO monomer in the injected solution.

Figure 31 is a plot of PDO monomer area counts versus the known masses of PDO monomer injected. The plot combines a total of 40 randomly ordered injections.

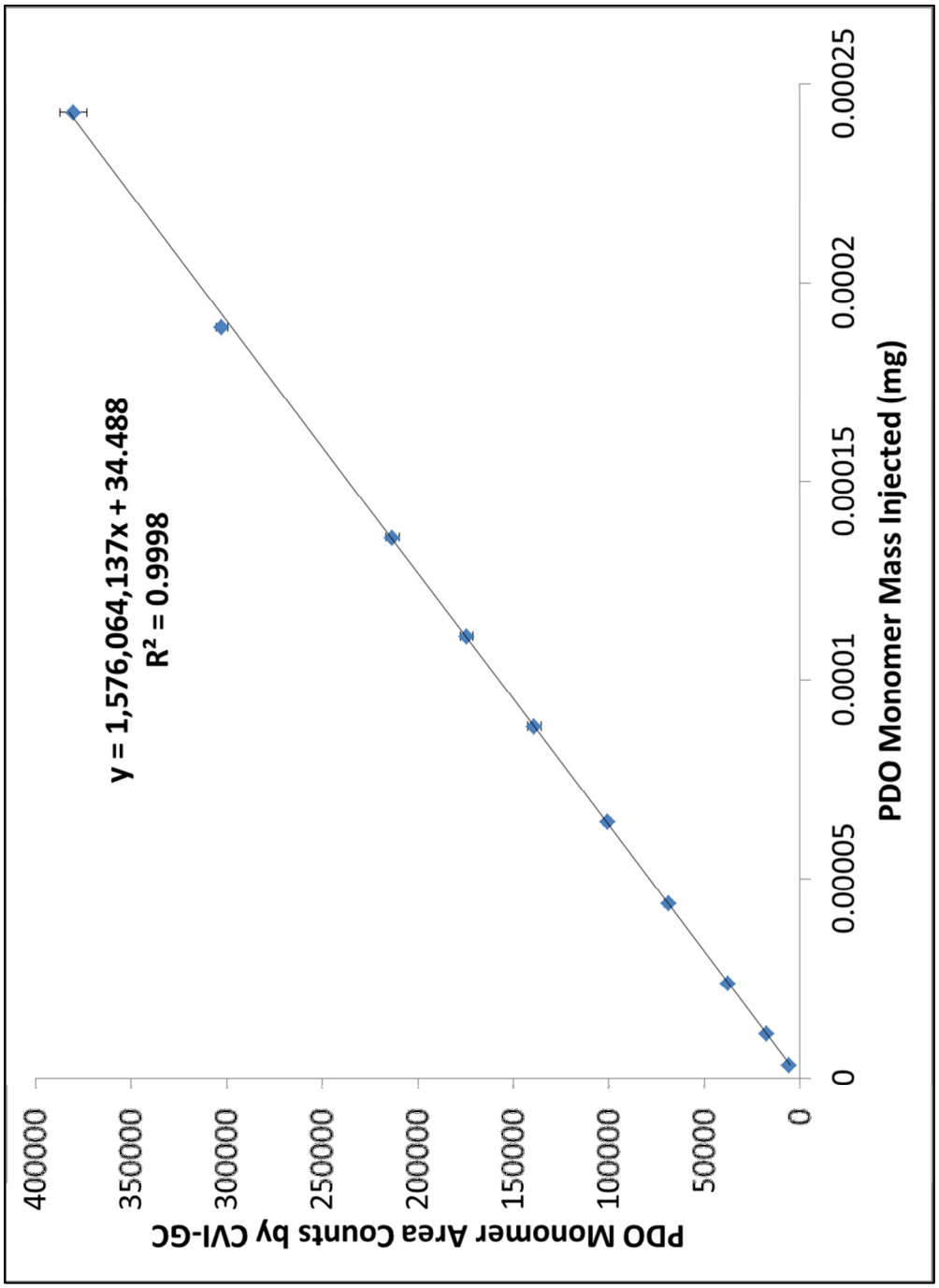


Figure 31: Plot of PDO Monomer Area Counts by CVI-GC versus the PDO Monomer Mass Injected (mg): For the Purpose of an External Calibration Curve (note – vertical bars represent one standard deviation based on n=4 injections)

The linearity of the standard curve confirmed the linear detector response over the relevant range of PDO monomer in polymer sample concentrations. The small standard deviation, as represented in the plot by the vertical bars above and below each point, associated with each concentration indicated the repeatability of the analysis. Repetition of this experiment generated similar curves. All slopes calculated were within 10% of the slope in Figure 31. Using the standard deviation of the four injections of the least concentrated of the solutions (σ), the slope of the calibration curve and the following equations for limit of detection (LOD) and limit of quantification (LOQ) the limits were calculated for the optimized for PDO monomer CVI-GC method.

$$LOD = \frac{3 * \sigma}{Slope}$$

$$LOQ = \frac{10 * \sigma}{Slope}$$

The LOD was calculated to be 0.36 ng of PDO monomer injected. Based on a nominal sample preparation with a PDO polymer mass of 150 mg in 10 mL of HFIP, the LOD is 0.0081%, or 81ppm, residual PDO monomer. The LOQ is 1.2 ng of PDO monomer injected. Based on a nominal sample preparation with a PDO polymer mass of 150 mg in 10 mL of HFIP, the LOQ is 0.027%, or 270 ppm, residual PDO monomer.

Standard Addition

Standard addition is a common technique to analyze unknown samples without the need for calibration or internal standards. It was used as a secondary quantification method to corroborate the results from the CVI method. This method involves preparing a series of samples with increasing known quantities of analyte added to the sample being analyzed. In this study different volumes of a single PDO monomer standard solution were added to the polymer. The amount of analyte added to each polymer solution was determined by triplicate injections of the PDO monomer standard solution.

Figure 32 is a plot of PDO monomer content determined by the optimized for PDO monomer CVI-GC method versus the PDO monomer content added to PDO polymer solutions. Linear regression analysis of the five standard addition samples provides an intercept that corresponds to the original amount of monomer in the polymer sample. Using the external calibration curve, three polymer solutions were analyzed by the CVI-GC method.

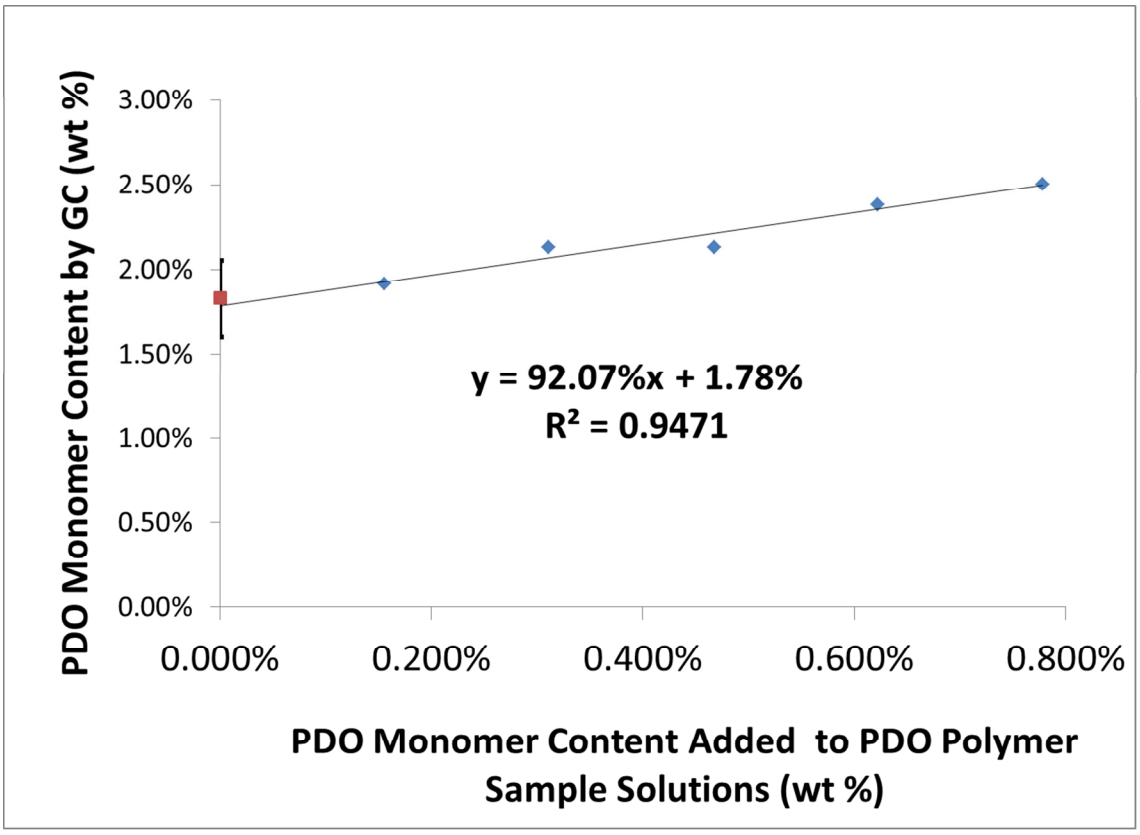


Figure 32: Plot of PDO Monomer Content Determined by GC versus the PDO Monomer Content Added to PDO Polymer Solutions: Accuracy Determination of CVI-GC Method by Standard Addition (Note – the bars above and below the square on the y-axis represent one standard deviation based on n=3 high monomer PDO polymer solutions)

The trendline associated with the five addition samples (blue diamonds) predicted a PDO monomer content in the high monomer PDO polymer of 1.78% (weight to weight). The CVI-GC method was used to determine a PDO monomer content in three PDO polymer solutions (red square) of 1.83% ±0.23% (weight to weight). The accuracy of the CVI-GC method is evidenced by the y-intercept of the standard addition curve intersecting well within one standard deviation of the measured mean for the polymer, thus demonstrating agreement between the two quantification methods.

Gage R&R Validation

Validation Gage R&R was performed with 2 operators and 10 monomer solutions (parts). Each solution was tested by each operator in duplicate for a total of 40 CVI-GC analyses. The ten parts were prepared as previously described in Figure 30. The samples were randomized and assigned to one of the two GC operators for analysis. Figure 33 is a column chart representation of the Gage R&R validation data. The data is presented as if the solutions were prepared with 150 mg of polymer and 10 mL of solvent. The Gage R&R was performed using standard monomer solutions to avoid the variability of the polymer.

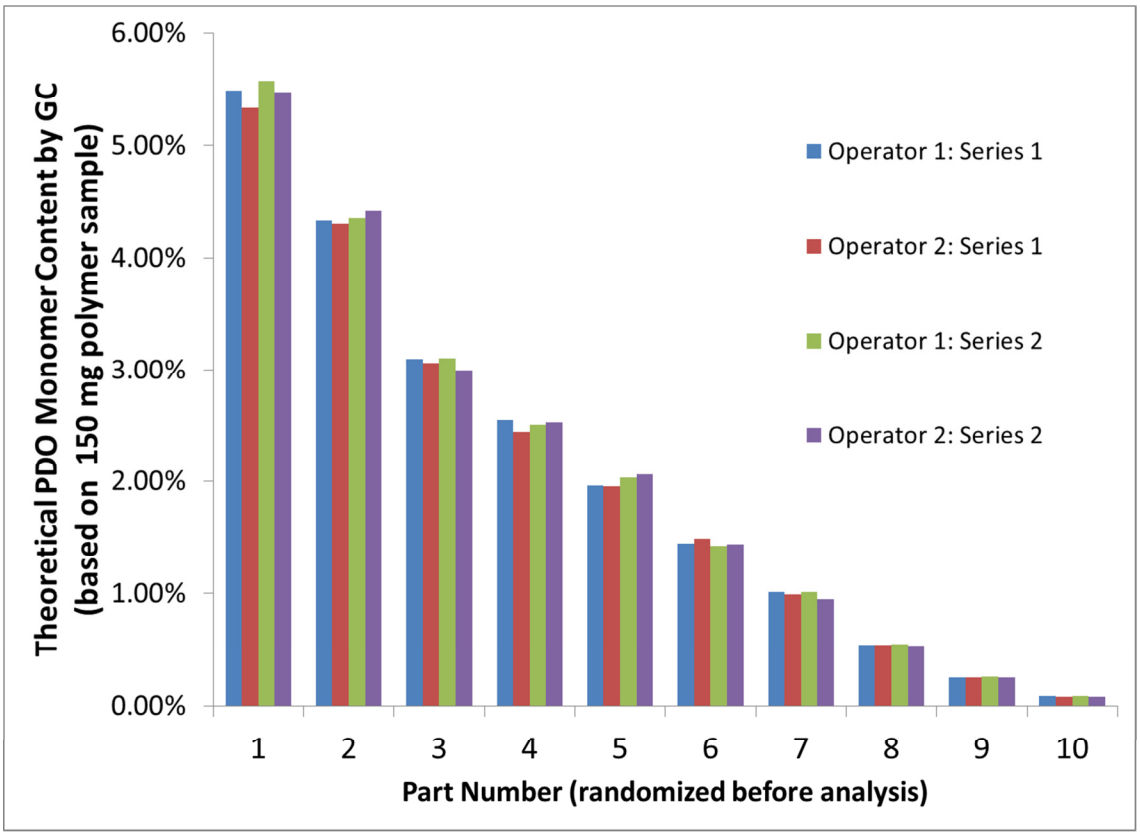


Figure 33: A Column Chart Representation of the Gage R&R Validation Data: Theoretical Residual PDO Monomer Concentration for Ten Monomer Standards as if Forty Individual Samples of Ten Different Parts, or Concentrations

The gas chromatography method for residual PDO monomer in PDO polymer was validated as extremely repeatable and reproducible. The analysis of the 40 samples did require replacement of one analysis that had an out of specification hexafluoroisopropanol (HFIP) peak and had to be rerun. This ensured an even better Gage R&R result.

The Gage R&R results provided by the Minitab software for the test method validation data are shown in Figure 34 and Table 7. The Gage R&R test analyzes the data to statistically determine the ability of the test to discern one sample from another with

97% confidence and calculates from this resolution how many different samples within the tested range could be resolved by the testing method.

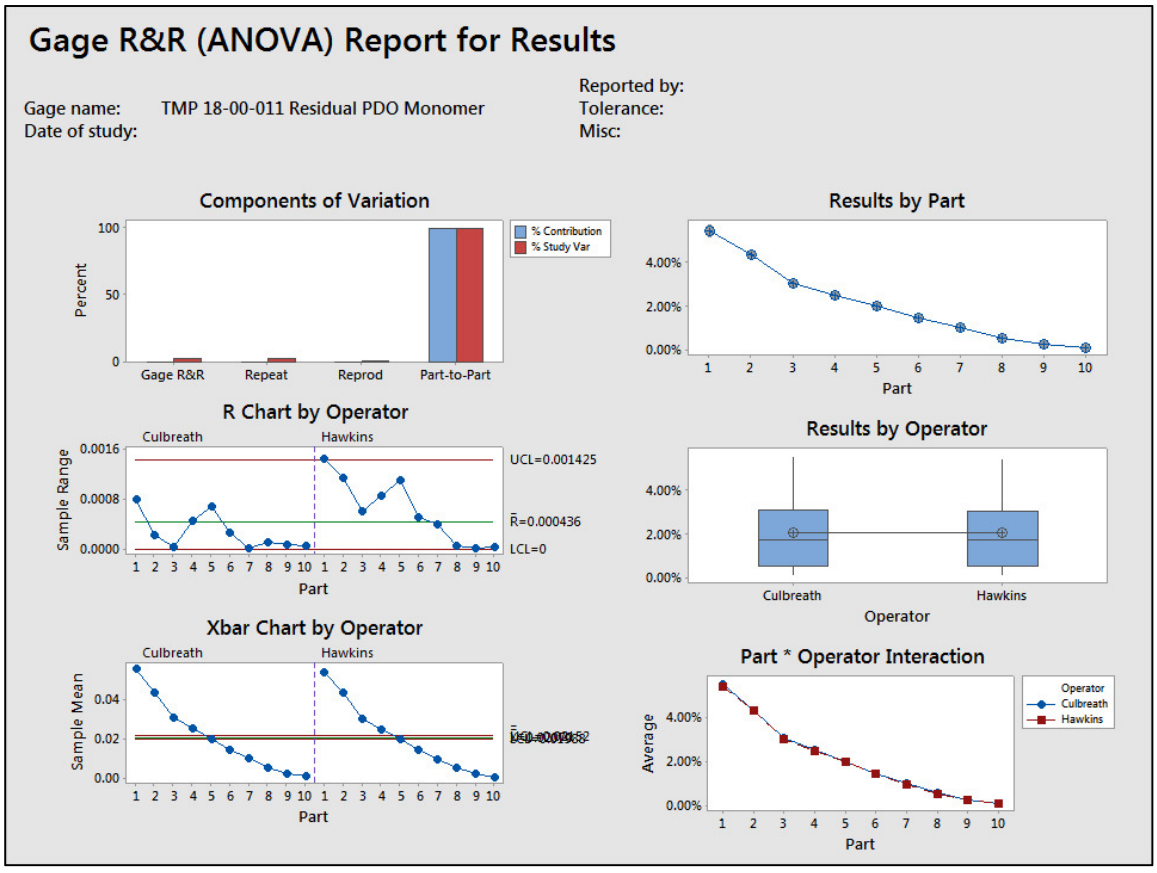


Figure 34: Summary of Gage R&R Analysis of Residual PDO Monomer by GC Test Method Validation Data

Table 7: Gage R&R Results for Residual PDO Monomer by GC Test Method Validation

Gage R&R Study - ANOVA Method

Gage R&R for Results

Gage name: TMP 18-00-011 Residual PDO Monomer
Date of study: 06/04/2015
Reported by: CJC
Tolerance:
Misc: PDO monomer in HFIP for TM 18-00-027

Gage R&R

Source	VarComp	%Contribution (of VarComp)
Total Gage R&R	0.0000002	0.07
Repeatability	0.0000002	0.06
Reproducibility	0.0000000	0.01
Operator	0.0000000	0.01
Part-To-Part	0.0003227	99.93
Total Variation	0.0003229	100.00

Source	StdDev (SD)	Study Var (6 × SD)	%Study Var (%SV)
Total Gage R&R	0.0004676	0.002805	2.60
Repeatability	0.0004449	0.002669	2.48
Reproducibility	0.0001438	0.000863	0.80
Operator	0.0001438	0.000863	0.80
Part-To-Part	0.0179626	0.107775	99.97
Total Variation	0.0179686	0.107812	100.00

Number of Distinct Categories = 54

The statistical conclusions of the Gage R&R were represented by the Total Gage R&R percentage and the Number of Distinct Categories reported in Table 7. A Total Gage R&R value of 2.6% indicated a very repeatable and reproducible testing method. With 54 distinct categories between 0.1% and 5.0%, the testing method was capable of statistically discerning two samples that differ by only 0.1%.

CHAPTER SIX

COMPARISON OF THE TESTING METHODS

Multiple headspace extraction (MHE) was noted to have a great deal of inconsistency when analyzing for residual monomer in bioresorbable polyesters. The extractions and purging should have reduced the volatiles content in the vial, but the peak areas often increased with each extraction, especially with larger weight samples. Classical Vaporization Injection (CVI) was found to be more accurate and extremely repeatable, for both monomer standards and polymer samples.

Table 8 is a summary of the weight percent of PDO monomer found in both the high and the low monomer PDO polymers analyzed with each GC injection method. The results for the high monomer PDO polymer are from a single lot of this polymer. However, the MHE-GC results for the low monomer PDO polymer are from a single lot of polymer and the CVI-GC results are an average of three material lots. One lot included in the three is the same material lot analyzed by MHE-GC. The old low monomer PDO polymer is the same lot of material used for MHE-GC but was made available for CVI-GC analysis approximately 24 months after the previous analyses were completed. It had been stored in the dark, at room temperature, under a nitrogen atmosphere in an effort to minimize polymer degradation.

Table 8: Poly(1,4-dioxane-2-one) Residual Monomer Analysis by Gas Chromatography

GC Method	MHE	CVI
PDO Polymer (high monomer)	5.86% ±8.55%	1.83% ±0.23%
PDO Polymer (low monomer)	0.49% ±0.45%	0.25% ±0.09%
PDO Polymer (old low monomer)	-	1.30% ±0.03%

The CVI method was found to be much more precise than the MHE method. The average monomer content found using MHE method is at least two times greater than the amount found using CVI method. Both of these results may be due to the thermal stability of the PDO monomer and/or PDO polymer. The increase in PDO monomer content in the low monomer PDO polymer from 0.25% to 1.30% in two years suggests that even at room temperature, the PDO polymer is not stable.

To further compare MHE and CVI, the methods were applied to another bioresorbable polymer and its monomer, polycaprolactone (PCL) and caprolactone. MHE-GC for PCL was performed using a thermostat temperature of 60°C and six extractions versus 100°C and four extractions. Caprolactone has a relatively large response factor and is removed from the bulk polymer quickly when compared to PDO monomer. A progressive thermostat time of 5 minutes was used versus the general method of 10 minutes.

Figure 35 is a plot of caprolactone area counts by HS-GC versus progressively increasing thermostat time for 0.5 gram samples of PCL.

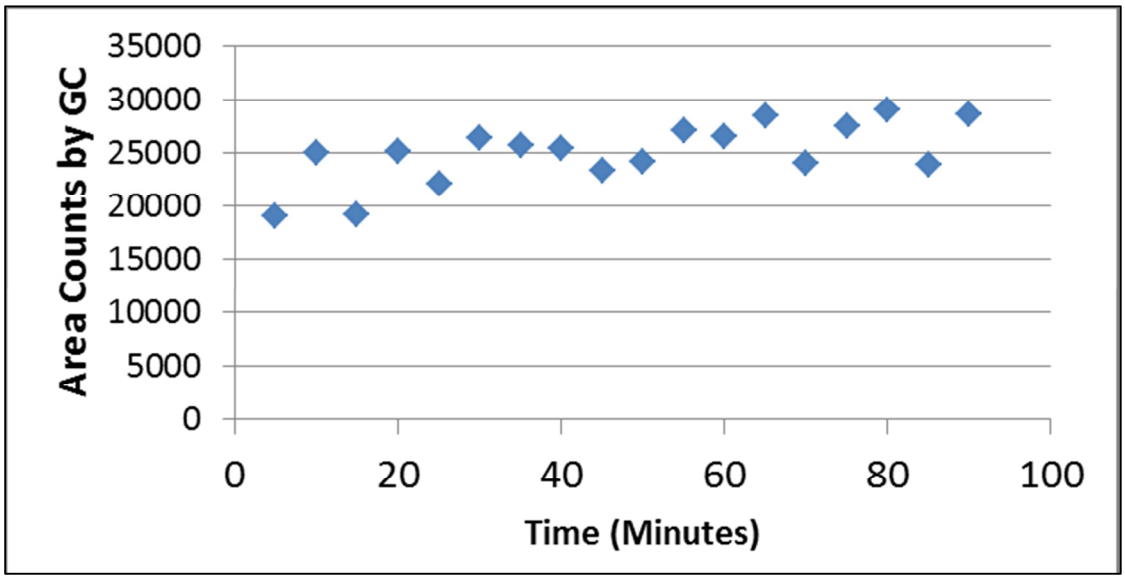


Figure 35: Plot of Caprolactone Area Counts versus Progressively Increasing Thermostat Time using 0.5 grams of PCL per Sample

PCL appears much more stable than PDO polymer, but the curve does not confirm equilibrium as expected or described in the literature. Variation beyond thermostat times of 30 minutes was approximately 24%. The acceptability of this variation would depend on the rate of extraction observed during MHE-GC. A calibration curve based on MHE analyses of caprolactone monomer was needed. Caprolactone monomer was aliquoted into headspace (HS) vials in solution via the general MHE monomer standard preparation method.

Figure 36 is a plot of the total area counts calculated by MHE versus the masses of the known caprolactone standards.

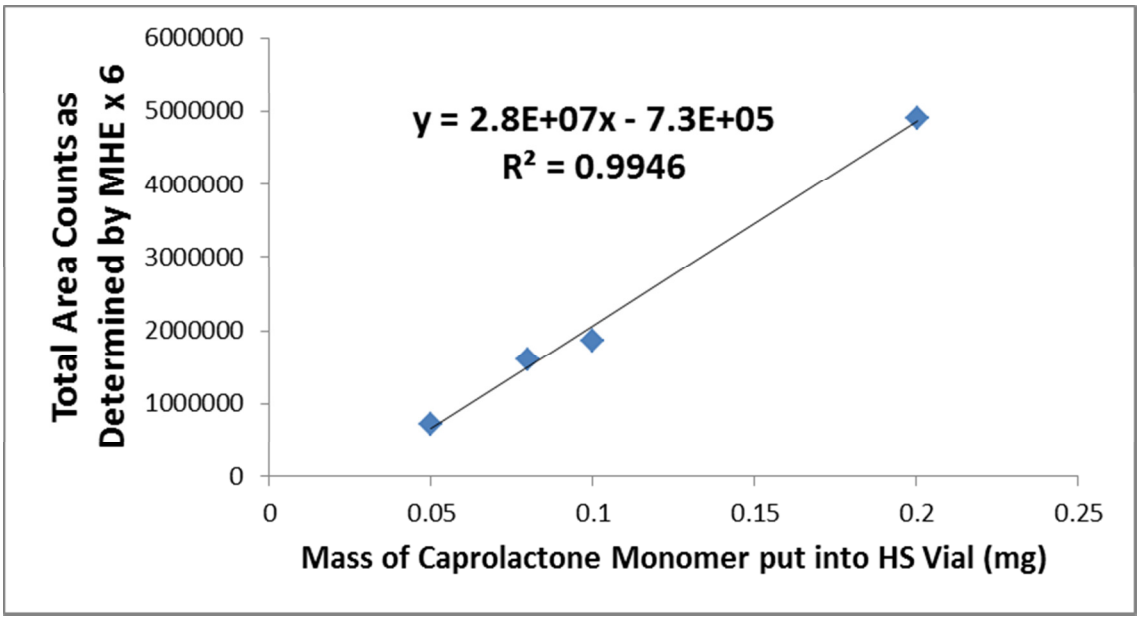


Figure 36: Plot of the Total Caprolactone Area Counts Calculated by MHE versus the Masses of the Known Caprolactone Standards: Caprolactone MHE-GC Calibration Curve

The calibration curve was utilized to calculate residual monomer content of PCL samples without further standards created or replicates analyzed despite the linearity and y-intercept.

Analysis using the MHE-GC general method amended for six extractions and a 60°C thermostat was completed for six polymer samples and four monomer standards. One of the polymer samples had water added to the vial by autopipette just before crimping.

Figure 37 is a representative plot of caprolactone area counts by MHE-GC versus number of extractions for a 25 mg PCL sample and a 0.08 mg monomer standard overlaid.

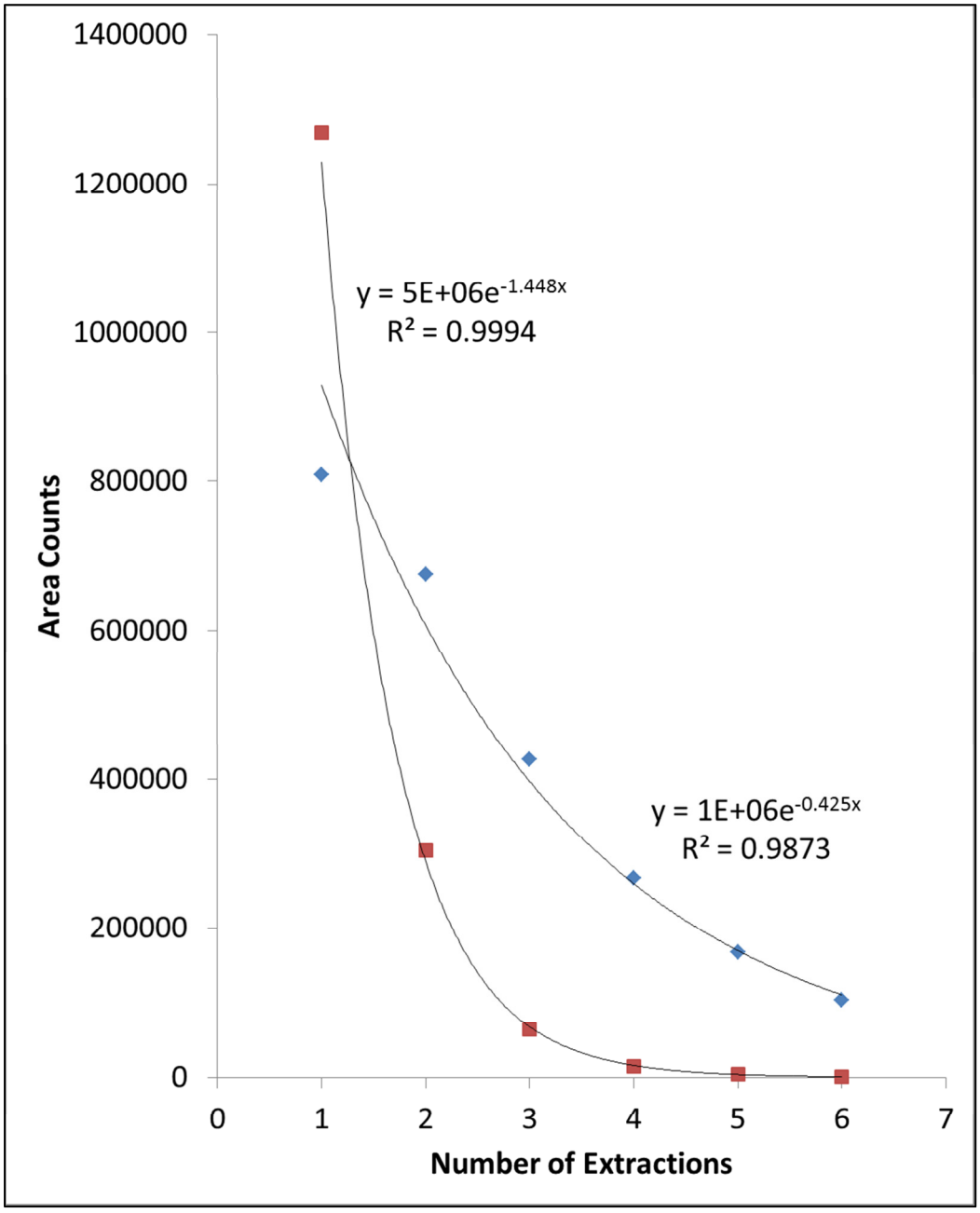


Figure 37: MHE Curves for PCL and Caprolactone Monomer
(Monomer Standard = Red Squares, PCL Sample = Blue Diamonds)

Representative MHE curves for both PCL and caprolactone monomer samples were observed. The overlay allows the rate of extraction of a monomer standard to be compared to the rate of extraction from monomer in a polymer matrix. The rate of

monomer extraction from the polymer sample HS vial was less than that of the standard. This could be indicative of the matrix effects. The MHE curves for the caprolactone monomer standards were analogous to those generated with PDO monomer, but the negative y-intercept was not as pronounced when the standard curve was generated and no material remained in the HS vials after analysis of the monomer standards.

Several different sample sizes of PCL were analyzed to investigate if the observations for PPDO would be repeated. The first extraction data point in Figure 37 was not as aligned with the remaining extractions as before with PPDO, but with the first data point removed, the calculated monomer content was shifted 0.01% , less than 2% of the reported values. With fewer extractions (MHE x 4, etc.), and thus less data, this deviation observed in the first extraction measurement did have a greater effect. MHE analysis of PCL was also performed on PCL with water added via autopipette just before the HS vial was crimped. The goal of this experiment was to investigate the sensitivity of MHE-GC to moisture contamination during sample preparation and if sample drying was a critical step in sample preparation when performing MHE tests on bioresorbable polymers.

CVI analysis of PCL began with generation of a basic caprolactone calibration curve. Injections of the monomer standards, as well as polymer samples, dissolved in HFIP were performed using the general CVI-GC method. Figure 38 is a plot of the caprolactone area counts versus the known masses of caprolactone injected.

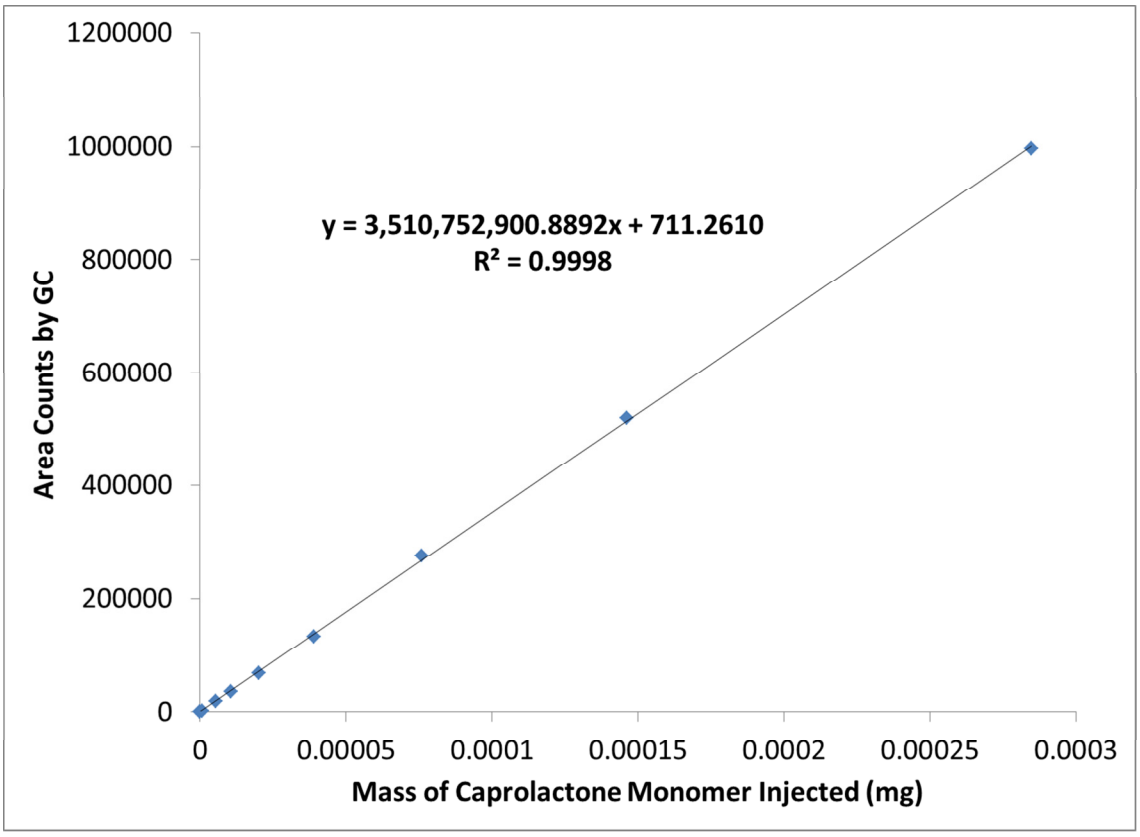


Figure 38: Plot of Caprolactone Area Counts by CVI-GC versus Known Masses of Caprolactone Injected (mg)

The calibration curve linearity and nearly zero y-intercept indicated that the CVI-GC method was promising in addition to the MHE-GC analysis of PCL.

Each result for residual caprolactone monomer in PCL was compiled and tabulated for comparison of the GC methods. Table 9 is the residual monomer results for PCL by both MHE and CVI GC methods. The MHE data also includes a sample that had water added just prior to crimping the cap onto the HS vial. The MHE average and standard deviation represent six samples of mass varying from 0.025 grams to 1.0 gram. The CVI average and deviation relate to three solutions ranging from 5 to 15 mg / mL concentrations.

Table 9: PCL Residual Monomer by GC Results

GC Method	MHE	CVI
PCL	0.56 ± 0.02	0.28 ± 0.02
PCL (326.3mg +1uL H2O)	0.78 (n=1)	-

The CVI-GC method determined a lower monomer content value for PCL just as it did for PDO polymers. The effect of moisture on the MHE-GC results was dramatic and suggests that moisture contamination could be a major contributor to the variability observed via MHE-GC determinations of residual monomer content in bioresorbable polyester materials.

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Two distinct sampling methods for gas chromatography were investigated and compared. Observations and research related to 1,4-dioxane-2-one (PDO) monomer and polymers were compiled. A combination of the previously described study and the review of the literature have led to the following conclusions.

Multiple headspace extraction (MHE) gas chromatography (GC) is not suitable for accurate quantification of residual monomer in equilibrium polymers that retain active end groups on the polymer chains. This is due to the reversible nature of the polymerization reaction at the temperatures, pressures and / or times required for extraction of monomer from the polymer in a headspace (HS) vial as they relate to the equilibrium reaction. Modification of the end groups on the polymer chains could prevent de-polymerization and make MHE a much more suitable technique for this type of analysis, but the method would still be subject to moisture sensitivity and the instability of the monomer during analysis and calibration.

Classical vaporization injection (CVI) GC is capable of extremely accurate, repeatable and reproducible quantification of PDO monomer. This conclusion applies to hexafluoroisopropanol (HFIP) solutions that can contain sufficient amounts of dissolved PDO polymeric materials for resolution and sensitivity to at least 0.1% by weight residual PDO monomer. It is hypothesized that this conclusion would apply to analysis of other equilibrium polymers that retain active end groups on the polymer chains. The

use of CVI methodology does incur certain repercussions related to the GC instrument and its components. HS methods are recommended to avoid the complications associated with injection of nonvolatile components such as the polymer. The use of a guard column may offset some of the costs associated with these complications, but a frequent need for injector liner replacement, injector cleaning, guard column trimming, column replacement, and /or the use of additional materials would not be avoided. Additional materials could include the use of a selective solvent to precipitate the polymer from solution prior to injection of the sample. This method of sample preparation would be subject to the tendency of a portion of the monomer to also precipitate and thus negatively affect analytical results.

Gage R&R analysis is a valuable statistical technique to compare and combine multiple operators', a method's and an instrument's abilities to successfully perform an analytical quantification method into a single reference value that can be applied to an applicable range of results required for quality control and / or process optimization monitoring. The results of the Gage R&R study are only relevant to the range of parts included in the study though. Gage R&R is only a measure of precision. Separate techniques must be employed to determine the accuracy of an analytical method.

The PDO monomer and its associated PDO polymer are relatively unstable, especially when polymer chains have active end groups that allow de-polymerization. This instability could lead to generation of nontrivial levels of PDO monomer during the processing phases and shelf life of PDO polymer. Due to the PDO polymer being capable of generating monomer at room temperature conditions, the residual PDO

monomer in PDO polymer test method developed in this study can only determine the current PDO monomer content in the polymer. The residual monomer content does affect processing, degradation rate and toxicity of the polymeric material, thus it is concluded that PDO polymeric materials should be analyzed for monomer content several times between polymerization and surgical utilization.

As a result of this work, a reliable monomer content quantification method made feasible the optimization of key PDO polymer processes in an industrial setting. This process optimization was critical to the further development of PDO polymer medical devices that will continue to replace current devices due to the PDO polymeric material's superior mechanical and bioresorption properties. This development of PDO based devices will directly translate to positive benefits for patient health. Monomer removal from the polymer was optimized so minimal time and resources were consumed during processing of the polymer. Optimized processes reduce costs and increase yields of superior products. These medical and industrial successes would not possible without the methodology developed during this work as well as the precision of the method.

Recommendations

The information and observations gathered during the course of this study have provided insight into the abilities and limitations of gas chromatography (GC), 1,4-dioxan-2-one (PDO) based polymers, and the general effects of impurities in a polymeric material. Even very small amounts of certain impurities can have drastic effects and thus the residual contents need to be quantified. The quantification and investigation of the impurities' effects should also be considered critical information for a polymeric material, especially when utilized for medical device implants.

The GC analytical method developed in this study and the confidence associated with it would greatly benefit from comparison of third party generated monomer content results to the classical vaporization injection (CVI) gas chromatography (GC) method. The accuracy, precision and detection ability of the additional method should be included in the comparison of the methodologies. By confirming the results with a third party, the CVI GC method could be considered a standard method for broad application and further recommendations. This third party analysis could be a separately developed GC method or another analytical technique sufficient to identify and quantify residual levels of PDO monomer.

Additional investigation related to the GC analytical method developed in this study should include verification of the chromatography. Retention time alone should not be considered sufficient for definitive identification of an analyte in any chromatographic methodology. The addition of mass spectrometry detection is a commonly applied technique to accomplish the goal of determining if coelution of peaks is confounding the

results of the analysis, but this would require a reference spectrum of PDO monomer for a conclusion. Performing the analysis with different column chemistry and a mass spectrometer could provide significant confidence. Coelution of materials for both stationary phases would be extremely unlikely.

The Gage R&R results provide excellent confidence in the range of 0.1% to 5.0% residual monomer in PDO polymer, such that an average resolution of 0.1% differentiation between parts was shown. If a smaller range of analysis is found to be needed, a new Gage R&R study should be performed to examine this range. It is hypothesized that the method would continue to provide exceptional precision, especially if the desired range would be the 0.1% to 0.5% residual PDO monomer contents. Visual inspection of the standard calibration curve does indicate relatively more variation and deviation from linearity in the data relating to higher masses of monomer injected. This would suggest that the method would be capable of very fine monomer content resolution when comparing low monomer content PDO polymeric materials.

Quantification of residual solvent content using multiple headspace extraction (MHE) GC should be investigated. Methods utilizing MHE GC to quantify residual solvent content would reap the benefits that led this study to investigate MHE without experiencing the limitations found. Some polymer materials could not generate solvent molecules of analytical interest via de-polymerization, thus the accuracy and precision of MHE GC would not be affected. Additionally, Gage R&R studies of the residual solvent method could offer valuable information about the applicability of the method.

Development of methodologies for non-volatile residuals such as “free-acid” content that can be applied to monomer and polymeric materials should be investigated. The linear forms of the monomers and oligomers associated with bioresorbable polyester materials have a carboxylic acid end group and an alcohol end group. These molecules may have similar effects on polymer properties as the ring form of the monomers but are not often volatile enough to be quantified by the current GC method. Sample preparation reactions can allow GC testing of carboxylic acids via esterification, but other methodologies such as titration should also be investigated.

Glass transition temperature and viscosity shifts due to residual impurities content should be investigated. While somewhat relevant to the end use patient, the bulk material property effects due to residual level impurities are of much greater importance to device manufacturing and in-process procedures. With the types of impurities identified and quantified, the effects on the glass transition of the material as well as the viscosity when melted should be investigated thoroughly to ensure proper handling of the material. This information in conjunction with in process determination of residual contents would allow determination of process parameters prior to processing versus reactive adaptations of the process that negatively affect productivity. These investigations would involve extensive rheological analysis of a wide array of residual contents in a material.

The rate at which PDO monomer, and other residual impurities, leaches into various relevant biological media from PDO polymeric materials should be investigated. The developed quantification methods would be integral to this study. Included in the study of PDO monomer exposure into biological environments should be the

toxicological effects of the PDO monomer and other residual impurities in those environments. These rates and toxic effects, once quantified, should be applied with sufficient safety factors to generate acceptable levels of residual content for each impurity and certain combinations if warranted depending on the results of the toxicology studies.

The tendency of the PDO polymer to generate PDO monomer under ambient room conditions despite typical moisture contamination mitigation techniques suggests that medical devices that contain PDO polymer should be regularly inspected throughout shelf life for residual PDO monomer content. The kinetics of this polymerization / de-polymerization reaction in relation to shelf life of PDO based medical devices should be investigated. Generation of PDO monomer in the device could lead to negative consequences for the patient's health. Investigation of current PDO based devices of different ages or storage conditions could also offer information regarding the use of end capped PDO polymers in the current market. A PDO polymeric material that displays consistent residual monomer content despite age or storage temperature would indicate that the effect of the equilibrium reaction on the polymer was alleviated in some way. Exposure to sufficient amounts of moisture would be expected to activate the ability to generate monomer from the new end groups created via hydrolysis of ester group linkages.

A combination of the observations in the above studies could be used to generate a comprehensive PDO polymer material standard that provides recommendations and regulations for medical devices that utilize PDO based materials. This standard was not

found to currently exist, but with the rising interest in PDO based systems for medical device development, this material standard is needed.

Validation of purity by GC methods for monomers and solvents should be performed. The methodologies for determination of monomer and solvent purity are currently standardized via ASTM [47]. Application of these standard methods should include Gage R&R studies to conclude the effective precision of these methods to allow for industrial implementation. Implementation of purity methods would allow inspection of process raw materials prior to use and potentially avoid costly production of unacceptably impure polymer.

It is recommended to apply the residual monomer content method to other polymers / monomers. The polyester family of bioresorbable polymers and copolymers should be well suited for adaptation of the CVI GC method. Modification of the general GC method may be necessary to optimize separation of multiple monomer types present in copolymer materials. Slower ramp rates of the GC oven should facilitate sufficient separation power. While slower ramp rates would increase the time required for each test, the several hours required for headspace (HS) methodologies would not be approached. More polar column technologies, such as polyether based stationary phases, could also provide the separation power required for copolymer analysis.

Continued use of the CVI GC method should also continue implementation of the solvent peak area check in the residual monomer methods. This would ensure less than ideal injections are discarded and repeated. The ability to discern proper application of

the GC method could continue to prove very valuable when precise results are required for costly decisions.

Lastly, use of HS / pyrolysis methodologies for investigation of residual content relationships to degradation of bioresorbables could be investigated. The temperatures required for HS detection of PDO monomer was hypothesized to generate monomer from the polymeric material, indicating polymer degradation. Careful consideration for the effect of temperature on the equilibrium reaction could allow for automated accelerated aging tests of polyester based bioresorbable materials in a HS vial. Currently the copolymers created as part of engineering a specific degradation rate are analyzed *in vitro* for 2 to 50 weeks to predict the rate of degradation *in vivo*. An accelerated GC method could possibly reduce this material screening time to hours. A much quicker degradation analytical method could allow more rigorous quality control of bioresorbable devices and materials produced.

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