MIGRATION STUDIES AND CHEMICAL CHARACTERIZATION OF SHORT CHAIN CYCLIC POLYESTER OLIGOMERS FROM FOOD PACKAGING LAMINATE ADHESIVES

By

ADITI SHRIKHANDE

A thesis submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of Master of Science

Graduate Program in Food Science

written under the direction of

Dr. Thomas G. Hartman

and approved by

_____

_____

_____

New Brunswick, New Jersey

May, 2012

Abstract of the Thesis

MIGRATION STUDIES AND CHEMICAL CHARACTERIZATION OF SHORT CHAIN CYCLIC POLYESTER OLIGOMERS FROM FOOD PACKAGING LAMINATE ADHESIVES

BY ADITI SHRIKHANDE

THESIS ADVISOR:

DR. THOMAS G. HARTMAN

Laminates are extensively used for food packaging applications such as retort pouches and retort packaging, boil in the bag, microwavable packaging, military meals ready to eat (MRE's), single serving dispensers, etc. Laminates are manufactured by bonding multiple layers of films together using adhesives, where each layer acts as a functional component and contributes to overall integrity of the package. Polyurethane adhesive, the most common choice of adhesive for flexible packaging, is the reaction product of polyurethane pre-polymer and/or diisocyanate with polyester. The polyester component reacts with isocyanate, forming urethane bonds and introduces soft chain segments into the final, cured polyurethane. During the formation of polyester, low molecular weight cyclic diesters and oligoesters are formed as unwanted byproducts. These low molecular weight species often migrate out of packaging into the contents of the package. Since these species are novel compounds, the safety and toxicological properties have not been investigated. Our research focused on studying the chemistry and migratory properties of these compounds. We conducted migration testing of laminates using USFDA recommended food simulants such as 10% Ethanol for aqueous and acid foods and 95% Ethanol for the fatty foods. Single side extraction cell assembly was used for the purpose of extraction which was conditioned at 100 ℃ for 30 min. In our research

GC-MS analysis was used to determine chemical structures, gas chromatography retention time indices and the average migratory concentration levels of ten short chain cyclic diesters and oligoesters. The chemical structures were deduced by analyzing the characteristic fragmentation pattern. Also to investigate the predicted metabolic fate of short chain cyclic diesters and oligoesters after their ingestion and potential absorption into the bloodstream, they were treated with non-specific porcine esterase enzyme at 37 ℃ for 1 h. In our research it was also shown that the enzyme treatment metabolized the short chain cyclic diesters and oligoesters back into their original corresponding diol and dicarboxylic acid precursors.

Acknowledgements

I would like to extend my sincere gratitude to my advisor and committee chair, Dr. Thomas G. Hartman for his invaluable guidance and direction, constant encouragement and mentoring on this research.

Also, I would like to thank the professors on my Master's Thesis Committee, Dr. Mukund V. Karwe and Dr. Chi-Tang Ho, for their insightful reviews and guidance.

My appreciation is extended to the students and staff of the Mass spectrometry laboratory at the Center for Advanced Food Technology for their assistance throughout my research.

I would specially like to thank Dr. Mukund V. Karwe for mentoring me in every possible way. I am extremely grateful to him for his constant support, guidance and encouragement throughout the time I spent at Rutgers.

I would like to express my sincere thanks to my wonderful parents, my fiancé and extended family, for their constant encouragement and endless love throughout this work.

Table of Contents

List of Figures

List of Tables

I. Introduction

Laminates are widely used in food packaging applications such as retort pouches and retort packaging, boil in the bag, military meals ready to eat (MRE's) and single serving dispensers etc. A laminate structure is used when individual thermoplastic material is unable to provide the functional needs that the package demands. Such laminates, made by bonding together at least two or more thermoplastic layers, can achieve the combination of desired functional traits, resulting in a package with desired functional, barrier and performance properties (Smith, 2004).

Laminate structures are prepared by bonding multiple plies of film together. Different layers of laminate structures include, but are not limited to, exterior layer, tie layer, barrier layer and sealant layer, which are shown in **Fig. 1**. The outermost exterior layer provides a dimensionally and structurally stable surface for printing. It protects the ink and the barrier layer and contributes to the burst strength as well as the tear-resistance of the package. The tie layer is the layer of the material which bonds two dissimilar material surfaces. It provides additional layer of protection to the barrier layer and bonds the outer layer to the barrier layer. The barrier layer, as the name suggests, prevents the moisture, light and oxygen transmission. Also it provides chemical resistance to the package. Finally, the innermost layer is the sealant layer, which makes the laminate structure heat sealable. It protects the barrier layer and provides the burst strength to the package (Packaging Solutions: Glenory, Inc.).

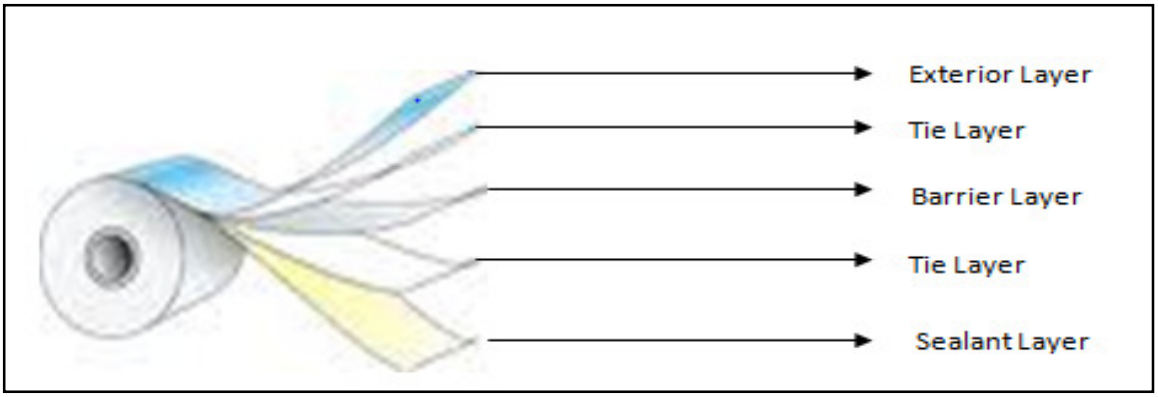


Figure 1: Different Layers of Laminate Structure (Packaging Solutions: Glenory, Inc.)

The different methods of constructing laminates include extrusion, co-extrusion and application of adhesives to bond multiple plies of film together. However, the focus of this research was to study the migratory properties of the compounds arising from the use of adhesives and hence other methods have not been discussed. The different layers of laminates are bonded together by applying a thin layer of adhesive between the two surfaces of the layers. **Figure 2** shows the cross section of the laminate from a Scanning Electron Microscope. The thin line indicated by the arrows represents the layer of adhesive binding two films.

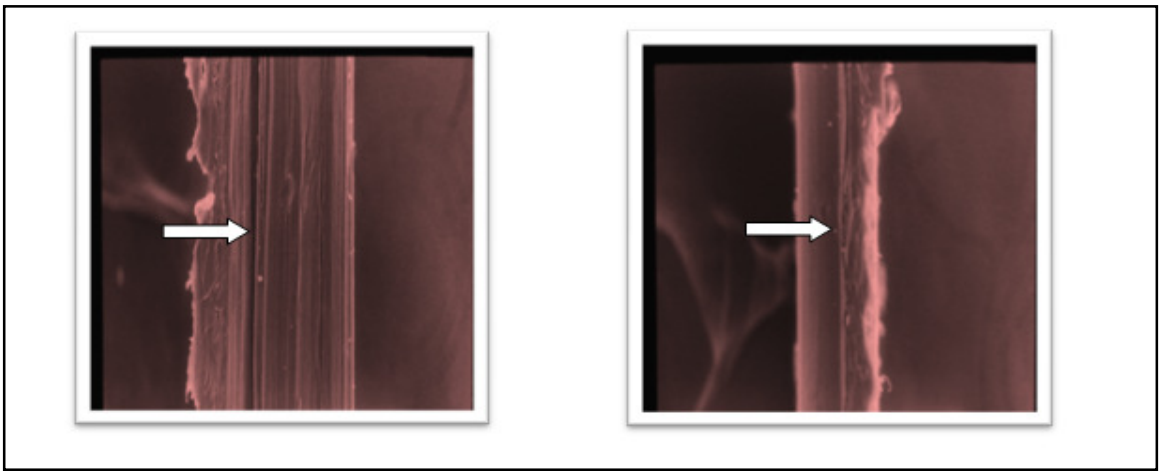


Figure 2: Laminate Cross Section from Scanning Electron Microscope (Abbott Nutrition, 2010)

Various adhesive systems have been used to design multilayer laminates such as solvent based, water based and two component adhesive systems (Bushendorf, 2008). Amongst this variety, Polyurethane, which is a two component adhesive system, is popularly used in the flexible food packaging industry as it offers excellent chemical resistance, abrasion resistance and flexibility to the package. Polyurethane-type adhesives are reaction products of polyurethane pre-polymers and/or diisocyanate with polyesters. The polyester component reacts with the isocyanate to form urethane bonds and introduces soft chain segments into the final cured polyurethane. These soft chain segments modulate the rheological properties of polyurethane. Polyesters are manufactured by esterification reaction of polyhydric alcohols (polyols) with dibasic aliphatic or aromatic acids. Unwanted byproducts associated with polyester synthesis are the low molecular weight short chain, cyclic diesters and oligoesters (Ionescu, 2005). The hydroxyl groups of low molecular weight cyclic diesters and oligoesters are blocked and not available for the reaction with isocyanate to build higher molecular weight polyurethane, as a result they remain behind as impurities. These low molecular weight species often migrate out of the packaging into the contents. The chemistry and the migratory properties of these compounds have not been extensively studied and was the focus of our research.

We have conducted migration testing of the laminate packaging and used mass spectrometry analysis to determine the chemical structures, gas chromatography, retention time indices and migratory concentrations of short chain cyclic polyester oligomers. Since these species are novel compounds, the safety and toxicological properties have not been investigated. However, the toxicological properties of monomers used for polyester synthesis have been extensively studied. We predict that if

these short chain cyclic diesters and oligoesters are absorbed into the bloodstream via dietary intake, they will be metabolized in vivo by non-specific esterase enzymes, which are ubiquitous in blood and tissues. In our research we have shown that treatment with porcine non-specific esterase enzyme metabolizes the short chain cyclic diesters back into their original corresponding diol and dicarboxylic acid precursors. We are hopeful that this in vitro metabolic fate data will assist toxicologists and regulatory scientists to assess the safety of this class of packaging-borne migrants.

II. Background

II.A. General Overview of Adhesive Systems

II.A.1. Introduction to Adhesives

An adhesive is any substance capable of holding at least two surfaces together in a strong and permanent manner. Adhesives generally have high sheer and tensile strengths. Intermolecular forces between two different substances responsible for adhesion are shown in **Fig. 3**. The area between an adhesive and an adherend is called "interphase region" which has different physical and chemical properties than the bulk from an adhesive or an adherend. The nature of the interphase region governs the properties and the quality of the adhesion bonds.

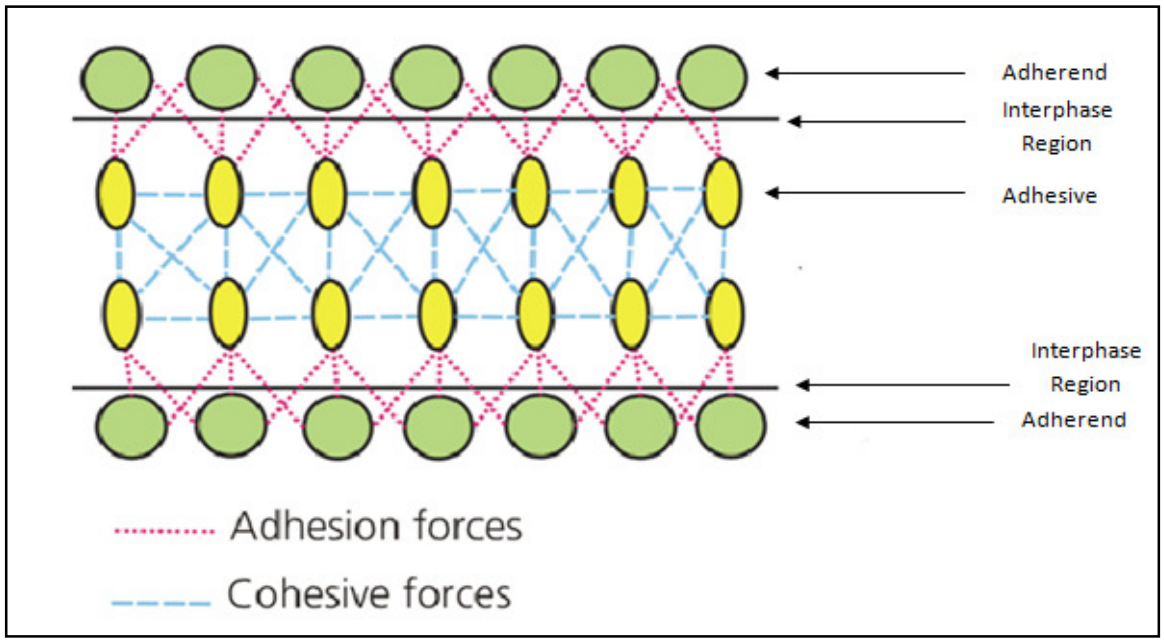


Figure 3: Adhesion and Cohesion Forces (The Adhesive and Sealant Council, Inc. 2010)

II.A.2. Classification of Adhesives

There are numerous adhesive formulations engineered for different applications. Thus, adhesives can be broadly classified by many ways and there can be hierarchical stages to the individual classification system. Adhesives could be classified as natural or synthetic adhesives based on materials from which they are constructed. Some adhesives are made of organic polymers while some find their origin in inorganic polymeric systems. Another way of classification is based on whether the adhesive is biodegradable or pulpable. All these categories mentioned are way too expansive for practical purposes. **Figure 4** below describes a more comprehensive way of classification of adhesives (Petrie, 2000).

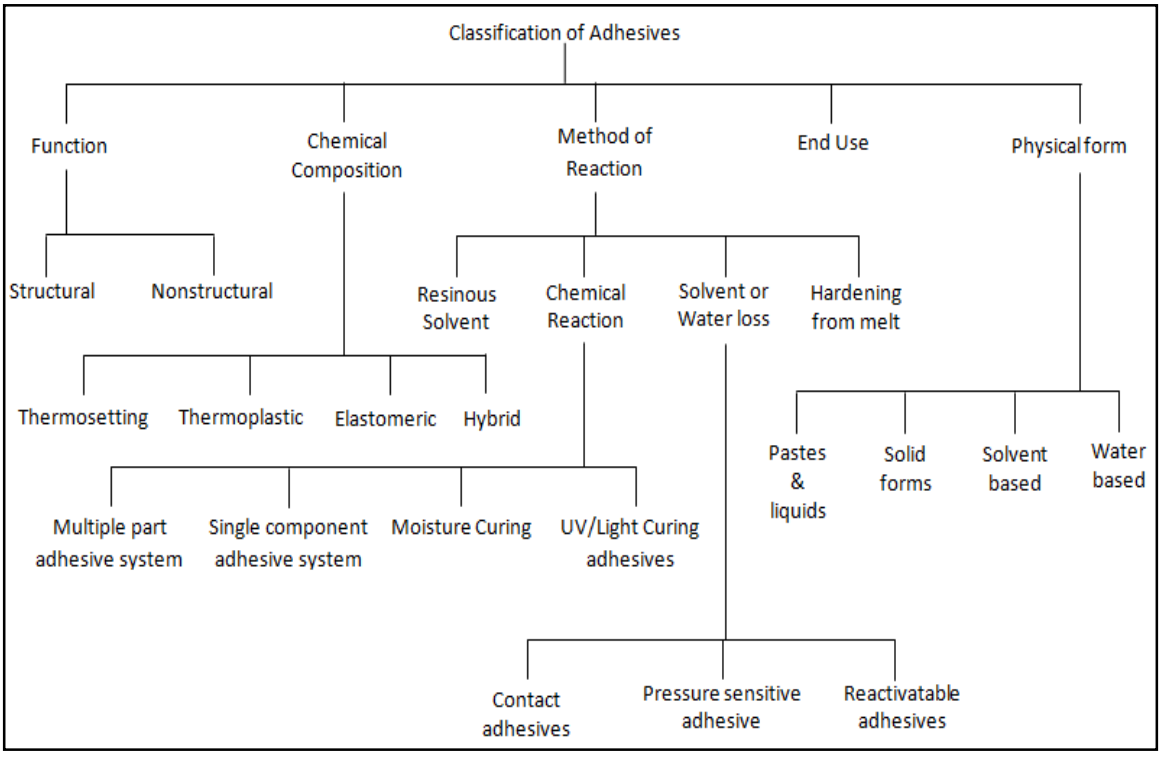


Figure 4: Classification of Adhesives (Petrie, 2000)

II.A.3. Advantages and Disadvantages of Adhesive Systems

Using adhesive systems to construct laminates has its own set of advantages and disadvantages compared to the extrusion technique for constructing laminates. Some of the distinct advantages include the application of adhesive to variety of different films. Adhesives can be applied at high speeds and adhesion can be achieved in shorter times as compared to the extrusion technique. They also protect the print from distortion. Also, they require lesser energy consumption compared to extrusion coated lamination. Adhesives can provide excellent mechanical resistance to the package.

In some cases, application of adhesives needs extensive surface preparation for the adhesives to wet the surface of the film. This incurs additional cost to the process of lamination. Another disadvantage is the long curing times of the adhesives. Environmental, health and safety considerations are necessary, while using adhesives for constructing food laminates, as they have to comply with the FDA regulations (Petrie, 2004).

II.A.4. Applications

Adhesives find their application in multidisciplinary areas, which are shown in **Fig. 5**. Adhesives are widely used in industries like food packaging, construction, textile, aircraft, etc. In food industry adhesive resins are used as permeation barrier in a bottle and container packaging for ketchup, meat, soup, beverages, cheese etc (Applications: Mitsui Chemicals America, Inc).

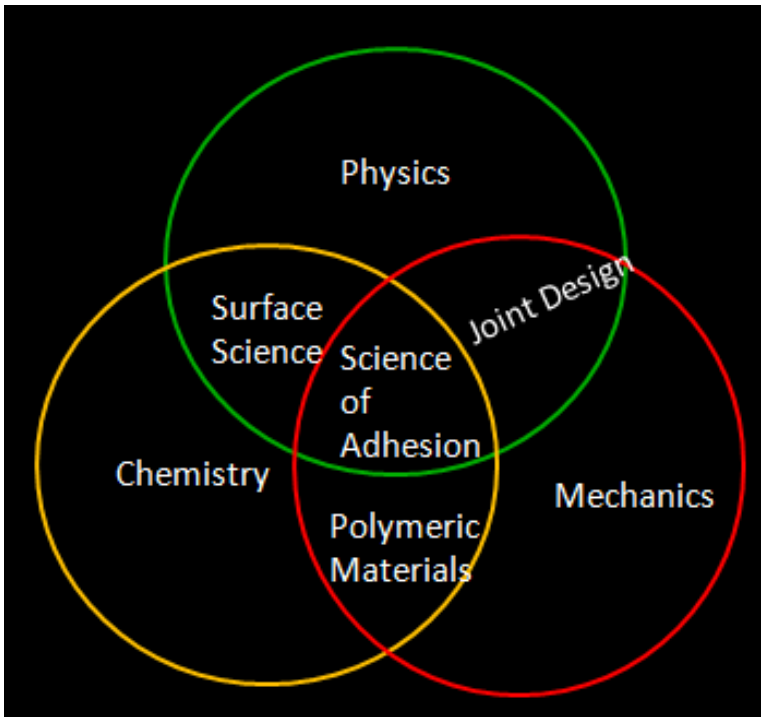
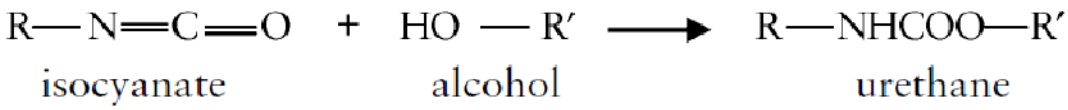


Figure 5: Use of Adhesives in Diverse Fields (Petrie, 2000)

II.B. Chemistry of Polyurethane Adhesives

Polyurethane adhesives belong to the class of reaction polymers which include epoxy compounds, unsaturated polyesters and phenolics (Oertel, 1985). Polyurethane, a two component system, is formed by reacting diisocyanate with polyols. The process of polyurethane polymerization contains the traits of both polycondensation and polyaddition reaction. Even though no simple molecule is eliminated, polymerization of polyurethane is considered to be a polycondensation reaction, as the reaction kinetics resembles more closely to the polycondensation reactions (Lamba et. al., 1997).

Polyurethanes can be synthesized by two methods, one-shot method and prepolymer method. One-shot method indicated below is the simplest one step method in which difunctional or multi fuctional isocyanates and diols are made to react with eachother. Materials cured from the one step process result in formation of elastomers.



In the prepolymer method **(Fig. 6),** linear hydroxy terminated diols react with excess of diisocyanate to form isocyanate terminated polymer called prepolymer. Prepolymer is further reacted with diols to form polyurethane polymer. The multistep synthesis of polyurethane allows the greater control over the reaction chemistry, thus influencing structure, reactivity and physical properies of the polyurethane. Hence, from the industry point of view, prepolymer method is more extensively used compared to one-shot method for the synthesis of polyurethane (Szycher, 1999).

Reaction:

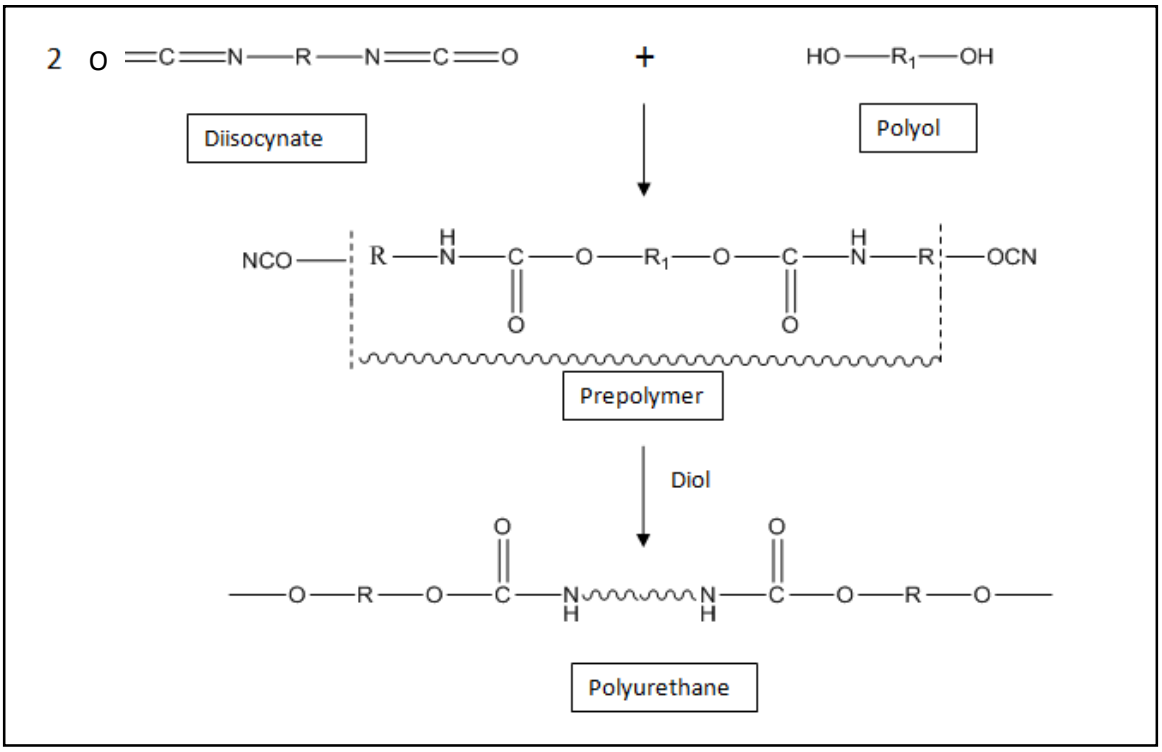


Figure 6: Preparation of Polyurethane by Prepolymer Method (Szycher, 1999)

High molecular weight polyols of low functionality, containing 2-3 hydroxyl groups, lead to formation of elastic polyurethanes. Similarly, low molecular weight polyols of high functionality, containing 3-8 hydroxyl groups, yield rigid and crosslinked polyurethanes. Urethane linkages contain 'hard segemts' due to hydrogen bonding. At the same time, high molecular weight polyols have higher mobility which introduces soft chains in the polyurethane linkages. These linkages are shown in **Fig. 7**. This structural complexity contributes to the rigid and elastomeric properties of polyurethane (Ionescu, 2005).

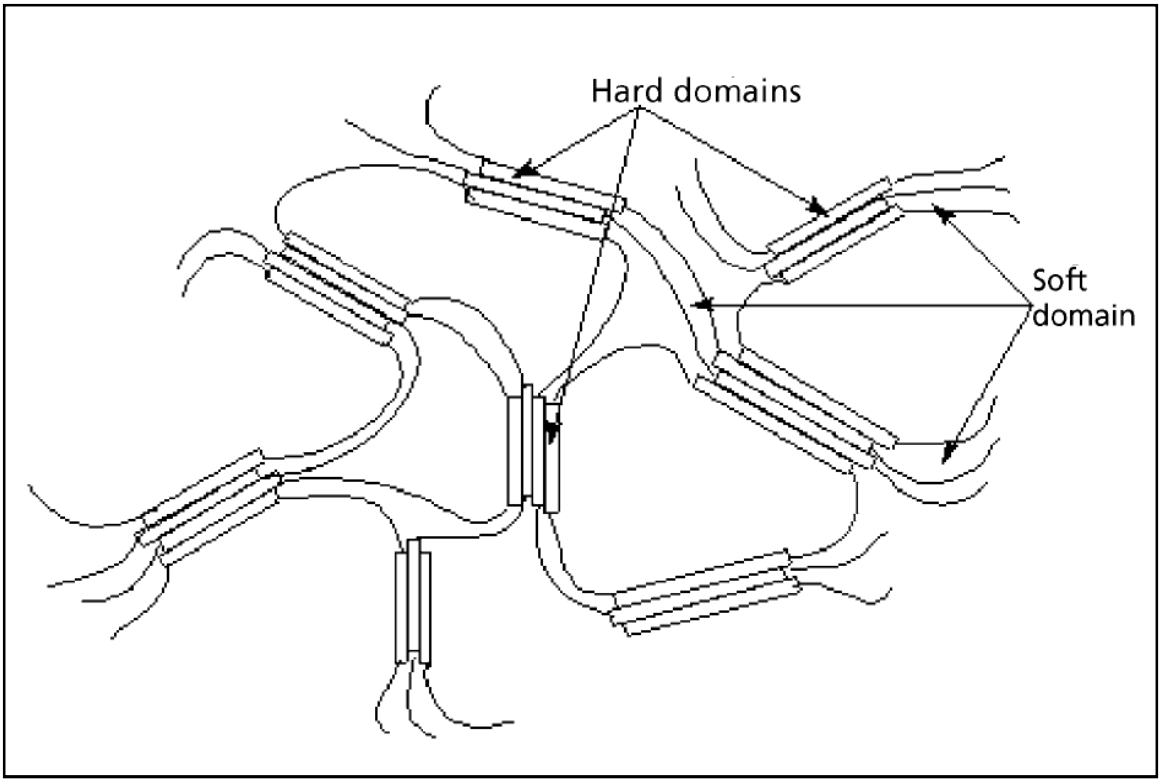


Figure 7: "Hard Segments" and "Soft Segments" of Polyurethane (Ionescu, 2005)

II.C. Formation of Polyester Oligomers

Polyurethane structure contains “hard segments” due to its ability to form hydrogen bonds. The rigidity associated with the hard segments in the polyurethane structure can cause it to crack during its processing. This could potentially restrict the use of polyurethane adhesives in the flexible food packaging applications. Hence, polyesters are used as raw materials in the synthesis of polyurethane. Polyesters react with diisocyanates to form urethane linkage and introduce the “soft chains”, thus breaking up the crystallanity associated with cross linking in polyurethane. Further, the polyester imparts the required flexibility to urethane due to the high mobility of hydroxyl groups associated with it and hence, can be used in flexible packaging industry (Wagner, 2010).

Polyesters are synthesized by the esterification reaction between diols or polyols and dicarboxylic aliphatic or aromatic acids, as shown in **Fig. 8**. It is a polycondensation reaction where the water molecules are removed. When the reaction proceeds under equilibrium conditions, ester groups, hydroxyl groups and carboxylic groups are in equilibrium with each other and the reaction conditions lead to the formation of linear polyester (Sasano & Igarashi, 2005).

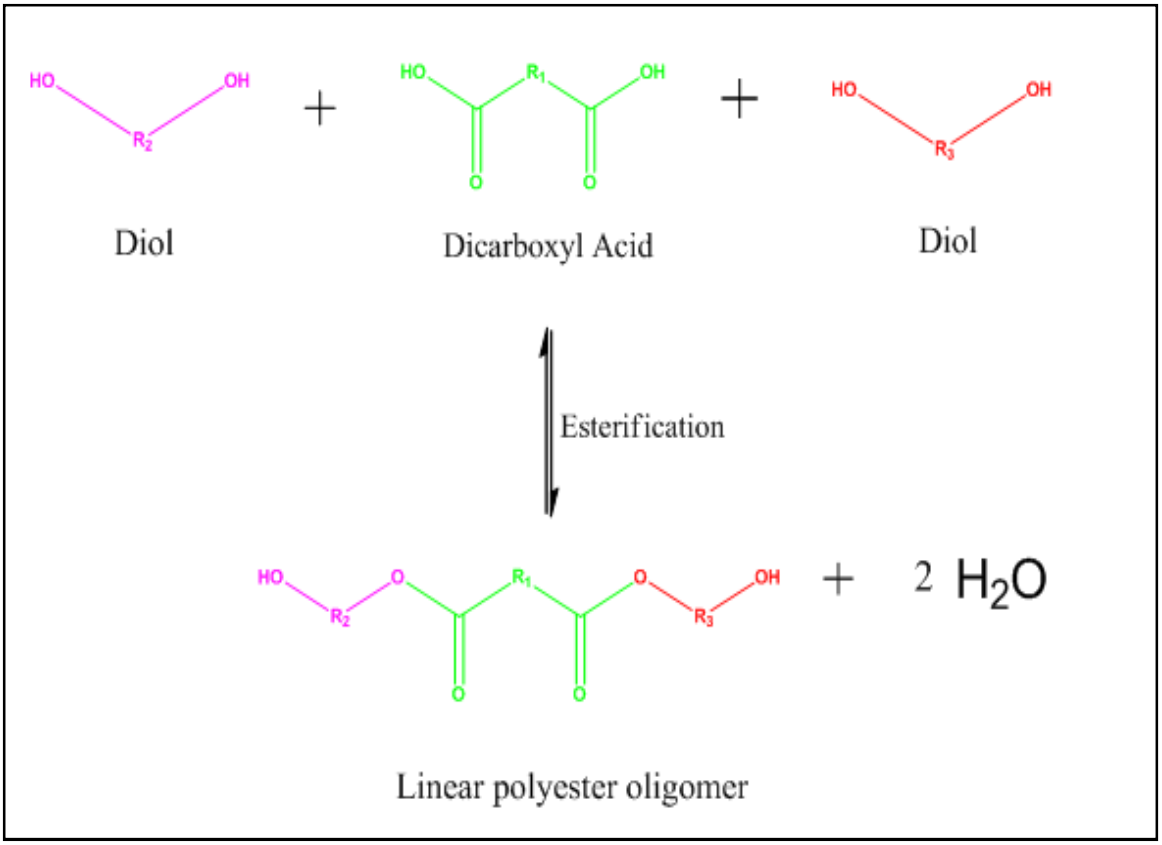


Figure 8: Formation of Linear Polyester

As the reaction continues, the polymeric mass begins to build up. This creates shift of equilibrium towards the right (Brugel, 2003). At this instance, the stoichiometry of the reaction favors the formation of short chain cyclic polyester oligomers over linear polyesters (Ionescu, 2005) as shown in **Fig. 9**.

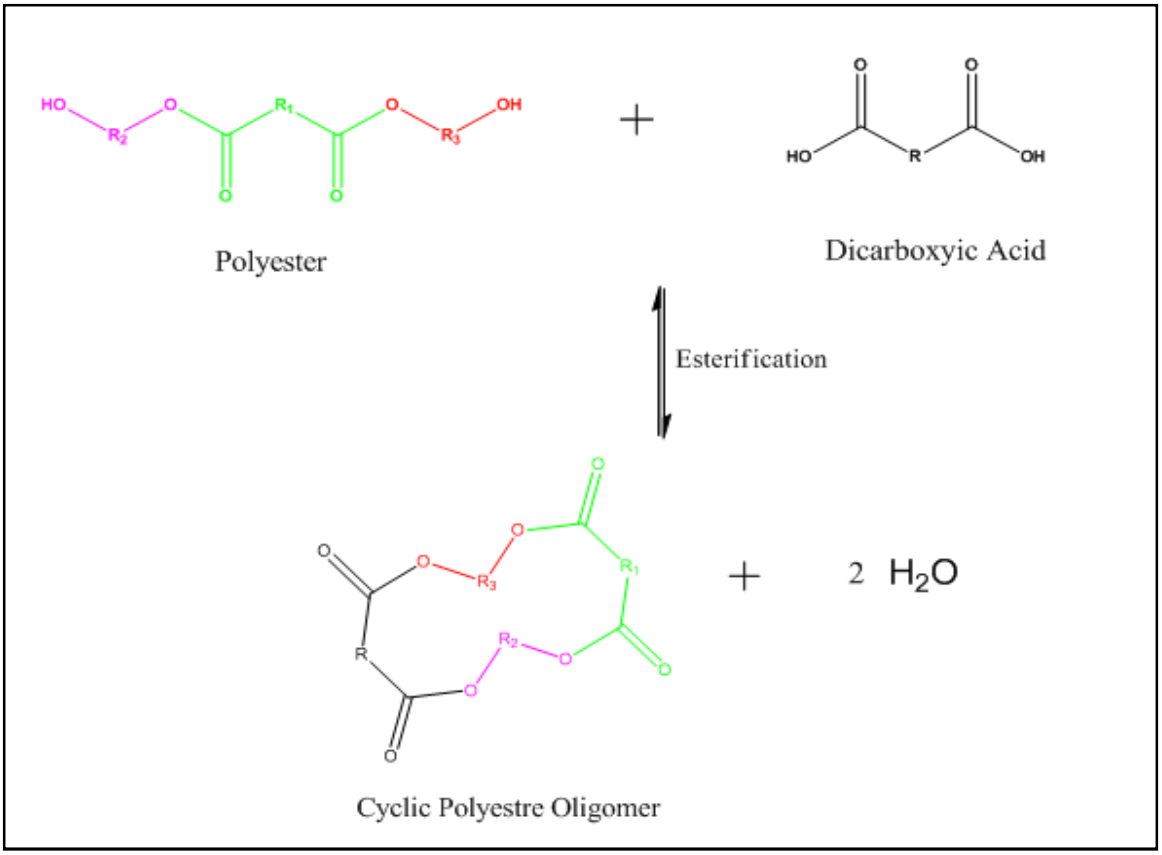


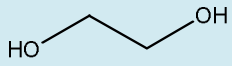
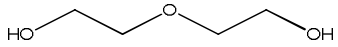
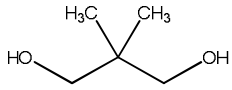
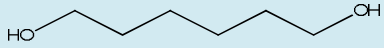
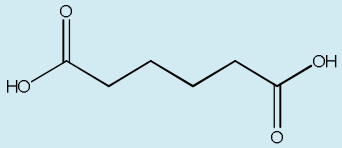
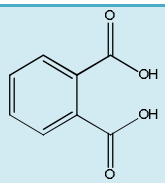
Figure 9: Formation of Cyclic Polyester Oligomer

The water generated as a product of the esterification reaction is removed from the system using heat or vacuums to obtain polyester with an increased molecular weight. Under the concentrated conditions, linear polyester is formed. On the contrary, under dilute conditions, short chain cyclic polyester oligomers are formed.

The formation of short chain cyclic polyester oligomers is undesirable from an industrial point of view for multiple reasons. Linear polyesters have terminal hydroxyl group which can react with diisocyanates to form high molecular weight polyurethane. On the other hand, the terminal hydroxyl group is no longer available due to cyclization and hence cannot react with diisocyanates to form high molecular weight polyurethanes. Presence

of cyclic esters oligomers affects the curing property of polyurethane adhesives. **Table 1** summarizes the raw materials used for the manufacture of polyesters.

Table 1: Raw Materials Used in the Preparation of Polyesters

	Polyol	**Formula**	**Molecular Weight**
1.	Ethylene glycol (EG)		62 g/mol
2.	Diethylene glycol (DEG)		106g/mol
3.	1,4-Butanediol (BD)		90 g/mol
4.	Neopentyl glycol (NPG)		104 g/mol
5.	1,6-Hexanediol (HD)		118 g/mol
	Aliphatic dicarboxylic acids		
1.	Adipic acid (AA)		146 g/mol
	Aromatic dicarboxylic acids		
1.	Phthalic acid (IPA)		166 g/mol

II.D. Migration

Food systems are chemically aggressive media which can interact with any material that they come in contact with. No food contact material is completely inert; hence, there is a possibility that it may influence the food system through the 'migration' of its chemical constituents from the food packaging material into the food system. Food packaging is comprised of either single packaging material (e.g. Paper, glass, aluminum and tin) or composite packaging with printing and varnish. Such materials used in food packaging leach small amounts of their chemical constituents when they are brought in contact with certain food systems. The term migration accounts for the movement of chemical entities from the packaging material into the food system contained therein. Scientifically, migration is defined as "mass transfer from external source into food by sub microscopic process" (Castle, 2005).

The process of migration can be explained on the basis of laws of diffusion. Chemical migration is a diffusion process subject to kinetic and thermodynamic control. Diffusion process is a function of time, temperature, thickness of the material, cohesive energy density, concentration of migrant in the material, diffusivity of migrant, partition coefficient and distribution coefficient. The kinetic parameter of the migration takes into account the rate of migration while the thermodynamic parameter governs the extent of mass transfer at the end of diffusion. The factors that trigger the chemical migration also include the nature of the food system, the contact conditions and the intrinsic properties of the packaging materials. **Figure 10** explains the process of chemical migration from the laminate film into the food system in contact with it (Castle, 2005).

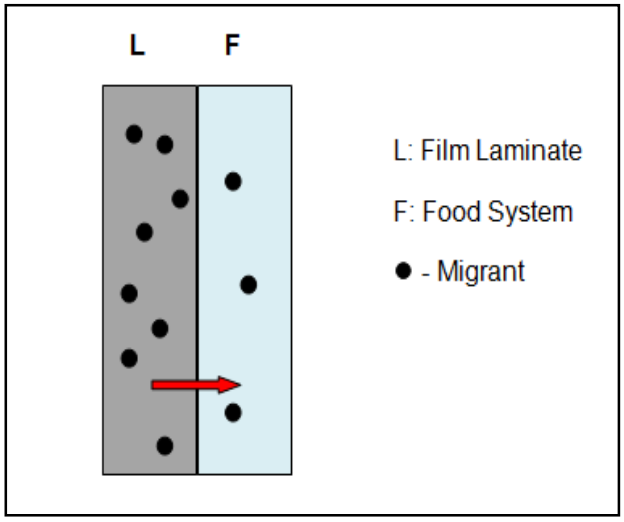


Figure 10: Chemical Migration

With this in mind, it is important to understand and control the chemical migration because of the two primary reasons.

1. **Food Safety:**

   Migration of the chemical constituents from the food contact material beyond the permissible levels possesses a great risk of introducing undesirable substances in food. These migrants, if ingested in large quantities, may be harmful.

2. **Food Quality:**

   Food packaging borne migrants can affect composition of food as well as the properties of the food such as color, odor, taste and appearance. These factors affect the shelf life of the product.

Food migration is not an inconsequential process. To ensure the safety of the food product consumed, different legislations have been enforced. Packaging must comply with such regulations. Also various guidelines, recommendations and rules on the use of individual substances have been set forth. For many substances, the specified area of

their use and the permissible limits for their migration has been specified. However, there are certain substances, like adhesives whose migration potential has not been assessed sufficiently. One of the chief reasons behind this is because they are used in very small amounts (Piringer, 1999).

The migratory properties and the potential toxicity associated with the short chain cyclic polyester oligomers are still unknown. However, they tend to induce the bitter off notes in the food systems, thus, compromising the quality of the food. Hence, their migration in the food is considered to be undesirable.

II.E. Regulations Regarding the Use of Adhesives

FDA Regulations

FDA allows the use of adhesives as a component of food packaging under certain prescribed conditions in compliance with certain regulations (21 CFR 175.105) Adhesives are separated from the food contact by the barrier layers and can be classified as indirect food additives.

According to No Migration exemption, clarified by the United States Court of Appeals, the term “food additive” has been defined as:

> “*Food additives include all substances not exempted by section 201(s) of the act, the intended use of which results or may reasonably be expected to result, directly or indirectly, either in their becoming a component of food or otherwise affecting the characteristics of food. A material used in the production of containers and packages is subject to the definition if it may reasonably be expected to become a component, or to affect the characteristics, directly or indirectly, of food packed in the container. "Affecting the characteristics of food" does not include such physical effects, as protecting contents of packages, preserving shape, and preventing moisture loss. If there is no migration of a packaging component from the package to the food, it does not become a component of the food and thus is not a food additive. A substance that does not become a component of food, but that is used, for example, in preparing an ingredient of the food to give a different flavor, texture, or other characteristic in the food, may be a food additive*.” (21 Code of Federal Regulations (CFR) 170.3.(e) Food Additives, Definitions)

The threshold limits for the use of adhesives should not exceed the prescribed limits of good manufacturing practices (21 CFR 175.105). Also where there are no specific limits specified for the use of same, the quantity should not exceed the reasonable amount required to accomplish the intended physical or technical effect (21 CFR 174.5).

At Code of Federal Regulations, Title 21, Parts 170.39, “Threshold of regulation for substances used in food-contact articles” of FDA stipulates the proposition for the substances which can be considered as safe on the basis of low dietary exposure. The

substance at extremely low levels, 0.5 ppb or below in the diet may be considered as GRAS.

The Code of Federal Regulations, Title 21, parts 175 provides information on the safe conditions and the limitations of use of the substances that may be employed as the constituents of adhesives. It was noted that there were no specific limits prescribed for the monomers used in the preparation of polyesters, which are constituents of polyurethane adhesive, including adipic acid, phthalic acid, neopentyl glycol, 1,6-Hexanediol, 1,4-Butanediol, Diethylene glycol, ethylene glycol etc (21 CFR 175.105).

European Union (EU) Regulations:

According to the article 3(1)(b) of Regulation (EC) No 1935/2004, “the release of substances from food contact materials and articles should not bring about unacceptable changes in the composition of the food.” Advance technologies engineer materials in particle size which possess chemical and physical properties significantly different from the large scale, causing difference their toxicological properties. Substances with molecular weight above 1000 Da cannot be absorbed in the body, hence possess minimum potential health risk. However, non-reacted or incompletely reacted monomers or other starting materials from the low molecular weight additives, migrated in the food may possess potential health risk. Therefore, the starting materials, monomers and additives should be subjected to potential risk assessment. Based on the risk assessment study, specific migration limits should be prescribed to ensure the safety of the final product (Commission Regulation (EU) N. 10/2011, 2011).

There are no specific legislation enforced on the use of coatings, printing inks and adhesives by European Union as yet and hence are not subjected to the requirement of declarations (Official Journal of the European Union, 2011). However, the use of

substances in coatings, printings and adhesives should comply with the established limits by the European Union. In the official journal of European Union, detailed information on authorized monomers, additives and polymers have been listed. The specific migration limit (SML) specified for the selected monomers used in the synthesis of polyesters for polyurethane adhesives are listed below **(Table 2**).

Table 2: Specific Migration Limit (SML) of Selected Monomers in Polyester Synthesis (EU Commission Regulation, 2011)

CAS No.	Chemical Name	Use as Additive	Use as Monomer	SML (mg/kg)
124-04-9	Adipic acid	Yes	Yes	
88-99-3	Phthalic acid	Yes	Yes	
110-63-4	1,4-Butanediol	Yes	Yes	
629-11-8	1,6-Hexanediol	No	Yes	0,05
126-30-7	Neopentyl glycol	No	Yes	0,05
111-46-6	Diethylene glycol	Yes	Yes	
107-21-1	Ethylene glycol	Yes	Yes	30

II.F. Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations

The Federal Food, Drug and Cosmetic Act (the Act) 2007 defines the food-contact-substance (FCS) in section 409 (h) (6) as “any substance that is intended for use as component of materials used in manufacturing, packing, packaging, transporting or holding food if the use is not intended to have any technical effect in the food” (FDA, 2007). As well as section 409 of the Act requires the food contact notification (FCN) or food act petition (FPN), which includes “sufficient scientific information to demonstrate that the substance that is the subject of the submission is safe under the intended conditions of use” (FDA, 2007).

The guidance for industry document outlines “FDA’s recommendations pertaining to chemistry information that should be submitted in a food contact notification (FCN) or food additive petition (FAP) for a food contact substance (FCS)” (FDA, 2007). One of the sections – Chemistry information for FCNs and FAPs illustrates migration testing and analytical methods in detail such as design of experiments (II D 1 a-e), characterization of test solutions and data reporting (II D 2), migration database (II D 3 a-e) and migration modeling (II D 5).

Under the design of migration experiments section, specification of migration cells, choice of food simulants, temperature and time combination tests for different materials have been thoroughly explained.

Table 3 describes the choice of recommended food simulants by FDA for the food types defined in 21 CFR 176.170(c). Similarly **Table 4** illustrates the food types and their respective definitions provided by FDA under Food Contact Substances (FDA, 2007).

Table 3: Recommended Food Simulants by US-FDA [21 CFR 176.170(c)]

Food-Type as defined in 21 CFR 176.170(c) Table 1	Recommended Simulant
Aqueous & Acidic Foods (Food Types I, II, IVB, VIB and VIIB)	10% Ethanol
Low and High-alcoholic Foods (Food Types VIA, VIC)	10 or 50% Ethanol*
Fatty Foods (Food Types III, IVA, V, VIIA, IX)	Food oil (e.g. corn oil), HB307, Migloyl 812 or others **

* Actual ethanol concentration may be substituted
** HB307 is a mixture of synthetic triglycerides, primarily C10, C12, and C14. Miglyol 812 is derived from coconut oil

Table 4: Food types and their Definitions (Food Contact Substances, 2007)

Food Type	Definition
I	Non acid, aqueous products; may contain salt or sugar or both (pH>5.0)
II	Acid, aqueous products; may contain salt or sugar or both, and including oil-in-water emulsion of low- or high-fat content.
III	Aqueous, acid or nonacid products containing free oil or fat; may contain salt, and including water-in-oil emulsions of low- or high-fat content.
IVA	Dairy products and modifications: water-in-oil emulsions, high- or low-fat.
IVB	Dairy products and modifications: Oil-in-water emulsions, high- or low- fat.
V	Low-moisture fats and oil
VIA	Beverages: Containing up to 8 percent alcohol
VIB	Beverages: Nonalcoholic
VIIA	Bakery products other than those included under Types VIII or IX of this table: Moist bakery products with surface containing free fat or oil
VIIB	Bakery products other than those included under Types VIII or IX of this table: Moist bakery products with surface containing no free fat or oil
VIII	Dry solids with the surface containing no free fat or oil
IX	Dry solids with the surface containing free fat or oil.

III. Literature Review

III.A. Critical Evaluation on Previous Investigation of Short Chain Cyclic Oligomers

Short chain cyclic polyester oligomers have attracted much attention recently as they possess the potential to migrate into the food systems from the packaging material. Many researchers have attempted to identify these compounds using different analytical techniques. This section critically evaluates the previous research conducted on this class of compounds.

- Schaefer et al., (2004)

Schaefer et al., (2004) focused on the identification and the quantification of cyclic ester migrants from can coatings. Cans were internally coated with polyester film to prevent the interaction of tinplate with chemically aggressive food media. Cyclic polyester oligomers arising from polyesters due to ring chain equilibrium tend to migrate as they were not integrated in the polymer during the process of curing. The analytical method chosen for the identification of cyclic polyesters was High Performance Liquid Chromatography (HPLC) coupled with Mass Selective Detector (MSD) and Ultraviolet Detector (UVD). The coated tinplate strips were extracted with 95% ethanol as the food simulant. The migrant was concentrated and then treated with NaOH to hydrolyze the polyesters into their monomers. The polyols were identified by gas chromatography with flame ionization detector. Similarly, dicarboxylic acids were analyzed by HPLC. With the knowledge of the monomers used in the polyesters, cyclic polyesters were tentatively identified. The amount of polyester oligomers migrated was determined by comparing their responses using different detectors, UVD, MSD and evaporative light scattering detector (ELSD) (Schaefer et al., 2004).

During the quantification of oligomers, it was found that the response of the ELSD was dependent on the molecular weight of the analyte, which reduced the accuracy of the detection method. The list of commonly used monomers (**Table 5**) and oligomers reported by Schaefer et al. are listed below in **Table 6**.

Table 5: List of Commonly used Monomers for Polyester Resins (Schaefer et al., 2004)

Polyols	Polyvalent carboxylic acid
1. Ethylene glycol	11. Trimellitic acid
2. 1,2-Propanediol	12. Phthalic acid
3. 1,3-Propanediol	13. Adipic acid
4. 1,3-Butanediol	14. Terephthalic acid
5. 2-Methyl-1,3-propanediol	15. Isophthalic acid
6. 2,2,-Dimethyl-1,3-propandiol (neopentyl glycol)	
7. 1,6-Hexanediol	
8. 2,2,4-Trimethylpentane-1,3-diol	
9. 1,1,1-Tris(hydroxymethyl)propane (trimethylolpropane)	
10. 1,4-*Bis*(hydroxy-methyl)-cyclohexane (cyclohexyldimethanol)	

Table 6: List of Cyclic Oligomers Identified in Polyester Coatings

(Schaefer et al., 2004)

	Polyester coating 1	Polyester coating 2	Polyester coating 3
Dicarboxylic acids (DA)	IPA, TPA	IPA, TPA	IPA, TPA
Polyols (PO)	PO-1, PO-2, PO-3	PO-1, PO-4	PO-3, PO-5, PO-6
Cyclic oligoesters	a. CYCLO (2DA 2PO-1) b. CYCLO (2DA 1PO-1 1PO-2) c. CYCLO (2DA 2PO-2) d. CYCLO (3DA 1PO-1 2PO-2) e. CYCLO (2DA 2PO-2 1PO-3) f. CYCLO (3DA 3PO-2) g. CYCLO (4DA 1PO-1 3PO-2) h. CYCLO (3DA 2PO-2 1PO-3) i. CYCLO (4DA 4PO-3)	CYCLO (2DA 2PO-1) CYCLO (3DA 3PO-1) CYCLO (2DA 1PO-1 1PO-4) CYCLO (3DA 2PO-1 1PO-4) CYCLO (2DA 2PO-4) CYCLO (3DA 1PO-1 2PO 4)	CYCLO (2DA 1PO-5 1PO-6) CYCLO (2DA 2PO-5) CYCLO (2DA 1PO-3 1PO-5) CYCLO (3DA 1PO-3 2PO-5)

- Laine et al., (2001)

The research by Laine et al., (2001) was focused on investigating the cyclic oligomers found in polyesters prepared from phthalic acid and diols such as 2-Butyl-2-ethyl-1,3-propanediol (BEPD). 2,5-Dihydroxybenzoic acid was used as a matrix compound, which was dissolved in methanol. The polyester synthesized from phthalic acid and BEPD was dissolved in Tetrahydrofuran (THF). The polyester sample and the matrix solutions were mixed in the ratio of 1:3. The method adopted to characterize these oligomers was gel permeation chromatography (GPC) interfaced with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS). Researchers found that the cyclic dimer peak was the strongest peak amongst the oligomers and the linear dimers slowly disappeared as the polymerization reaction proceeded. The cyclic dimer peak remained intact owing to its non-reactivity. The research also found that the ratio of diacid to diol had an impact on the amount of cyclic oligomers formed during the

polymerization. In the mass spectra obtained, different oligomers were separated on the basis of their molecular masses (Laine et al., 2001)

This research focused on the use of single diol (BEPD) as a starting monomer and hence only a narrow range of oligomers were analyzed. The oligomers analyzed are listed below in **Table 7**.

AA- Adipic acid PA- Phthalic anhydride IPA- Isophthalic acid TPA- Terephthalic acid

Table 7: List of Cyclic Oligomers Identified by MALDI-TOF-MS (Laine et al., 2001)

No.	Cyclic Polyester Oligomer
1.	AA-BEPD
2.	PA-BEPD
3	IPA-BEPD
4.	TPA-BEPD

- Lawson et al., (1999)

Lawson et al., (1999) investigated the migrants from a range of polyurethane adhesives used to bond different films to form a laminate. The migration experiments were conducted on commercially prepared PET-PE films heat sealed into pouches. The food simulant used for extraction was Millipore (18 MΩ) water. The method adopted for identification of the cyclic oligomers was matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The results from the migration tests of the polyester based laminate showed no detectable evidence of the anticipated oligomers. The only migrants observed were those of polyester polyols based on adipic acid and diethylene glycol in the mass range 450-900 (Lawson et al., 2000).

Although MALDI-MS, soft ionization method, provided nominal mass of the migrants, it did not provide any information on the fragmentation pattern of these migrants. Fragmentation pattern aids in elucidation of the structure of the unknown compounds. It was also noted by the authors (Lawson et al., 2000) that MALDI-MS measurements could not be quantified easily, thus driving the need to develop a derivatization protocol to facilitate the HPLC quantification. MALDI mass spectrum reported by the authors has been listed below **(Fig. 11).**

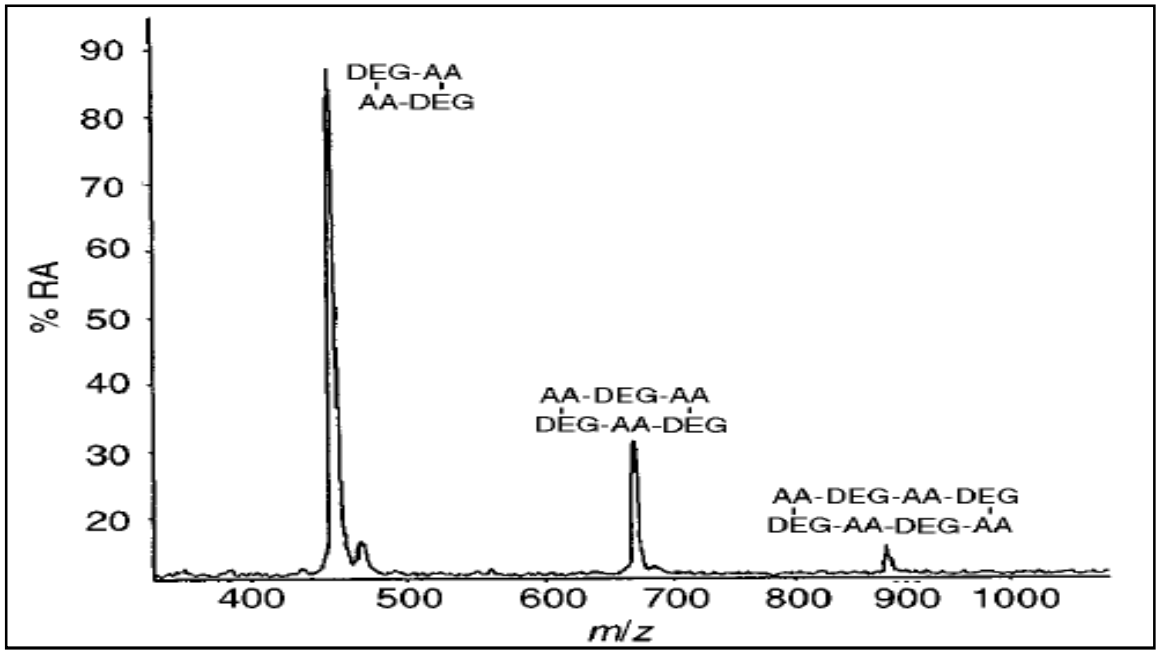


Figure 11: MALDI Mass Spectrum of Migrants from Laminate Bonded with PU containing Simple Polyester Based Polyol (Lawson et al., 2000)

- Bradley et al., (2009)

Bradley et al., (2009) focused on analytical approaches to identify potential migrants in the polyester polyurethane can coatings. The polyester-polyurethane coating containing a polyester resin and a block polyisocyanate resin was chosen for the study. The starting monomers used in the preparation of the polyester were not specified and hence all the

possible combinations of diacids and diols commonly used in polyester resins were considered for identifying the migrated polyesters. The samples were industrially stoved at 190 ℃ for 12 min. The analytical techniques used for the study included headspace GC-MS analysis and liquid chromatography-time-of-flight mass spectrometry (LC-TOF-MS). However, it was observed that no substances were detected in headspace GC-MS analysis. The TOF-MS data obtained was processed using Agilent Molecular Feature Editor Software. Using the software a database of the potential structures was prepared by combining different diols and diacids. The molecular weights of the potential oligomers were calculated. Using the software combined with the instrumentation, masses of the peaks in the chromatograms were compared with masses of the oligomers in the database (Bradley, 2009).

However, all the peaks in the chromatograms could not be identified by merely comparing the masses to the structures in the databases. With this method, the linear oligomers could be identified accurately, but the identity of the cyclic oligomers was tentatively proposed.

- Ahjopalo et al., (2000)

Ahjopalo et al., (2000) studied the distribution of cyclic oligomers in saturated polyesters. The raw materials used for the study included adipic acid (AA), phthalic anhydride (PA), Isophthalic acid (IPA), terephthalic acid (TPA), 2 butyl-2-ethyl-1,3-propanediol (BEPD) and neopentyl glycol (NPG). Polyesterification reactions were carried out in an oil jacketed glass reactor. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF) was employed to study the molecular weight distribution and to identify the cyclic oligomers. It was found that PA and AA showed the highest intensity for cyclic dimers (two diols and two acids) while common cyclic oligomers of IPA were trimers. TPA formed cyclic dimers and tetramers (Ajhopalo, 2000).

Researchers observed that TPA and PA formed stable cyclic dimers with BEPD. Dimers were non-reactive and could be migrated to the surface. However migratory properties of the dimers were not reported.

- Thie'baut (2007)

Thie'baut et al., (2007) studied the emission of volatile organic compounds in polyester based magnetic tapes, using headspace solid phase micro-extraction. The tapes were coated with poly(urethane ester). The compounds identified during the analysis included fatty acid ester lubricants, compounds from antioxidant and manufacturing byproducts like cyclic polyester oligomers. It was found that large amount of 1,6-dioxacyclododecane-7,10-dione was detected in few samples, suggesting that it was a byproduct of polyester based urethane manufacturing process (Thiebaut, 2007). Chemical structure and the chromatographic peak of the cyclic diester reported by the authors are listed below in **Fig. 12**.

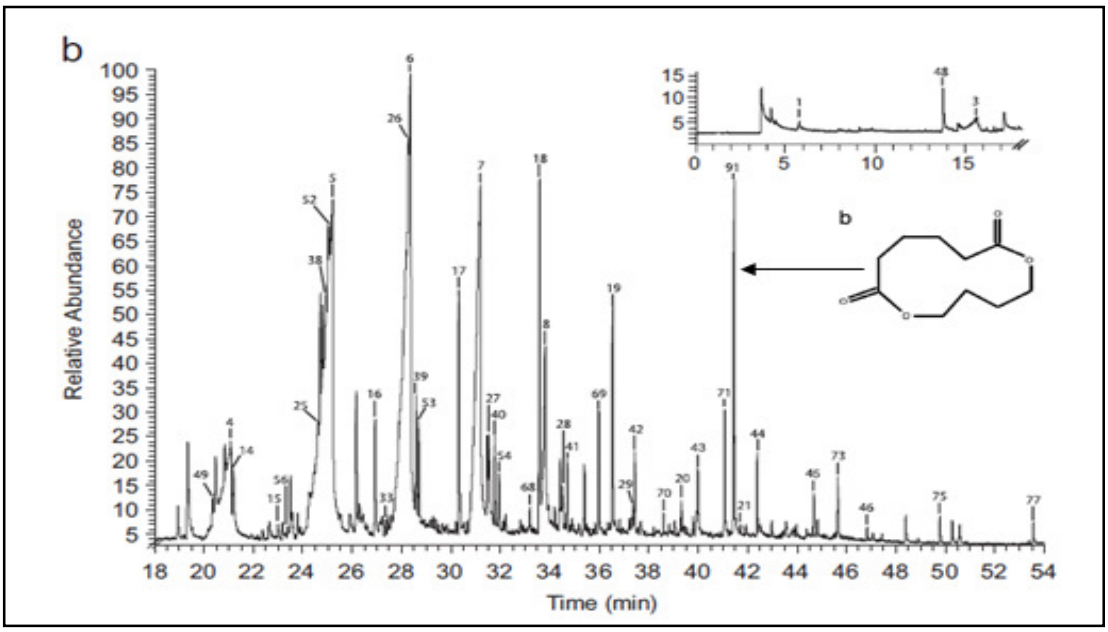


Figure 12: GC Profiles of SPME Extract from the Magnetic Tapes (Thiebaut et al., 2007)

- Lattimer et al., (1998)

Lattimer et al. (1998) analyzed the pyrolysis products from a segmented polyurethane containing 4,4'-methylenebis-(phenylisocyanate) (MDI), poly(butylene adiapate) (PBA) and 1,4-butanediol (BDO). Pyrolysis was carried under argon flow in the temperature range 250 ℃ to 325 ℃. The residue left behind after the pyrolysis tube was analyzed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The presence of cyclic polyester oligomers was reported in the study. The series B in the MALDI MS spectrum represent the cyclic polyester oligomers (**Fig. 13**). However, there was no information reported on the chemical structures and individual identification of these polyester oligomers (Lattimer, 1998).

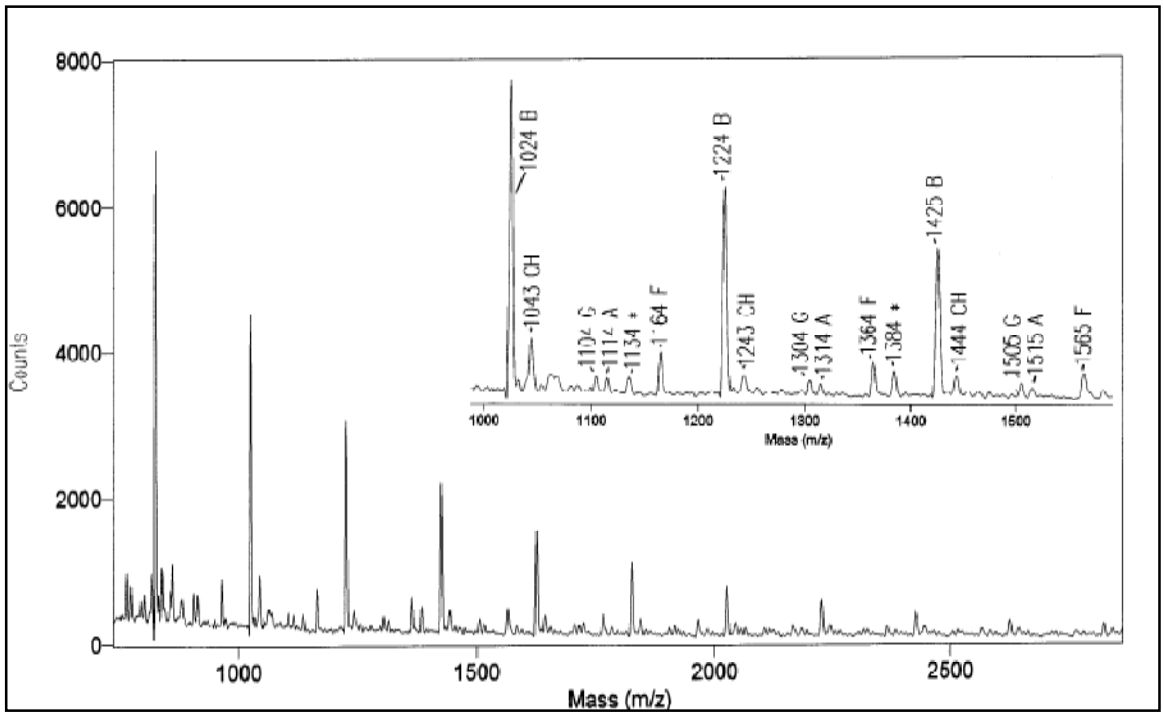


Figure 13: MALDI-MS of Segmented Polyurethane Depicting Series B of Individual Cyclic Polyester Oligomers (Lattimer et al., 1998)

- Laine et al., (2000)

Laine et al. (2000) studied qualitative and quantitative end group analysis of small molecular weight polyester by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). They also discussed the method to identify the presence of cyclic oligomers. The polyester used in study, poly(2-butyl-2-ethyl-1,3-propylene phthalate) was prepared by step reaction polymerization from phthalic acid and 2-butyl-2-ethyl-1,3-propanediol as instructed in the previous literature. The presence of cyclic oligomers formed during the polymerization was verified by deuterium exchange of the exchangeable protons of the polyesters. The polyester under the study was deuterated using deuterated solvents. For the basis of comparison, undeuterated samples of polyester in the organic solvents were prepared. The exchange of hydroxyl and carboxyl protons brought a change in the isotopic patterns of the signals of linear oligomers in the MALDI spectrum. Comparison of the deuterated and undeuterated samples revealed the signals of the cyclic oligomers, as they do not have exchangeable protons. The signals of the cyclic oligomers remain unchanged. However, the identities of the individual cyclic oligomers were not reported in the literature (Laine, 2000).

- Kim et al., (2012)

Kim et al., (2012) focused on determination of monomers and oligomers in polyethylene terephthalate trays and bottles for food use. Polyethylene terephthalate (PET) was synthesized by condensation polymerization of monoethylene glycol (MEG) and terephthalic acid (TPA). During the synthesis of PET, low molecular weight cyclic oligomers were formed. The analytical technique adopted to identify these oligomers was high performance liquid chromatography (HPLC) coupled with electrospray ionization mass spectrometry (ESI-MS). The solvents used for the purpose of extraction

were made with 50% acetonitrile and dichloromethane. The oligomers reported (**Fig. 14**) by the authors are tabulated below (Kim, 2012) in **Table 8**.

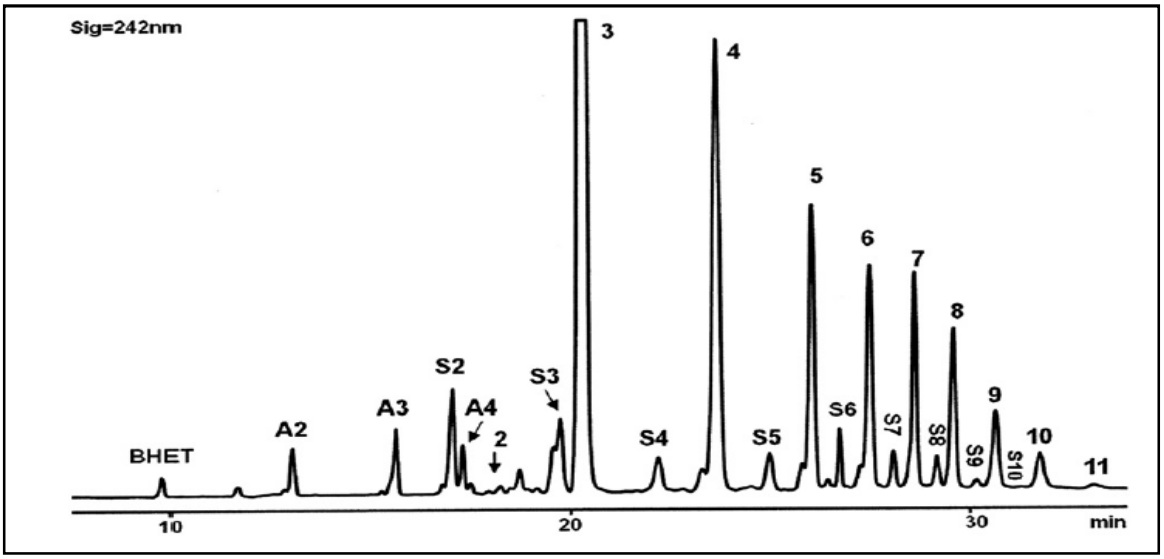


Figure 14: HPLC Chromatogram of PET Oligomers Obtained by Accelerated Solvent Extraction Method from PET Containers (Kim et al., 2011)

Table 8: Chemical Identities of Oligomer Series Detected from PET Containers. The numbers in the Table correspond to the peak numbers in the HPLC chromatogram (Fig. 14) (Kim et al., 2011)

No	Oligomer	Structure[a]	Molecular formula	MW[b]
BHET	Bis(2-hydroxyethyl) terephthalate	H-[TG]$_1$G-OH	$C_{12}H_{14}O_6$	254.08
A2	Second series acyclic dimer	H-[TG]$_2$G-OH	$C_{22}H_{22}O_{10}$	446.12
A3	Second series acyclic trimer	H-[TG]$_3$G-OH	$C_{32}H_{30}O_{14}$	638.16
A4	Second series acyclic tetramer	H-[TG]$_4$G-OH	$C_{42}H_{38}O_{18}$	830.21
S2	Second series cyclic dimer	[TG]$_2$G	$C_{22}H_{20}O_9$	428.11
S3	Second series cyclic trimer	[TG]$_3$G	$C_{32}H_{28}O_{13}$	620.15
S4	Second series cyclic tetramer	[TG]$_4$G	$C_{42}H_{36}O_{17}$	812.20
S5	Second series cyclic pentamer	[TG]$_5$G	$C_{52}H_{44}O_{21}$	1004.24
S6	Second series cyclic hexamer	[TG]$_6$G	$C_{62}H_{52}O_{25}$	1196.28
S7	Second series cyclic heptamer	[TG]$_7$G	$C_{72}H_{60}O_{29}$	1388.32
S8	Second series cyclic octamer	[TG]$_8$G	$C_{82}H_{68}O_{33}$	1580.36
S9	Second series cyclic nonamer	[TG]$_9$G	$C_{92}H_{76}O_{37}$	1772.41
S10	Second series cyclic decamer	[TG]$_{10}$G	$C_{102}H_{84}O_{41}$	1964.45
S11	Second series cyclic hendecamer	[TG]$_{11}$G	$C_{112}H_{92}O_{45}$	2156.49
2	First series cyclic dimer	[TG]$_2$	$C_{20}H_{16}O_8$	384.08
3	First series cyclic trimer	[TG]$_3$	$C_{30}H_{24}O_{12}$	576.13
4	First series cyclic tetramer	[TG]$_4$	$C_{40}H_{32}O_{16}$	768.17
5	First series cyclic pentamer	[TG]$_5$	$C_{50}H_{40}O_{20}$	960.21
6	First series cyclic hexamer	[TG]$_6$	$C_{60}H_{48}O_{24}$	1152.25
7	First series cyclic heptamer	[TG]$_7$	$C_{70}H_{56}O_{28}$	1344.30
8	First series cyclic octamer	[TG]$_8$	$C_{80}H_{64}O_{32}$	1536.34
9	First series cyclic nonamer	[TG]$_9$	$C_{90}H_{72}O_{36}$	1728.38
10	First series cyclic decamer	[TG]$_{10}$	$C_{100}H_{80}O_{40}$	1920.42
11	First series cyclic hendecamer	[TG]$_{11}$	$C_{110}H_{88}O_{44}$	2112.46

- Holland et al., (2001)

Holland et al., (2001) analyzed comonomer content and cyclic oligomers from poly(ethylene terephthalate) (PET). Comonomers like diethylene glycol (DEG) and isophthalic acid (IPA) were added to PET to reduce the rate of crystallization during the processing. Cyclic oligomers were extracted from commercial PET samples using chloroform. The mixture of oligomers was separated using high performance liquid chromatography (HPLC). Further, the oligomers were analyzed using electrospray mass spectrometry (ES-MS). It was observed that the yield of cyclic oligomers increased with the concentration of DEG segments. The oligomers reported (**Table 9**) by Holland et al. have been listed below (Holland, 2002) in **Fig. 15 and Fig. 16**.

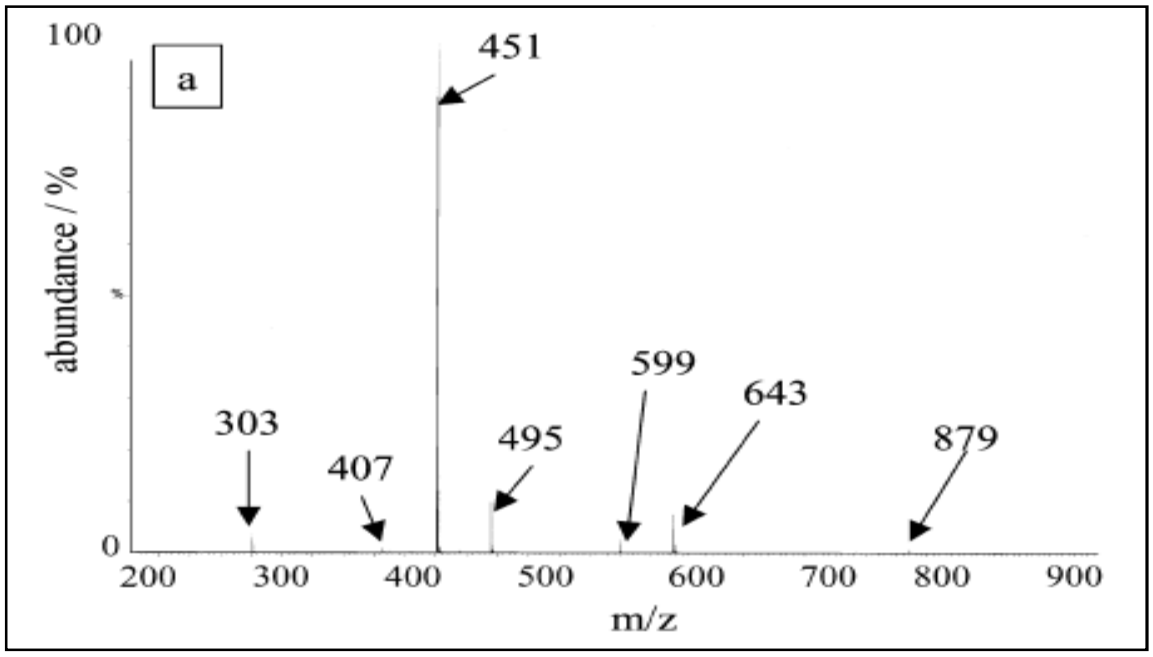


Figure 15: ES MS Spectrum of Individual Oligomers of PET Identified in Table 9 (Holland et al., 2001)

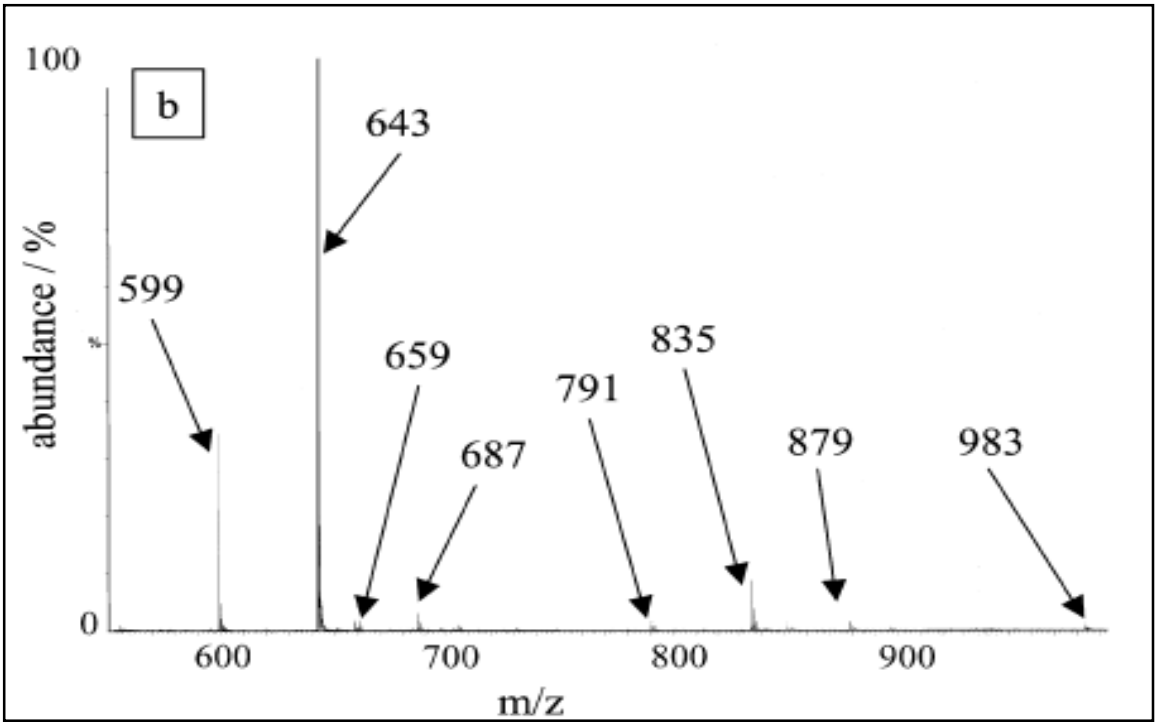


Figure 16: ES MS Spectrum of Individual Oligomers of PET (Holland et al., 2001)

Table 9: Assignment of ES MS Mass Spectrum of Oligomers Extracted from PET (Holland et al., 2001)

m/z (Da)	Intensity (%)	Assignment
303	2.5	(Cyclic monomer + two EG) + Na
407	1.0	Cyclic dimer + Na
451	100.0	(Cyclic dimer + one EG) + Na
495	10.0	(Cyclic dimer + two EG) + Na
599	2.5	Cyclic trimer + Na
643	7.3	(Cyclic trimer + one EG) + Na
659	0.1	(Cyclic trimer + one EG) + K
687	0.2	(Cyclic trimer + two EG) + Na
791	0.1	Cyclic tetramer + Na
835	0.7	(Cyclic tetramer + one EG) + Na
879	0.1	(Cyclic tetramer + two EG) + Na
983	< 0.1	Cyclic pentamer + Na

- Lim et al., (2002)

Lim et al. (2002) focused on isolation and identification of cyclic oligomers of poly(ethylene terephthalate) (PET) and poly(ethylene Isophthalate) (PEI). Cyclic oligomers were extracted from synthesized PET-PEI copolymer and were separated by HPLC. The oligomers were identified by employing H NMR, differential scanning calorimetry and mass spectrometry. Chemical structures of the reported oligomers have been listed below in **Fig. 17, Fig. 18 and Fig. 19** (Lim, 2002).

G: Ethylene glycol T: Phthalic acid I: Isophthalic acid

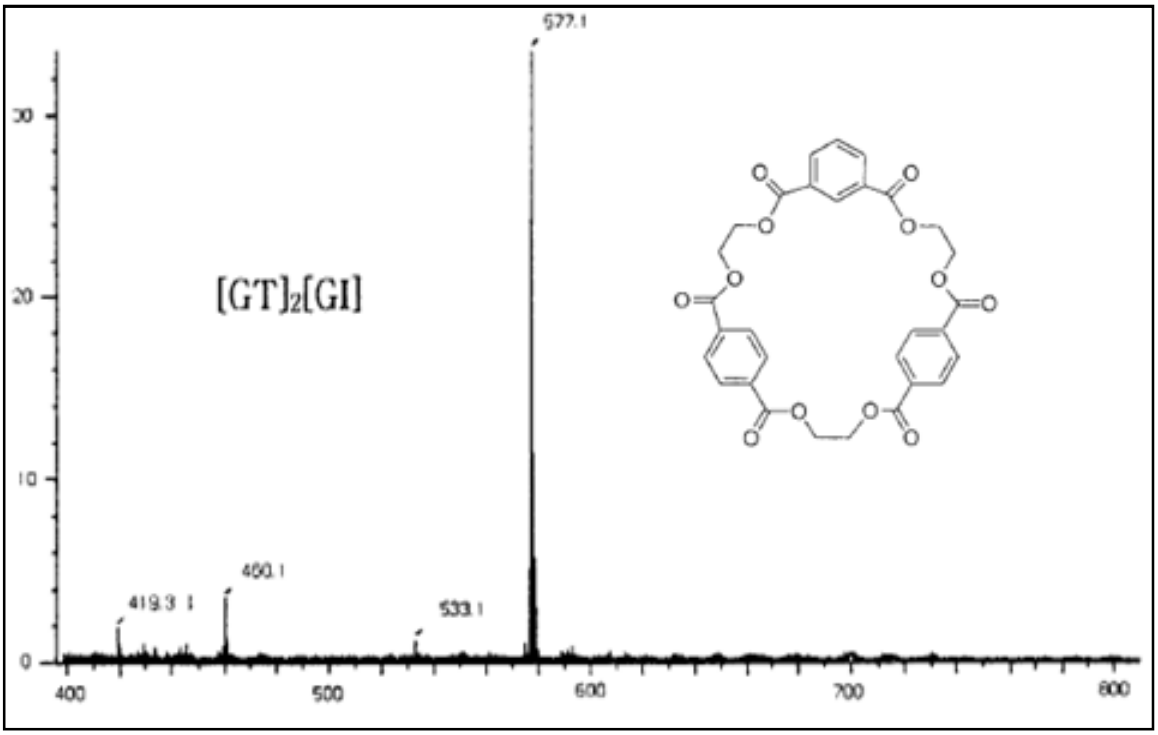


Figure 17: Fast Atom Bombardment (FAB) Mass Spectrum of Tricyclic Oligomer: [GT] $_2$[GI] (Lim et al., 2002)

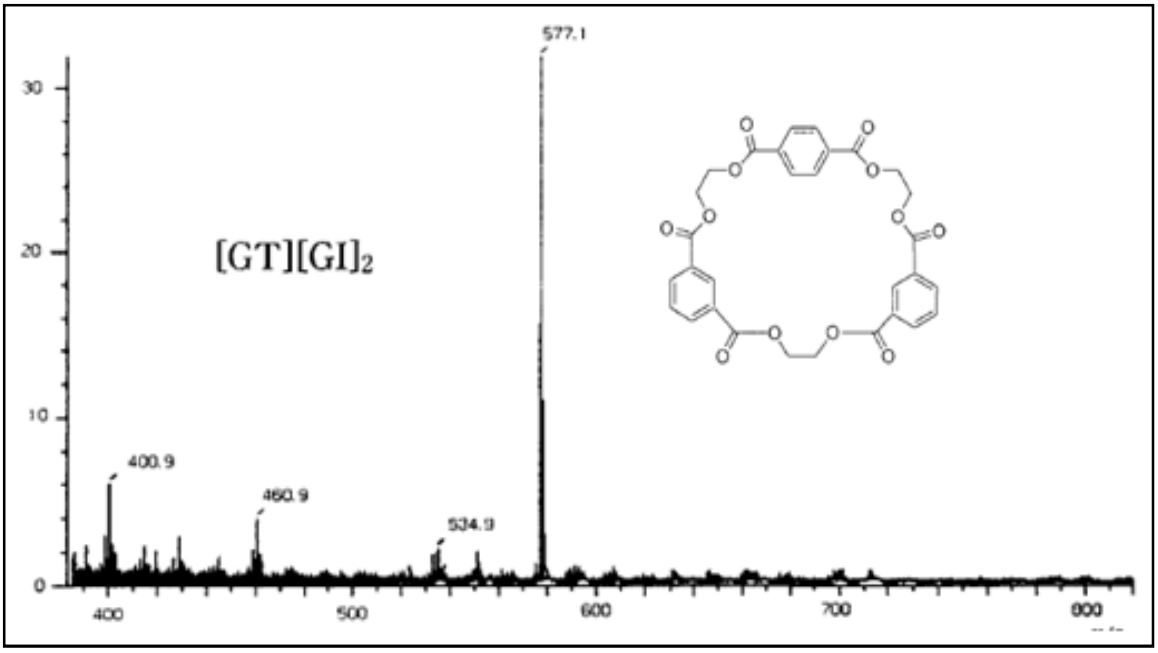


Figure 18: Fast Atom Bombardment (FAB) Mass spectrum of tricyclic oligomer: [GT][GI]$_2$ (Lim et al., 2002)

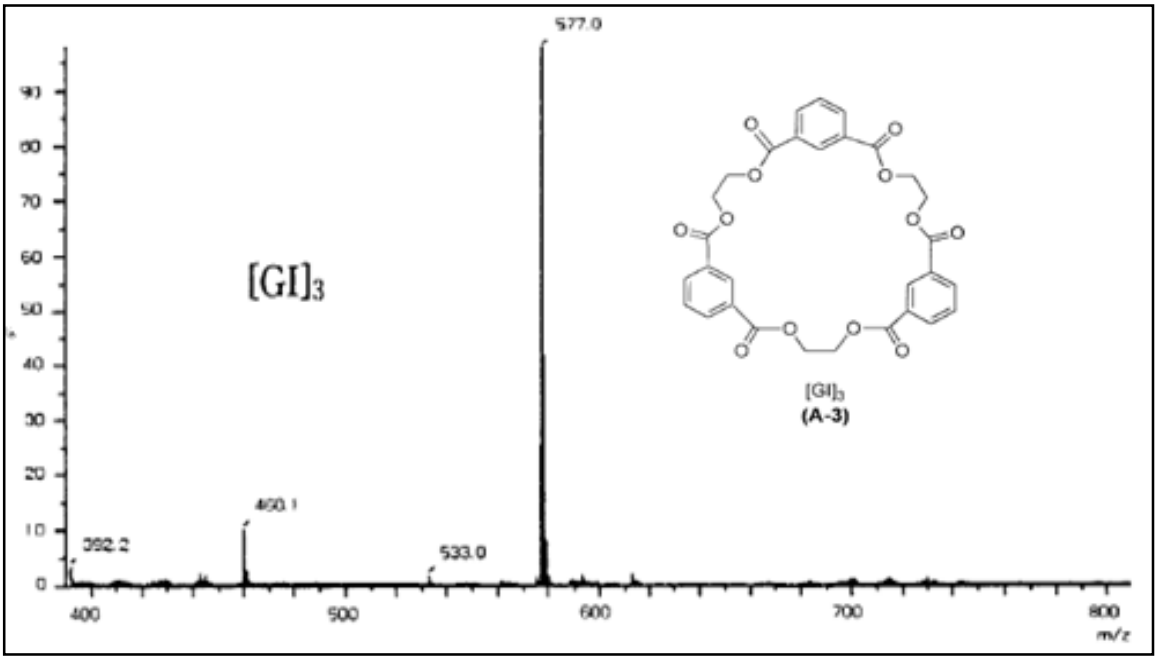


Figure 19: Fast Atom Bombardment (FAB) Mass Spectrum of Tricyclic Oligomer: [GI] $_3$ (Lim et al., 2002)

- Harrison et al., (1996)

Harrison et al., (1997) analyzed cyclic oligomers of poly(ethylene terephthalate (PET) by liquid chromatography and mass spectrometry. The field desorption mass spectra of the identified cyclic oligomers of PET $[CO.C_6H_4.CO.O.CH_2.CH_2.O]_x$ with x=3-8 were reported by the authors. Also, the structural information for fragmentation of cyclic oligomers was obtained by liquid chromatography/tandem mass spectrometry (Harrison, 1997). The reported mass spectra have been included below in **Fig. 20** and **Fig. 21**.

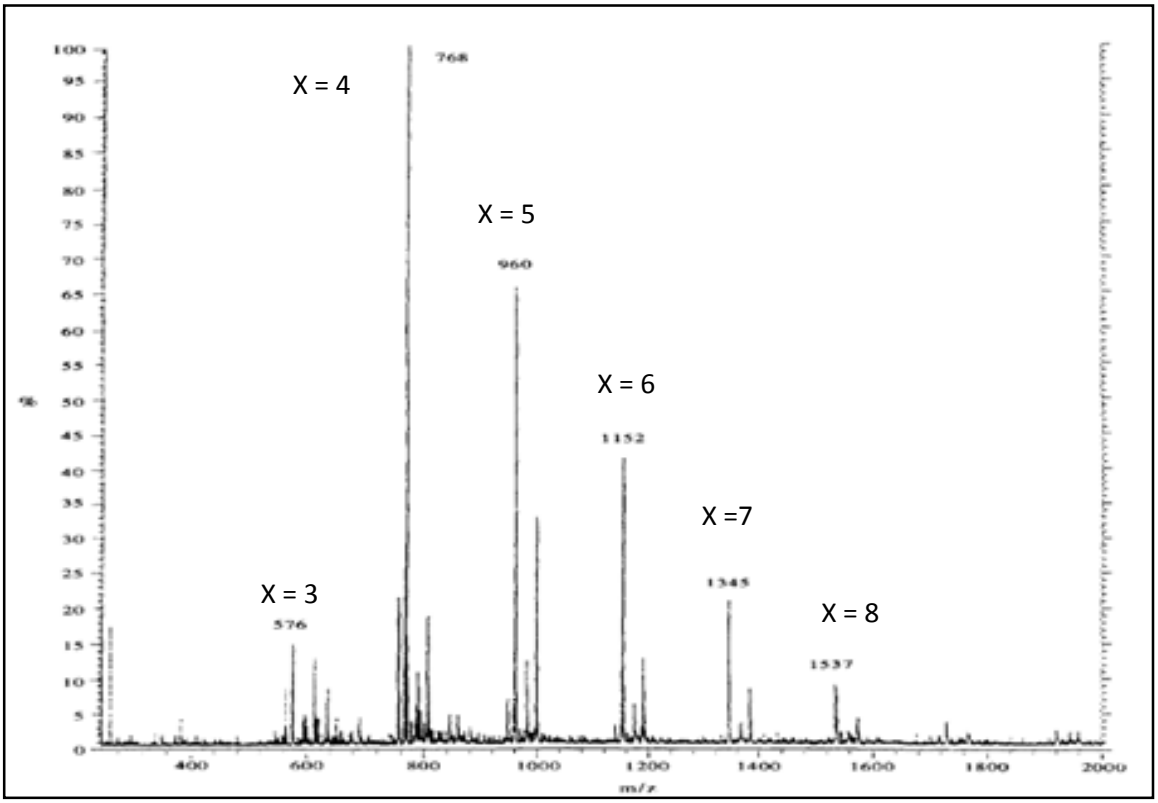


Figure 20: Field Desorption Mass Spectrum of Individual Cyclic PET Oligomers $[CO.C_6H_4.CO.O.CH_2CH_2O]_x$ where x=3-8 (Harrison et al., 1997)

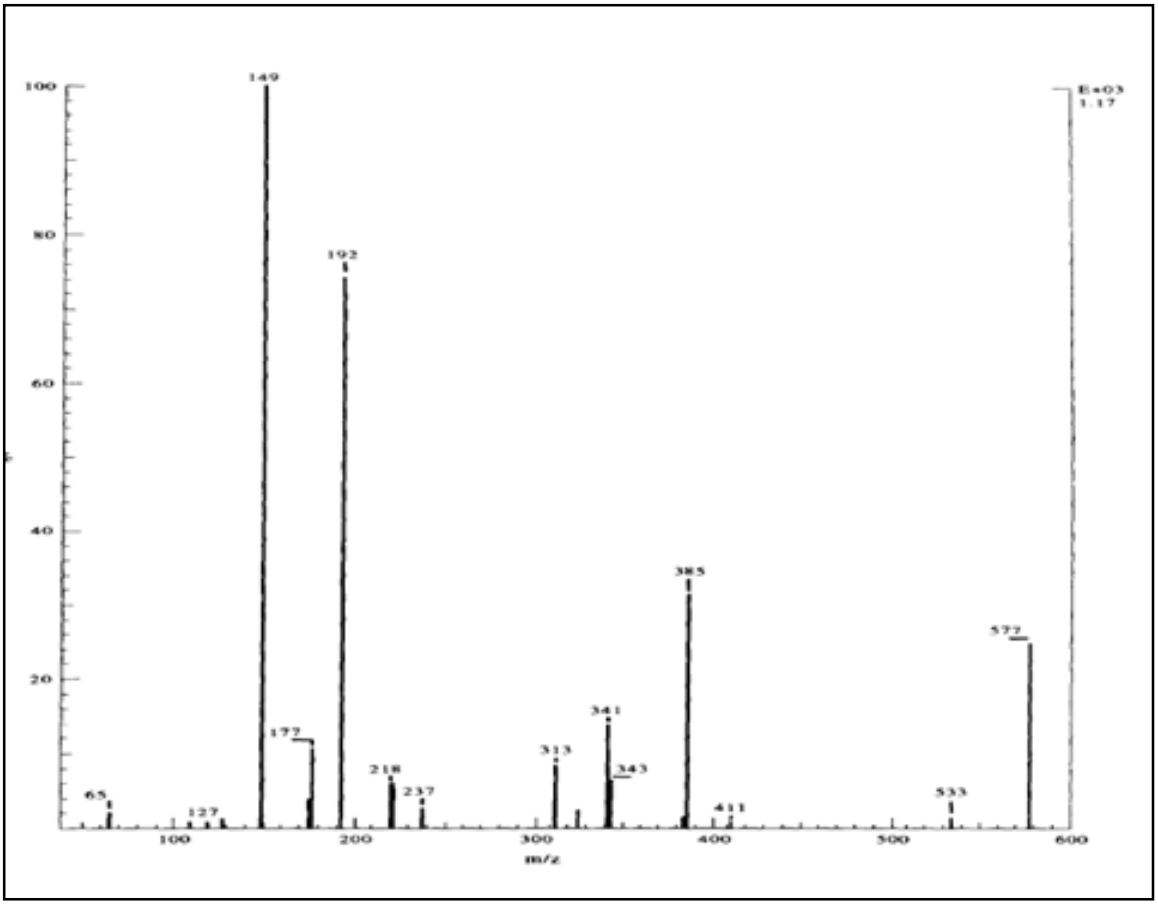


Figure 21: Fast Atom Bombardment (FAB) Mass Spectrum of Cyclic PET Trimer (Harrison et al., 1997)

- Nasser et al., (2005)

Nasser et al., (2005) identified the oligomers in polyethylene terephthalate (PET) bottles for mineral water and fruit juice. The oligomers were extracted in dichloromethane and identified using electrospray mass spectrometry (ESI-MS). The structural identification was confirmed by using NMR technique. The structures reported by Nasser et al. are listed below along with their chromatograms (**Fig. 22, Fig. 23 and Fig. 24**). However, the mass spectra of the compounds were not discussed (Nasser, 2005).

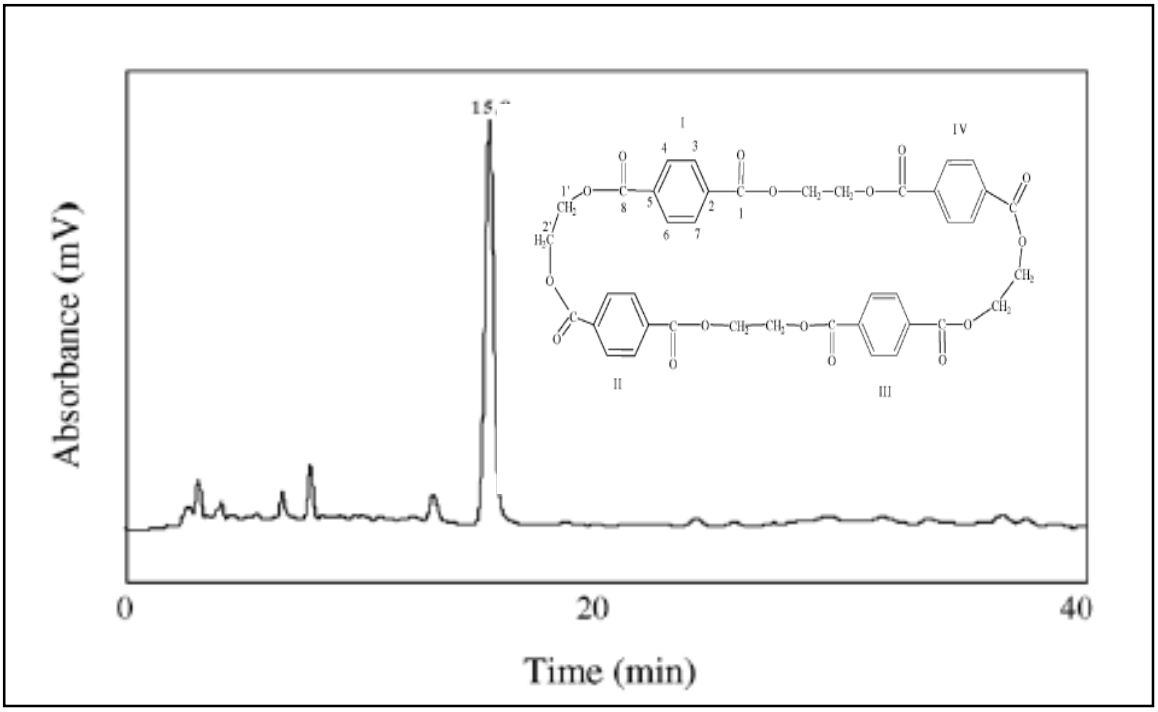


Figure 22: HPLC Chromatogram of Cyclic Tetramer in PET Bottles (Nasser et al., 2005)

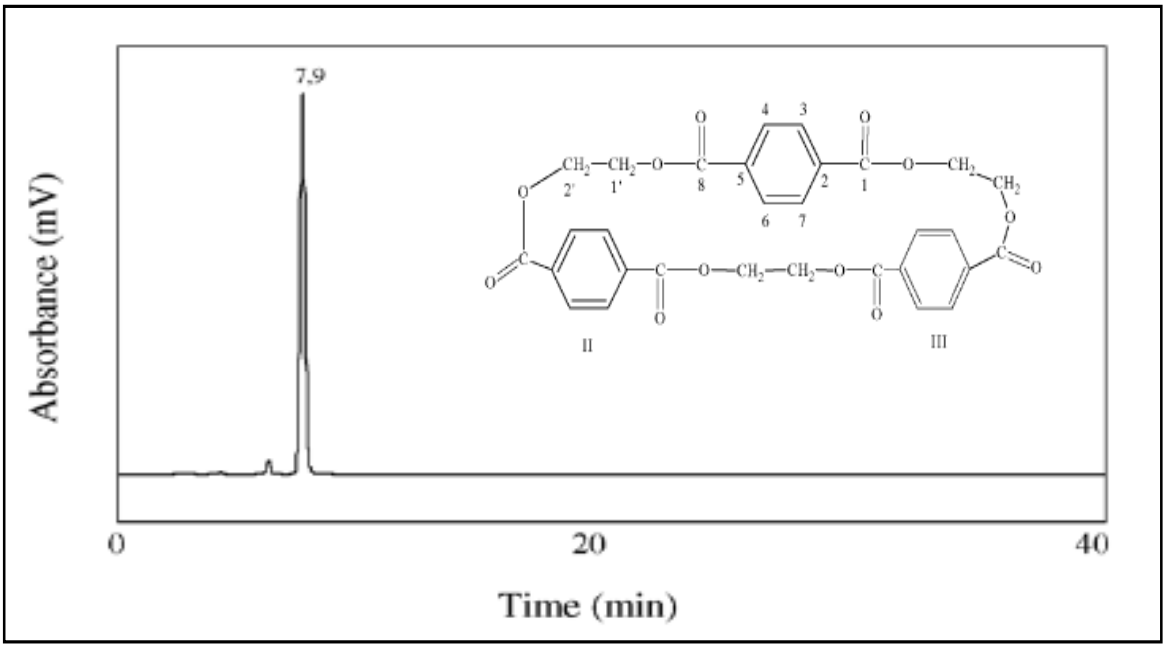


Figure 23: HPLC Chromatogram of Cyclic Trimer in PET Bottles (Nasser et al., 2005)

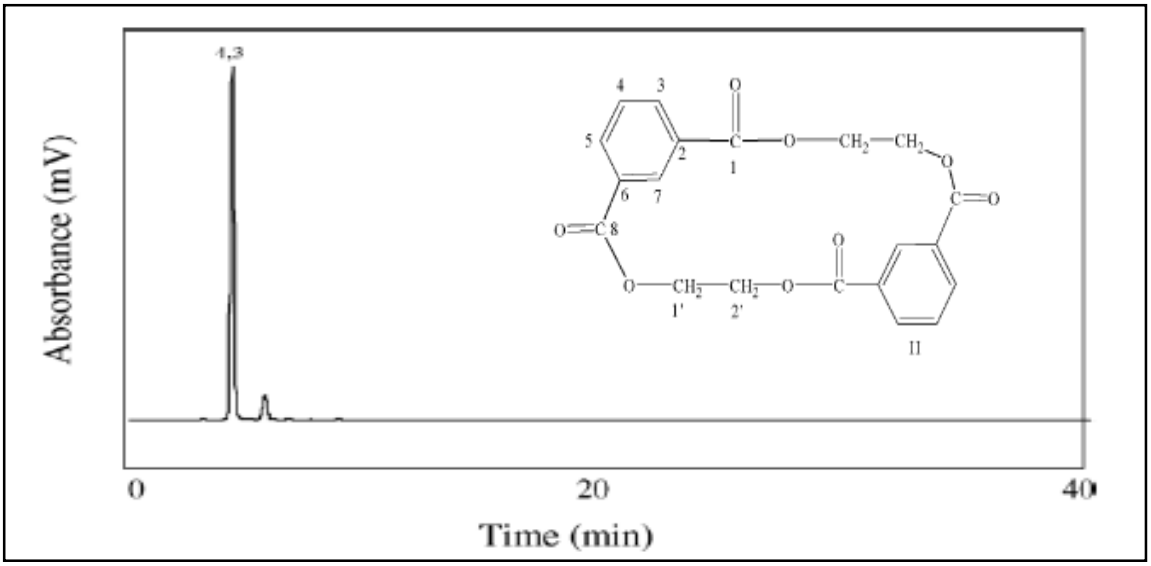


Figure 24: HPLC Chromatogram of Cyclic Dimer in PET Bottles (Nasser et al., 2005)

In the previous studies, researchers adopted analytical techniques like HPLC, GPC, MALDI-TOF-MS, LC-MS to identify and analyze cyclic polyester oligomers. It was reported that, the GPC could not accurately identify the high molecular weight, large cyclic and linear polyester oligomers (Laine et al., 2001). Although MALDI-MS, soft ionization method, provided nominal mass of the migrants, it did not provide any information on the fragmentation pattern of these migrants. It was also noted by the authors (Lawson, 2000) that MALDI-MS measurements could not be quantified easily, thus driving the need to develop a derivatization protocol to facilitate the HPLC quantification.

In some studies the polyesters were synthesized from the raw material and the cyclic oligomers so formed were characterized and quantified as opposed to using the commercial polyester samples. Commercial packaging samples contain definite amount of starting materials such as polyesters, dicarboxylic acid and diols as stipulated by the

regulations. Therefore, using commercial packaging samples would yield better results in terms of quantification of the migrants leaching from the sample. Using the synthesized samples would make it difficult to quantify the migrants consistently. Also it was noted that the migration of these compounds in the food was not thoroughly studied.

It was noted that the, during the previous investigations, cyclic oligomers of aromatic acids especially phthalates, isophthalates and terephthalates were reported. But the cyclic oligomers of the aliphatic acid were not reported extensively. The cyclic oligomers in the mass range above 400-1000 Da were reported in the literature, however, not many oligomers in the mass range 150-450 Da were found to be reported in the literature.

According to EU commission packaging migrants below 1000 Da are important from the toxicological point of view as they could be absorbed in the gastrointestinal tract. Hence, it is crucial to assess the safety of these compounds. However, toxicological properties and their safety related information has not been investigated yet.

The cyclic oligomers were reported to be undesirable in polyesters as they migrated and caused hazy surface deposits (Brugel et al., 2003). Also they lacked the functionality and hence could not participate in the polymerization reaction.

III.B. Previous Work on Hydrolysis of Short Chain Polyester Oligomers

Toxicological properties of the monomers used in polyesters synthesis have been well studied and documented. However, short chain cyclic polyester oligomers are considered to be novel compounds; their safety and toxicological properties have not been investigated before.

Studies on hydrolysis of flavoring esters under simulated gastrointestinal fluids, fresh preparations of rat liver and intestine and pancreatic rat lipase have been previously reported in literature to assess the hydrolytic fate of esters (Mattson et al., 1969; Longland et al., 1977; Harrison et al., 1989). Flavoring esters prepared by combining variety of acids and alcohols are extensively used in foods and cosmetics. It is crucial to assess the safety of these compounds as human exposure to this class of compounds can occur from wide variety of sources. The Flavoring Group Evaluation and general information on these compounds indicated that flavoring esters were rapidly absorbed in the gastrointestinal tract, metabolized and finally excreted. It was also anticipated that the esters would undergo hydrolysis to yield corresponding carboxylic acid and alcohol ((EC) 2000).

Longland et al., (1977) studied the rates of hydrolysis of 16 esters, used extensively as constituents of artificial flavors, in simulated gastrointestinal juices and fresh preparations of rat liver and small intestine. Artificial gastric and intestinal juices were prepared as mentioned in Pharmacopoeia Helvetica while the tissue homogenates were prepared from the rat organs viz. liver and intestine. The esters were hydrolyzed and the reaction kinetics was studied. The results of the ester hydrolysis indicated that the simulated gastrointestinal juices had a limited ability to hydrolyze the flavoring esters. As

opposed to that, the tissue homogenates from rat liver and small intestine proved to be extremely efficient in hydrolyzing the esters. In the light of these findings it was noted that studies involving liver and small intestinal preparations would be a more accurate way to investigate the hydrolytic fate of esters in in-vitro conditions to evaluate their toxicological properties (Longlang 1977).

Previously, simulated gastric and intestinal hydrolysis studies have been conducted on polymeric plasticizers, in order to study their likely metabolic fate after their ingestion through contaminated food. During the analysis, the parent molecule ion was not found, indicating the occurrence of partial hydrolysis under simulated gastric conditions; with low molecular weight oligomers being most susceptible. Further, it was deduced that polymeric plasticizer contained ester linkages which were more resistant to the gastric hydrolysis conditions; hence, complete breakdown of the ester backbone yielding acid and alcohol was not observed (Castle et al., 1993; Hamdani et al., 2002). However, plasticizers have not been exposed to the simulated enzymatic hydrolysis conditions to determine their potential in vitro metabolic fate.

Human non-specific esterase enzyme is ubiquitous in nature and is found in human serum and tissues and bone marrow (Bisswanger, 2004). Non-specific esterase enzyme does not require a specific binding site, as opposed to certain specific enzymes, to elicit its effect. Also, non-specific esterase enzyme, being a protein, carries ionic groups, hydrogen bonds and hydrophobic regions, which enable the substrate to bind to the enzyme (Bisswanger, 2004).

Short chain polyester oligomers contain ester linkages whose metabolic fate after exposure to the enzyme treatment has yet to be discovered. We predict that, if these short chain cyclic polyester compounds are absorbed into blood stream via dietary

intake, they will be metabolized in vivo by non-specific esterase enzymes present in the blood stream or tissues. The effect of enzyme treatment on short chain cyclic polyester oligomers is discussed in detail under results and discussion section.

IV. Rationale and Research Objective

Polyurethane offers excellent adhesion properties, good chemical and abrasion resistance; hence, it has been a common choice of adhesive used in the food industry. Polyesters are the starting raw materials in the manufacture of polyurethane and they impart the required flexibility to polyurethane for food packaging. The short chain cyclic polyester oligomers formed during the manufacture of polyesters are low molecular weight, non reactive species and tend to migrate out of the packaging into the contents.

There is considerable concern regarding the safety of the compounds migrating from the adhesives into the food systems, including short chain cyclic polyester oligomers. To ensure the safety of the food, different legislations have been enforced on the use of adhesives by USFDA and European Union (EU). However, it is noted that very little work has been reported in the literature on the analysis of the migrants from food packaging adhesives (Lawson, 2000).

In the previous studies, the cyclic polyester oligomers were synthesized and extracted in organic solvents and quantified (Nasser et al., 2005; Harrison et al., 1997; Lim et al., 2002). However, only little work has been reported on the migration on these compounds in food systems using the food simulants recommended by the USFDA. The migration in the organic solvents is markedly different from that in food systems as the organic solvents tend to excessively swell the polymer, which accelerates the rate of migration.

During the previous investigations on cyclic polyester oligomers, analytical methods employed included mainly the chromatography methods like gel permeation chromatography (GPC) and high performance liquid chromatography (HPLC),

colorimetric analysis interfaced with soft ionization technique, matrix-assisted laser desorption/ionization (MALDI). The time of flight (TOF) mass spectrometer coupled with MALDI was a preferred choice of method used as the oligomers analyzed were in the mass range 400 Da to 900 Da. However, no literature was found on short chain cyclic oligomers in the mass range 150 Da. – 400 Da. Also, the methods mainly used for the identification of these oligomers were based on comparing them with the previously synthesized, anticipated oligomers (Bradley et al., 2009; Ahjopalo et al., 2000; Laine et al., 2000). It was also noted that electron ionization and chemical ionization mass spectrometry have not been exploited enough in the identification of cyclic polyester oligomers. The previous studies on oligomers also attempted to quantify the amount of migrants; however, no data about their migratory concentrations have been reported in the literature.

According to the European Commission, packaging migrants below the mass 1000 Da are important from a toxicological point of view as they can be absorbed in the gastrointestinal tract (Commission Regulation (EU) N. 10/2011, 2011). It was reported in the literature that absorption of molecules above 1000 Da was less than 1% (Schaefer, 2004). Previously conducted simulated gastric and intestinal hydrolysis studies on polymeric plasticizers have been reported in the literature indicating the occurrence of partial hydrolysis (Castle et al., 1993; Hamdani et al., 2002). The esterase enzymes in the bloodstream and tissues can potentially hydrolyze the absorbed polyester oligomers. The hydrolysis products can enter the cell, thus disturbing the cell environment (Ikada, 2000). Hence, it is crucial to assess the toxicological parameters of these compounds. It was noted that, there was no literature reported on the enzymatic hydrolysis of polyester oligomers in vivo condition. The potential safety of this class of compounds still remains uninvestigated due to lack of information. Although the safety and toxicological

properties of monomers used for polyester manufacture have been well studied, there is no safety or toxicology database established for this class of compounds.

The objective of the research was to study the chemistry and the migratory properties of short chain cyclic polyester oligomers using gas chromatography (GC) interfaced with mass spectrometric techniques. This research also focused on studying the predicted in vivo metabolic fate of this class of compounds under in vitro enzymatic hydrolysis conditions.

The chemical structures deduced in this research, would aid chemists to investigate structural properties of this class of compounds, thus providing a good understanding about their potential interactions in the food systems. Retention time indices obtained from the GC-MS analysis coupled with fragmentation patterns, would be a useful tool to characterize the short chain cyclic polyester oligomers. The in vitro metabolic fate data would assist the regulatory scientists and toxicologists to assess the safety of these packaging borne migrants.

V. Materials and Methods

V.A. Materials

Reagents and Reference Standards

Anthracene-d_{10} (CAS No. 1719-06-8, Lot 04021HO, 98% Atom D) used as an internal standard for the study was purchased from Aldrich Chemical Co. 1,6-Hexanediol (CAS No. 629-11-8, 99%), Diethylene Glycol (CAS No. 111-46-6, 99,5%), 1,4-Butanediol (CAS No. 110-63-4, 99%), Adipic Acid (CAS No. 124-04-9, 99%),Phthalic Acid (CAS No. 88-99-3, 99.5%) and Neopentyl Glycol (CAS No. 126-30-7, 99%) used as reference standards in the enzyme study was obtained from Aldrich Chemical Co. Sylon BFT derivatizing agent (BFTSA + TMCS, 99:1, 0.1 ml ampoules) was purchased from Supelco. Esterase enzyme from porcine liver (CAS No. 9016-18-6), used to predict the in vitro metabolic fate of short chain cyclic polyester oligomers, was ordered from Sigma Aldrich. The ethanol used in the study (USP grade) was purchased from Pharmco Products. Dichloromethane solvent (Optima grade) was bought Fischer Scientific. Distilled and deionized water used in the study was from “in house” and was prepared by double distillation in a glass-lined still followed by activated carbon filtration and deionization treatment in a Waters Milli-Q Nanopure TM system.

Single side extraction cells were used for the migration testing of short chain cyclic polyester oligomers. The extraction cells have been designed according to the specification of FDA for the food contact polymer migration and have been developed by Hartman (Hartman, 2010).

Single side extraction cell assembly (**Fig. 25**) comprises of 10 cm x 15 cm stainless steel (SS) plates (top and bottom) with Teflon spacer gasket sandwiched between the

plates. The assembly is held firmly by bolting 12 SS cap screws around the perimeter. The top stainless steel plate contains SS tube ports of outer diameter ¼ inches for filling and emptying the contents. The ports are sealed with ¼ in. SS Swagelok® caps with Teflon ferrules. Teflon gasket isolates 7.9 in^2 or 51 cm^2 of only food contact surface area and can hold different volumes (30 ml, 62.5 ml, and 120 ml) of food stimulants depending on the size of the gasket.

A sample film was placed on top of the bottom stainless steel plate in such a way that the food contact surface was facing in the upward direction. Teflon gasket was placed on top of the food contact side. Teflon gasket cavities allow the food stimulant to reach the food contact surface of the film. The top stainless steel plate was placed on the Teflon gasket and the assembly was tightened together with the screws. Through the port on the top stainless steel plate, the food stimulant was introduced to the cell. The ports were sealed tight with caps so as to retain the simulant solvent and volatile compounds, if any. The cells are then conditioned at suitable temperature.

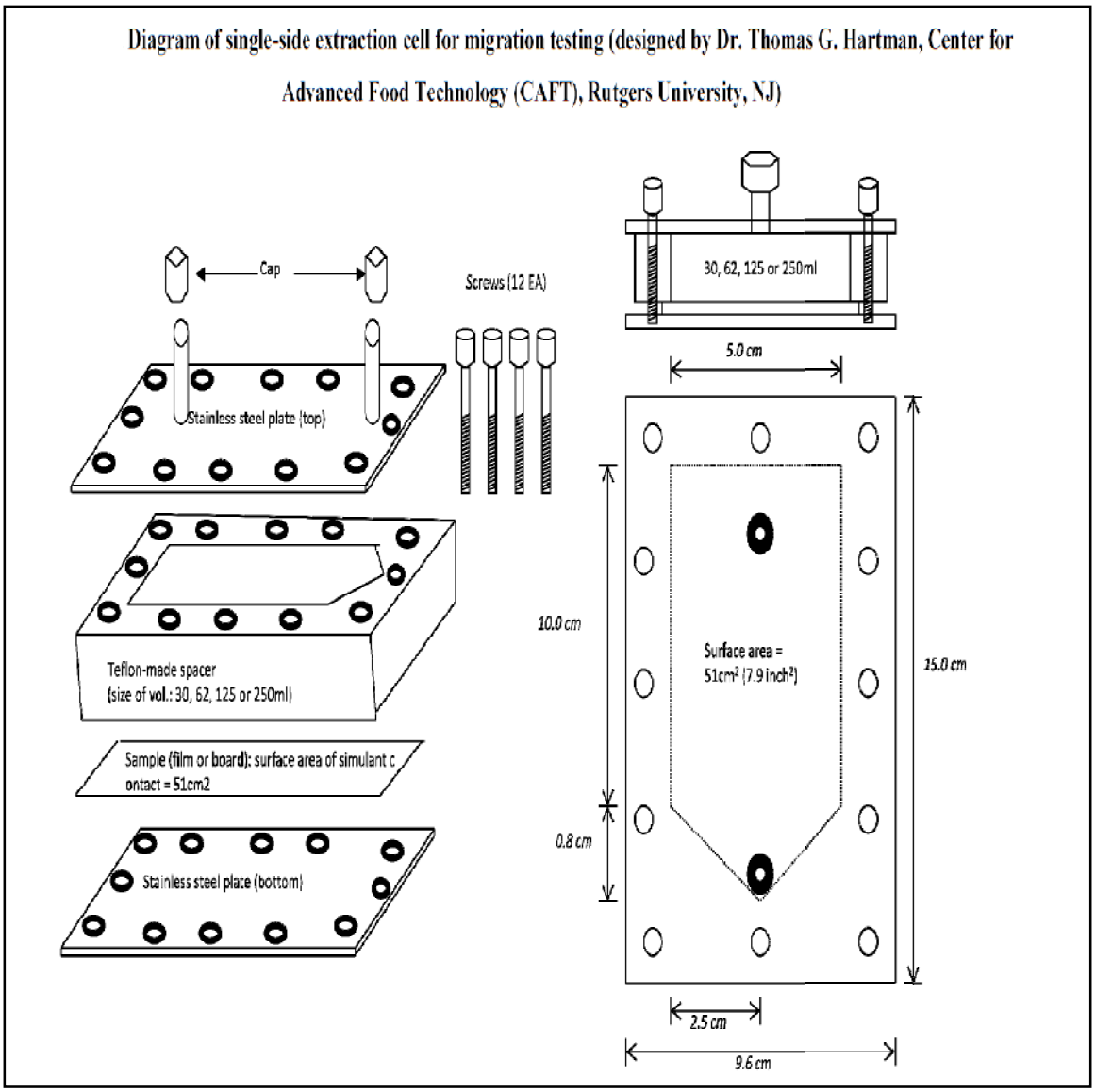


Figure 25: Single Side Extraction Assembly (Hartman, 2010)

V.B. Methods

The scope of the research work included studying the chemistry and migratory properties of the short chain cyclic polyester oligomers and determining their in vivo metabolic fate. The methods are divided into two subparts.

1. Migration testing of laminate packaging
2. Enzymatic hydrolysis of short chain cyclic polyester oligomers

V.B.1. Migration Testing of Laminate Packaging

The methods used for conducting the migration testing of laminate packaging are validated by USFDA. Since these cyclic oligomers were not highly soluble in water, organic solvents were used for extraction purpose.

The sample preparation with acidic food simulant (10% EtOH) and fatty food simulant (95% EtOH) is discussed below, as per the USFDA recommendations.

a) Sample Preparation with 10% Aqueous Ethanol

The single side extraction cell was thoroughly cleaned with water and subsequently with acetone and dichloromethane, in the decreasing order of polarity, so as to remove organic as well as inorganic impurities. The cell was then baked out in the oven at 100 ℃ for 15 min. The Teflon spacer was cleaned with warm water and kept in the oven at 40 ℃ for 15 min to dry out.

The commercially manufactured laminate packaging films were used for the experiments. The food contact side of the film was made from Polyethylene, while the outside layer of the film was Polyethylene Terephthalate (PET). The laminate film was placed on the bottom stainless steel plate of the extraction cell assembly in such a way

that the food contact side or the sealant side was facing in the upward direction. The Teflon gasket was placed above the film, followed by the top stainless steel plate. The entire assembly was tightened with stainless steel screws. 30 ml of 10% aqueous ethanol was introduced in the single side extraction cell through the stainless steel tube port which was then sealed with stainless steel caps having Teflon ferrules. The extraction cell was placed in the oven at 100 ℃ for 30 min.

The extraction cell was cooled down and was allowed to attain room temperature. The content of the cell was then emptied in test tubes with Teflon-lined cover. 100 ppb of internal standard was spiked in the simulant to quantify the concentration of the short chain cyclic polyester oligomers. The internal standard chosen for the experiment was Anthracene d-10 in dichloromethane. The advantage of using deuterium labeled standards was to achieve good isotopic purity. The test tube was vortexed to ensure the thorough mixing (Chapman, 1993).

The dichloromethane was added to the test tube to extract the short chain cyclic polyester oligomers. The organic solvent forms an immiscible layer with the 10% Ethanol; the cyclic polyester oligomers are partitioned between the two phases. The test tubes were centrifuged for 30 min at 2000 rpm. Centrifugation allowed the two phases to separate and facilitated the extraction of short chain cyclic polyester oligomers into the organic phase. The dichloromethane layer was then separated and was concentrated with a nitrogen stream. 1 µl of the extract was injected in the GC system for the analysis.

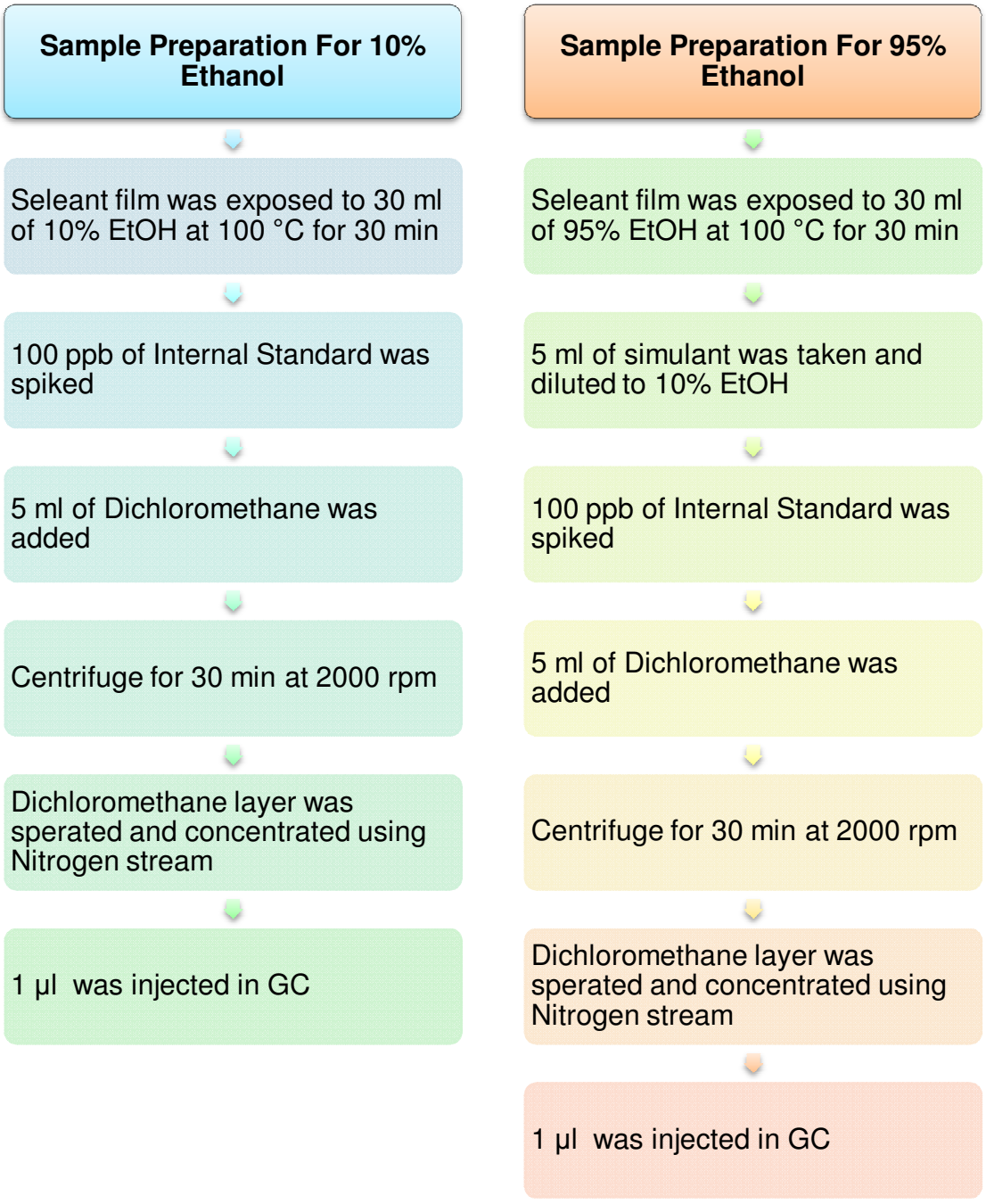
Sample Preparation with 95% Ethanol

Previously cleaned single side extraction cells with water and organic solvents were baked out in the oven at 100 ℃ for 30 min. The sealant side Polyethylene (PE) film was

placed in the extraction cell and 30 ml of 95% ethanol was introduced into the extraction cell assembly via stainless steel tube port. The cell was conditioned at 100 ℃ for 30 min.

After 30 min the extraction cells were cooled to room temperature. The simulant was transferred into glass test tube. 5 ml was pipetted out and was transferred into another glass tube. 47.5 ml of water was added to the test tube to make up 10% aqueous ethanol solution by dilution. The organic solvent, dichloromethane, added to extract the polyester oligomers in the organic phase was miscible with 95% ethanol and immiscible with 10% ethanol. The dilution allowed the extraction of cyclic polyester oligomers into the organic phase.

Anthracene d-10 was chosen as an internal standard for the experiment. 100 ppb (0.1 mg/ml) of the internal standard was added to the test tube. 5 ml of Dichloromethane was added to the test tube and it was centrifuged at 2000 rpm for 30 min. The bottom organic layer was transferred into small glass vials. The layer was concentrated using nitrogen stream at room temperature. 1 µl of the extract was injected in the GC system.



Flowchart 1: Sample Preparation Overview for Migration Testing

V.B.2 Enzymatic Hydrolysis of Short Chain Cyclic Polyester Oligomers

This section deals with methods to prepare sample extracts for the enzymatic hydrolysis by non-specific porcine esterase enzymes. The methods used for preparing the derivatized extracts are also discussed.

a) Preparation of Standard Stock Solutions:

As discussed previously, the short chain cyclic polyester oligomers are formed during the manufacture of polyesters, which are the integral components of polyurethane adhesives. If these short chain cyclic polyester oligomers were to be ingested, they would be acted upon by non-specific esterase enzymes in the saliva, liver and blood stream. The complete metabolisms of these compounds by enzymatic action would lead to the formation of the original constitutional monomers. The stock solutions of raw materials used in the preparation of polyesters were prepared in acetone (1 mg/ml) as shown in the following **Table 10**.

Table 10: Standard Stock Solutions of Monomers

Compound Name	Concentration of Stock Solution in Acetone
1. Phthalic Acid	1 mg/ml in acetone
2. Adipic Acid	1 mg /ml in acetone
3. Neopentyl Glycol	1 mg/ml in acetone
4. Diethylene Glycol	1 mg/ml in acetone
5. 1,4-Butanediol	1 mg/ml in acetone
6. 1,6-Hexanediol	1 mg/ml in acetone

b) Preparation of Trimethylsilane (TMS) Derivatives

Some compounds cannot be analyzed by a particular analytical method if they exist in the form which does not comply with the said analytical method and procedures. Derivatization modifies the chemical structure of the compounds in such a way that compounds become amenable to the desired analytical technique. It also enhances detection sensitivity of the compounds.

Compounds of interest contain functional groups with active hydrogen (e.g. dicarboxlic acid and diols) which possess a great tendency to form intermolecular hydrogen bonds and interact with column packing material or active sites in the analytical system. This in turn leads to poor peak resolution. To avoid the difficulty in the analysis, samples were derivatized.

The derivatization method chosen for the experiment was silylation and the reagent used was Sylon BFT. Sylon BFT is a combination of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) in the ratio 99:1, respectively. **Figure 26** shows the chemical structures of these reagents.

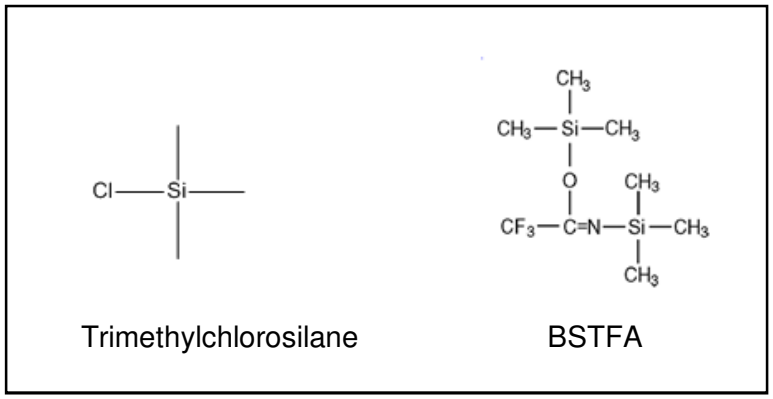


Figure 26: Structure of TMCS and BSTFA

10 µl of the previously prepared stock solution was taken into a small glass vial. 80 µl of acetone was then added to the vial. The contents of the vials were then vortexed to ensure uniform mixing. The silylation reagent, Sylon BFT (BSTFA + TMCS, 99:1) in pyridine solvent, was added to the vial to conduct the derivatization reaction. The vials were tightly stoppered and were placed on a heating block for 1 h at 80 ℃ for the completion of the reaction. 1 µl was then injected into the GC-MS System. The results were used as a reference for the enzyme study.

c) Sample Preparation to Study the In-Vitro Fate of Short Chain Cyclic Polyester Oligomers

The enzyme selected for the study was Porcine Esterase enzyme, obtained from Sigma Aldrich. Enzymatic Assay Protocol was followed for preparing a suitable buffer solution and the esterase enzyme solution for the in vitro study.

Preparation of 0.01 M Borate Buffer

The reaction was carried out in the buffered solution as the buffer set the initial pH of the solution and resisted or minimized the changes in its pH 0.01 M Borate buffer was prepared by dissolving 0.0309 g of Boric Acid in 50 ml deionized water at room temperature. The pH was adjusted to 8.0 using 1 M NaOH.

Preparation of Esterase Enzyme Solution

As per the enzyme assay instructions, 50 units/ml of esterase solution in cold borate buffer was prepared by dissolving 147.05 mg solid enzyme powder in 50 ml buffer solution.

Sample Preparation for the Enzymatic Hydrolysis

The previously prepared sample extracts (either 10% EtOH or 95% EtOH) in vials were completely evaporated to dryness using gentle nitrogen stream. 10 µl of pure ethanol was added to dissolve the samples and serve as co-solvent for the enzyme and buffer solution.

To the vials, 50 µl of previously prepared esterase enzyme was added. The vials were then incubated at 37 ℃ for 1 h with periodic vortexing. After an hour, the vials were taken out to attain room temperature. Further, the vials were then evaporated to dryness using a metal heating block to remove the water, as the silylation reagents are moisture reactive and must be protected from moisture. Finally 50 µl of acetone and 20 µl of Sylon BFT reagents were added to the vials followed by vortexing. The vials were then kept on the metal heating block at 80 ℃ for 1 h for the derivatization reaction to complete. 1 µl was then injected into the GC-MS system.

V.C. Instrumentation

Gas chromatography, when combined with mass spectrometry, becomes a powerful analytical tool in the identification and quantization of the unknown compounds. Alone, GC can separate volatile or semi volatile compounds with a good resolution but cannot identify them. On the other hand, Mass Spectrometer provides detailed structural information of the compound. In this research, the GC-MS system was used to determine the molecular weights, chemical structures and the migratory concentration of short chain cyclic polyester oligomers.

GC-MS analysis was performed on a Varion 3400 gas chromatograph that was directly interfaced to Finnigan MAT TSQ7000 mass spectrometer.

V.C.1. GC Conditions

1. Column selection for GC:

The optimized chromatographic separation begins with choosing the right column for the analysis. The factors which govern the selection of the column are phase polarity, internal diameter (I.D.), film thickness and the length of column. Phase polarity is a critical factor in selecting the column as it controls the selectivity or the ability of the column to separate the components of the sample. The polar column is used for the compounds with polar functional groups. High efficiency of the capillary column is achieved by decreasing the internal diameter of the column. At the same time, it is important to note that sample capacity is dependent on I.D. of the column. Extremely low column I.D. will result in very low sample capacity. Increasing the sample capacity of the column will result in the skewed peaks and decrease in the resolution. Columns with reduced film thickness will provide sharper peaks, reduced column bleed and improved signal to noise ratio. It also increases the maximum operating temperature of the

column. Selecting the length of the column is a compromise between speed and head pressure one hand, and peak resolution on the other hand. Longer the length of the column, higher is the peak resolution. However there are practical limits to increasing the length of the column.

Considering above factors, 30 m × 0.32 mm I.D. × 0.25 µm film thicknesses – SLB™-5ms Capillary GC Non-Polar Column was selected for the analysis **(Fig. 27)**. Additionally, the column provided low bleed which lowered the detection limit of the analyte.

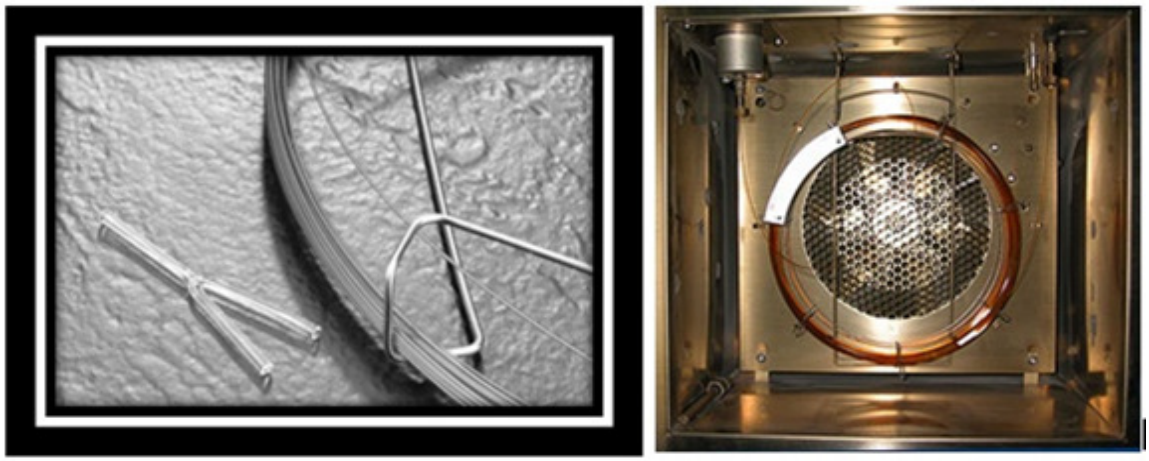


Figure 27: Supelco SLB™-5ms Capillary GC Column

2. Sample Injection:

The instrument is provided with sample injection port which introduces the analyte on the head of the column. The injection port consists of a rubber septum through which a syringe needle is inserted to inject the sample. The temperature of the port is higher than the boiling point of the least volatile component of the analyte. This ensures easy vaporization of the analyte so that it can comply with the method.

A calibrated micro-syringe shown in **Fig. 29** was used to inject 1µl of the sample through the rubber septum into the vaporization chamber of the GC instrument. The injections were made in the split less mode **(Fig. 28)** followed by a septum purge 100:1 split after 5 min. The temperature of the sample injection chamber was maintained at 300 ℃.

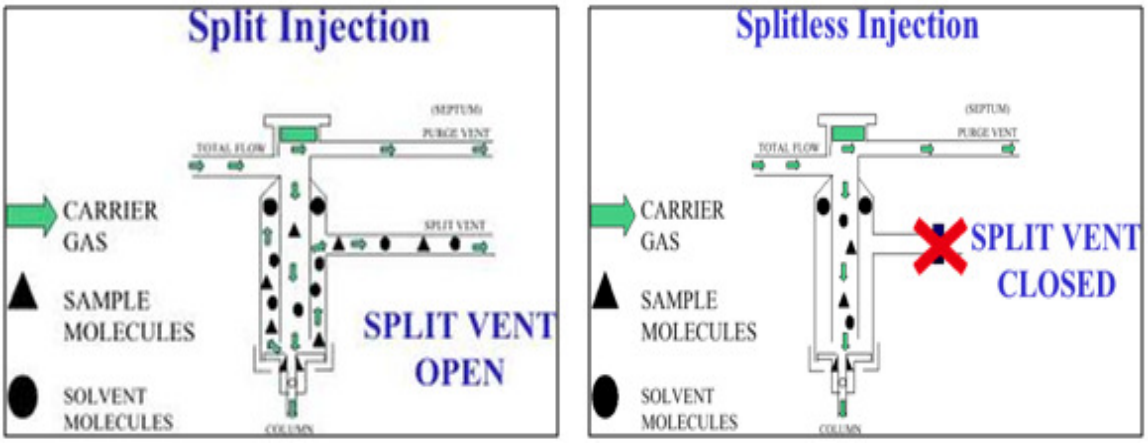


Figure 28: Split injection and Splitless Injection Mode (SGE Analytical Science)

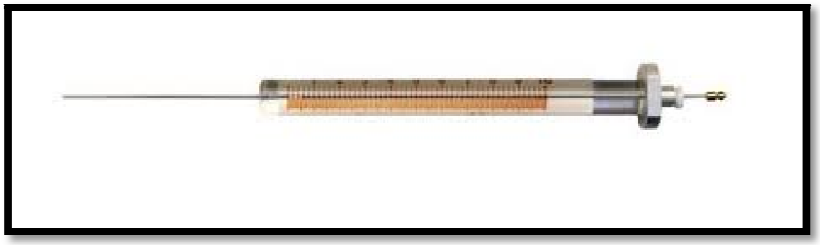


Figure 29: Calibrated Micro Syringe

3. Carrier Gas:

The selection of the carrier gas depends on the type of detector used in the analysis, sample matrix, purity of the gas. The carrier gas linear velocity or the flow rate has an important role in influencing retention time and efficiency. The efficiency and the reproducibility of the analysis are obtained by controlling the head pressure of the carrier

gas. The pressure adjustment depends on the type of the gas, length of the column and the column temperature.

Being an inert gas, Helium was used as a mobile gas phase in the GC-MS analysis. Helium has low density and diffuses the solutes rapidly and improves the rate of mass transfer in the mobile phase, thus regulating the column efficiency.

4. Column Temperature and the Temperature program Control

The rate at which the analyte travels the length of the column is directly proportional to temperature of the column. Higher the column temperature, faster would be the elution time of the analyte. However, higher temperature leads to lesser interaction of analyte with the stationary phase, as a result of which the retention characteristic of the analyte is affected. However, the use of temperature control program allows heating of the oven at the controlled rate, thus reducing the peak broadening and improving the retention times of the solute.

A linear temperature program was implemented. The initial column temperature was set at 50 ℃ with initial hold time of 3 min. The hold time improves the resolution of the earlier eluting peaks. The final column temperature 320 ℃ was achieved with a ramp rate of 10 ℃/min for 30 min.

V.C.2. Mass Spectrometer Conditions

In mass spectrometer, the sample in the gaseous state is bombarded with a beam of electron to produce the molecular ion of the original molecule. Since the molecular ion is unstable it breaks into fragments. The fragmented ions are the separated by their m/z ratio and detected by the detector. The ion signal is then converted into mass spectrum, which is a plot of m/z vs. intensity of the fragments. The different components of mass spectrometer are: Ion Source, Mass Analyzer and Detector.

1. Ion Source:

The ion source converts the gaseous analyte molecules into ions by bombarding them with a beam of electrons. Electron ionization and chemical ionization modes were used during the research experiments. Chemical ionization helps in identifying the accurate molecular weight as the analyte does not fragment and the molecular ion remains intact. The electron ionization mode was operated with a 35-750 scanning range, while the chemical ionization mode with a 100-600 scanning range.

2. Mass Analyzer

The mass analyzer separates the ions according to m/z ratio. There are many types of mass analyzers which utilize static or dynamic field, magnetic field and electric field. The analyzers used in the instruments were double focusing magnetic sector and quadrupole mass analyzer.

The double focusing magnetic sector consisted of electric and magnetic fields to bend the trajectories of charged ions as they passed through the mass analyzer according to m/z ratio (Cottrel, 1986).

VI. Results and Discussion

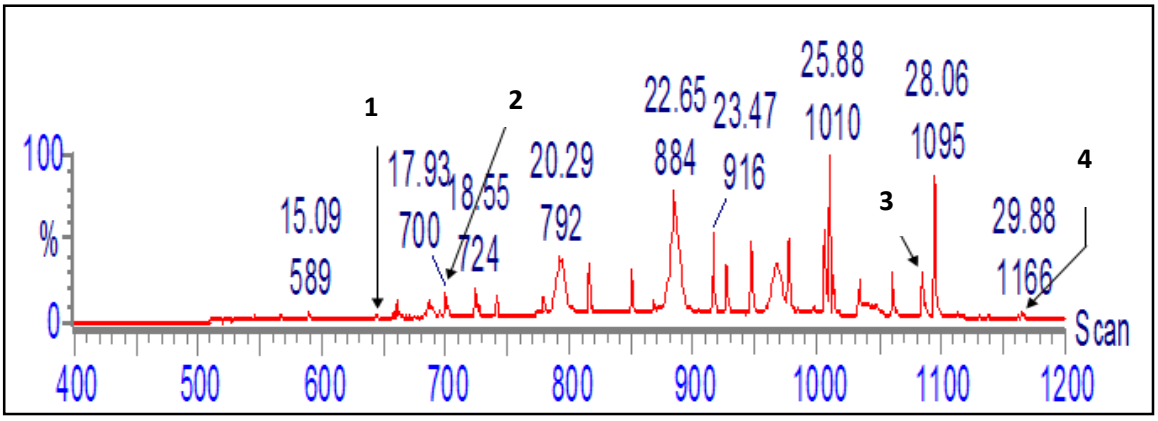
VI.A. Data Analysis of Migrants from Laminate Packaging

From the single side cell extraction of food grade laminates, complying with FDA recommended testing conditions, it was noted that many compounds migrated from the laminate into the food simulant system. However, the focus of the research was to study the migration potential of short chain cyclic polyester oligomers, only the analysis of these compounds have been discussed in detail in this section. The short chain cyclic polyester oligomers detected in 10% EtOH and 95% EtOH sealant-side laminate extracts are listed in **Table 11.**

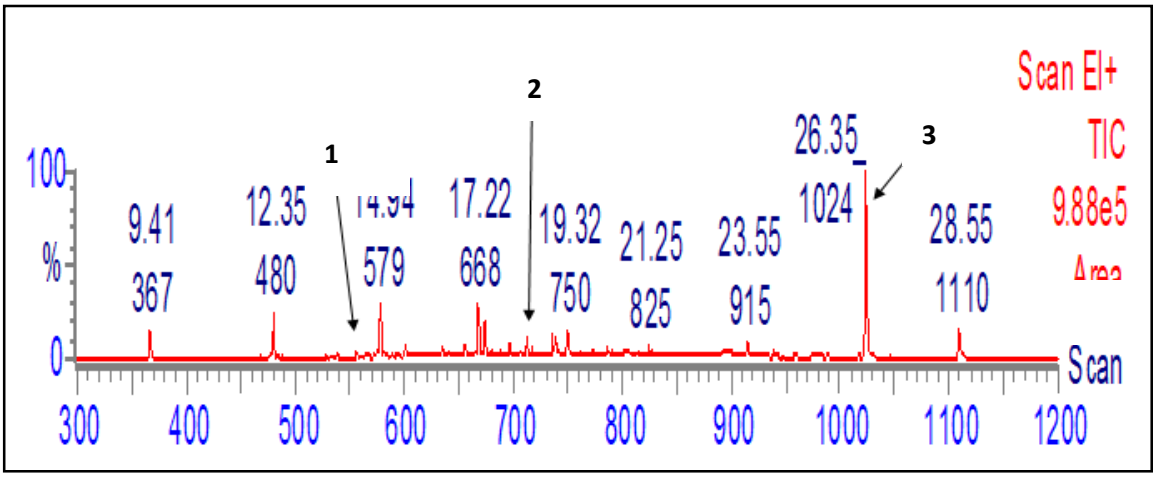
Table 11: Short Chain Cyclic Polyester Oligomers Detected in 10% and 95% EtOH Sealant-Side Laminate Extracts

	Molecular Weight (g/mol)	Name of the Compound
1	200	1,4-Butanediol, adipic acid, cyclic diester
2	216	Diethylene glycol, adipic acid, cyclic diester
3	228	1,6-Hexanediol, adipic acid, cyclic diester
4	236	Diethylene glycol, phthalic acid, diester
5	400	Di-(1,4-Butanediol, adipic acid, cyclic diester)
6	214	Neopentyl glycol, adipic acid, cyclic diester
7	428	Di-(Neopentyl glycol, adipic acid, cyclic diester)
8	432	Di-(Diethylene glycol, adipic acid, cyclic diester)
9	472	Di-(Diethylene glycol, phthalic acid, cyclic diester)
10	172	Ethylene glycol, adipic acid, cyclic diester

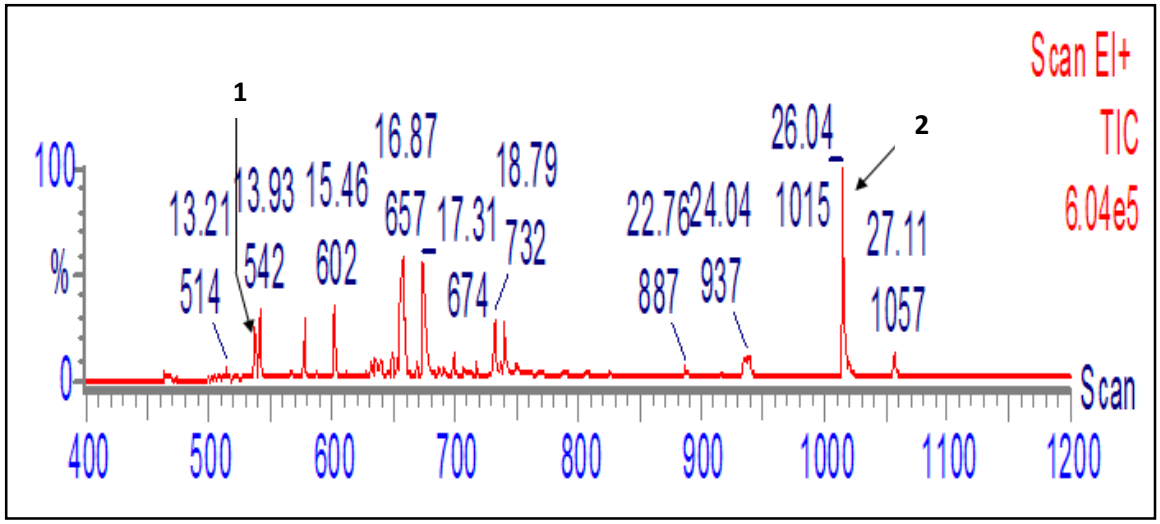
Chromatograms 1, 2, 3, and **4** depict the peaks of the short chain cyclic polyester oligomers detected in 10% Ethanol and 95% Ethanol (along with molecular weights in g/mol and the retention time in min) which are summarized in **Table 11**. In the chromatograms, X- axis represents the scan numbers, while the Y-axis represents the percentage intensity of the peaks.



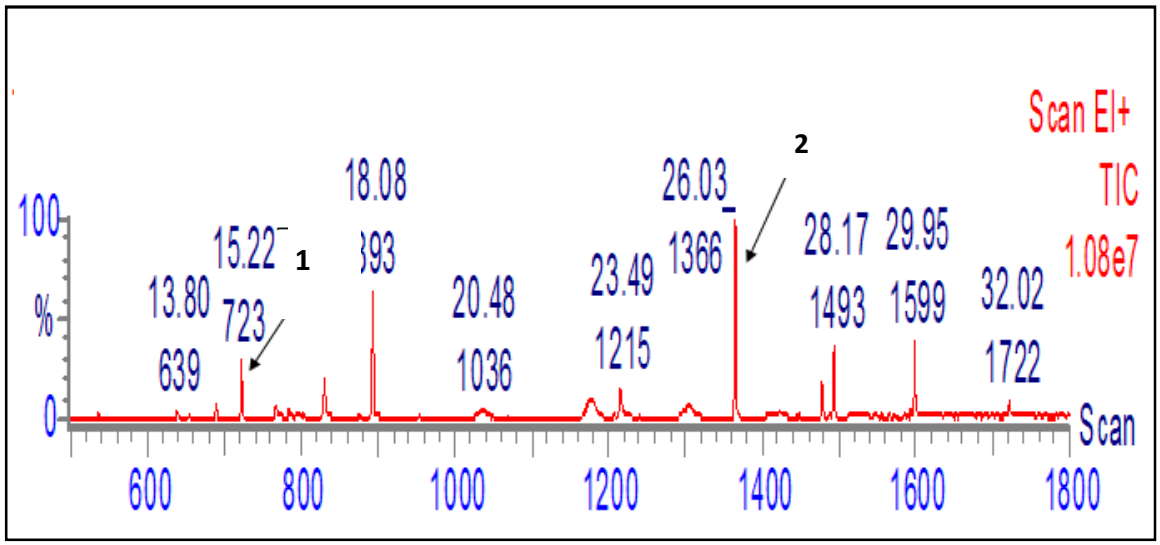
Chromatogram 1: GC-MS-TIC Chromatogram of 10% EtOH Sealant-Side Laminate Extract Showing Peaks of 1,6-Hexanediol Adipate Cyclic Diester (1), Diethylene Glycol Phthalate Cyclic Diester (2), Di-(Diethylene Glycol Adipate Cyclic Diester) (3), and Di-(Diethylene Glycol Phthalate Cyclic Diester) (4)



Chromatogram 2: GC-MS-TIC Chromatogram of 95% EtOH Sealant-Side Laminate Extract Showing Peaks of Neopentyl Glycol Adipate Cyclic Diester (1), Diethylene Glycol Phthalate Cyclic Diester (2) and Di-(Neopentyl Glycol Adipate Cyclic Diester) (3)



Chromatogram 3: GC-MS-TIC Chromatogram of 95% EtOH Sealant-Side Laminate Extract Showing Peaks of 1,4-Butanediol Adipate Cyclic Diester (1) and Di-(1,4-Butanediol Adipate Cyclic Diester) (2)



Chromatogram 4: GC-MS-TIC Chromatogram of 10% EtOH Sealant-Side Laminate Extract Showing Peaks of Diethylene Glycol Adipate (1), Di-(Neopentyl Glycol Adipate Cyclic Diester) (2)

It was noted that the short chain cyclic polyester oligomers migrated from the commercial laminate packaging were mainly comprised of ingredients like 1,4-butanediol, 1,6-hexanediol, neopentyl glycol, ethylene glycol, diethylene glycol, adipic acid, phthalic acid and isophthalic acid. This showed that high percentages of commercial laminates were prepared using these monomers which were listed in the Journal of European Commission Regulations with specific migration limits (SML) (Please refer to section II.E). Most of these compounds were novel compounds with the exception of 1,4-Butanediol Adipic Acid Cyclic Diester which was listed in NIST database and 1,6-Hexanediol Adipic Acid Cyclic Diester which was already reported by Thiebaut et al., 2007. However they were included since they migrated in the food simulant and belonged to the class of short chain cyclic polyester oligomers.

VI.B. Chemistry and Migratory Properties of Short Chain cyclic Polyester Oligomers

Under this section, chromatogram peak, mass spectrum, chemistry and migratory properties of individual short chain cyclic polyester oligomers, listed in **Table 10,** have been discussed. Further, some of the fragments formed in the mass spectrum have been analyzed and discussed in this section.

VI.B.1 1,4-Butanediol, Adipic Acid, Cyclic Diester

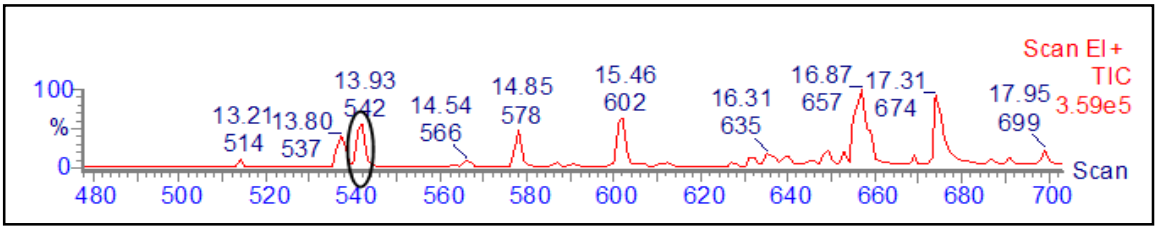


Figure 30: GC-MS-TIC Chromatogram of 10%EtOH Sealant-Side Laminate Extract of 1,4-Butanediol Adipic Acid, Cyclic Diester Peak

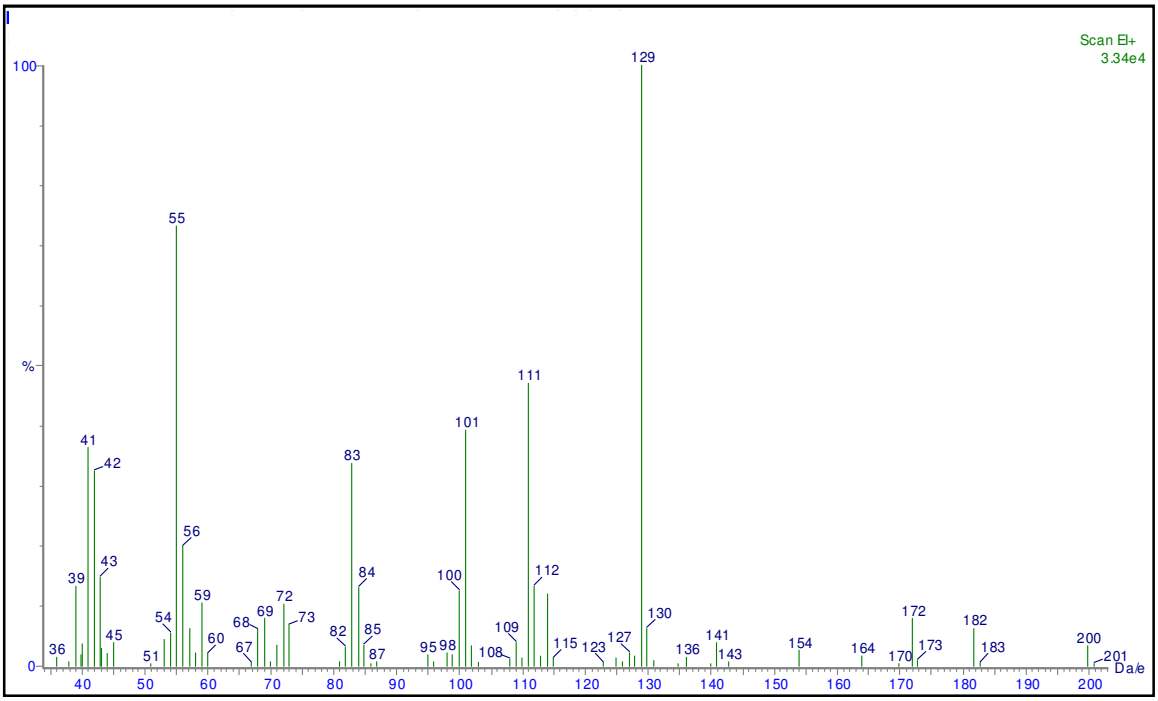


Figure 31: Mass Spectrum (EI) of 1,4-Butanediol, Adipic acid, Cyclic Diester

10 Highest Peaks

129 (100) 55 (73.29) 111 (46.94) 101 (39.17) 41 (36.15)

83 (33.57) 42 (32.28) 56 (19.76) 43 (14.63) 39 (13.19)

Table 12: Chemistry and Migratory Properties of 1,4-Butanediol, Adipic Acid, Cyclic Diester

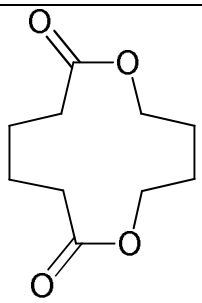
Common Name	IUPAC Name	M.W. g/mol	Structure	Retention Index	Concentration
1,4-Butanediol Adipate Cyclic Diester	1,6-dioxacyclododecane-7,12-dione	200		1514	**Range**: ppb:- 105 -116 ng/cm^2- 61.7 – 68.2 **Avg** = 110.7 ppb **σ** = ± 4.4 ppb **N** = 4

Figure 31 depicts the EI spectrum of 1,4-Butanediol Adipate Cyclic Diester. The X-axis of the mass spectrum represents the m/z ratio of the ions while Y-axis represents the intensity of the ion fragments. The ion fragment with m/z ratio 129 is the base peak with assigned abundance of 100 as it has the highest intensity. The 10 highest peaks of abundance have been listed for the EI mass spectrum **(Fig. 31)**. The characteristic fragmentation pattern aids in deducing the chemical structure of the compound, and the presence of molecular ion helps in identifying the molecular weight of the compound.

Retention time index is a concept in GC used to convert the retention time into system-independent constants (Kováts, 1958). Retention time is the measure of time taken by the analyte to elute from the column and hence it is dependent on the length of column, film thickness, carrier gas velocity, carrier gas pressure and void time. Under different GC conditions, retention times vary and hence they cannot be used as an identification

measure. However, retention time index is independent of these parameters and allows comparing the different types of analysis under varying GC conditions. Hence RI is a useful aid in identification by GC-MS especially when mass spectra obtained are ambiguous or in trace amounts. The linear retention time indices were calculated by normalizing the retention time to the closely eluting C-7 through C-35 n-paraffin standard mixture (Seong Jae Yoo, 2004). The following formula proposed by H. van den Dool and P.D. Kratz was used to calculate the retention indices (Van Den Dool, 1963)

$$RI = 100[Rt(x) - Rt(z)]/[Rt(z+1) - Rt(z)] + 100z \quad \ldots\ldots(1)$$

Where,

Rt(x): Retention time (min) of the compound of interest

Rt(z): Retention time (min) of the normal alkane before the compound

Rt(z+1): Retention time (min) of the normal alkane after the compound

z: Number of carbon atoms

The concentration of the analyte was calculated using the correlation of direct dependence of concentration of the analyte peak to its integrated area. By integrating the mass spectrum, the peak areas of the analyte and the internal standard (Anthracene d-10) were determined. Further, the unknown concentration of the analyte was calculated in ppb which represented parts per billion on a weight to volume (w/v) basis in the EtOH extraction solvent. The results were expressed in ppb as FDA uses the same unit of concentration for the food contact evaluation results. Also it is more important to express the concentration data in terms of extractable concentration per unit surface area, as it can predict the amount of migrant coming out from a sealant side. Therefore, concentration data are also expressed in ng/cm^2. The migratory concentration range was found to be 105 ppb -116 ppb in the food simulant, equivalent to 61.76 ng/cm^2 - 68.2

ng/cm^2 of the surface area exposed to extraction. The average concentration was found to be 110.7 ppb with a standard deviation of ±4.4ppb

Additionally, the molecular weight of the compound was confirmed from chemical ionization (CI) spectrum **(Fig. 32).** During EI mode, the molecules of the analyte are bombarded with high energy electrons. Sometimes the molecular ion so formed is highly unstable and it fragments instantaneously and hence it is hard to detect it. During the CI mode, the molecular ion remains intact and does not undergo extensive fragmentation. And hence, it is the most important tool to confirm the molecular weight of the compounds.

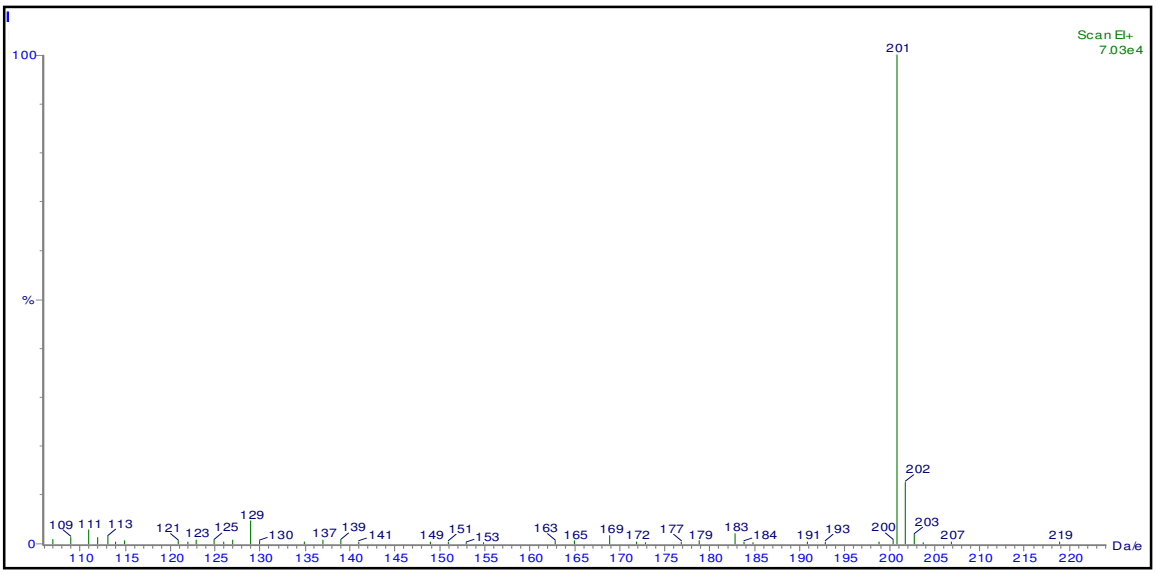


Figure 32: Mass Spectrum (CI) of 1,4-Butanediol, Adipic Acid, Cyclic Diester

Formation of 1,4-Butanediol,Adipic Acid, Cyclic Diester

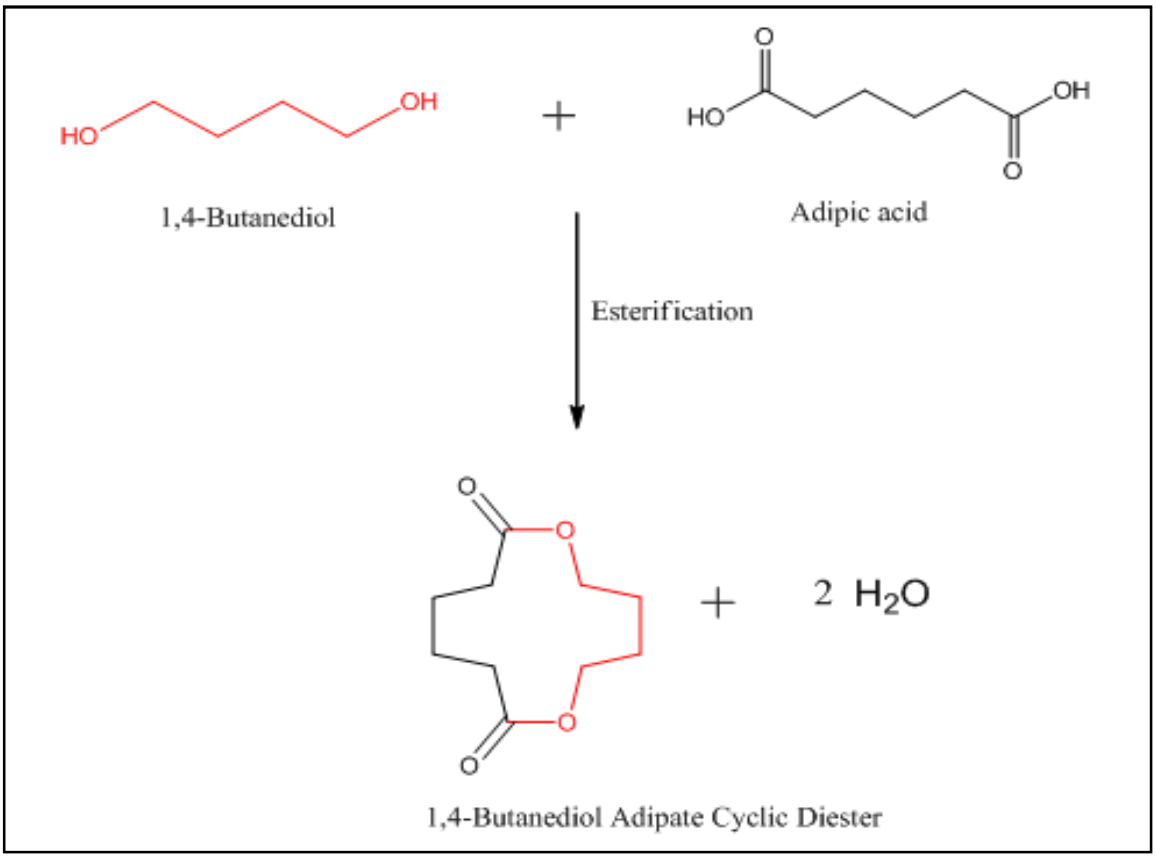


Figure 33: Formation of 1,4-Butanediol, Adipic Acid, Cyclic Diester

Fragmentation Analysis of 1,4-Butanediol, Adipic Acid, Cyclic Diester

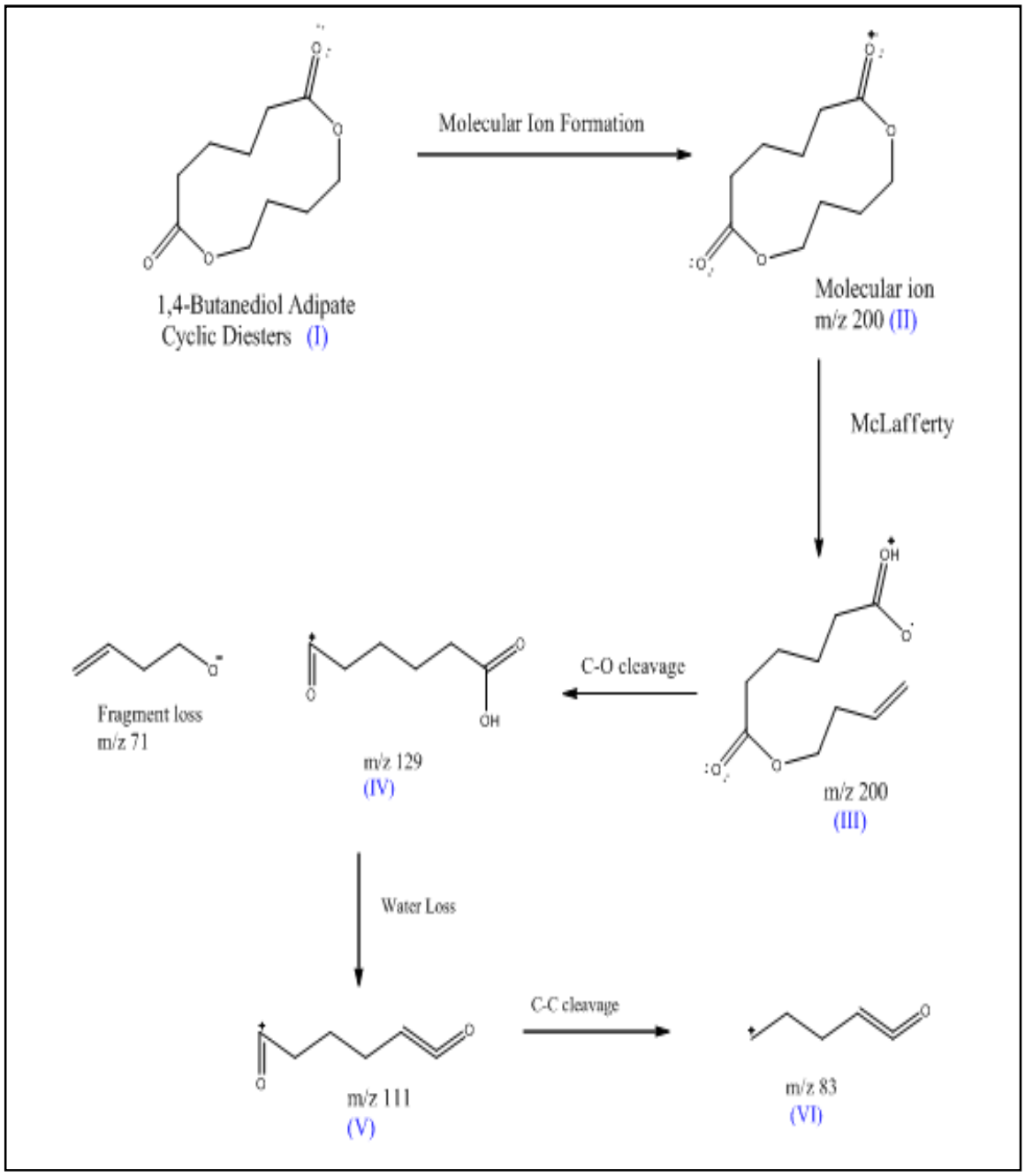


Figure 34: Fragmentation Pattern of 1,4-Butanediol, Adipic Acid, Cyclic Diester

When the sample is injected in the ionization chamber, the molecules are bombarded with high energy electron beams. These electron beams knock off the lone electron to form a molecular ion with m/z ratio same as that of molecular weight of the molecule, 200 (II). The molecular ion yields valuable information as its mass and elemental composition depicts the structural fragments in the mass spectrum. Some of the ion fragments formed have been discussed and the possible fragmentation mechanism has been suggested in **Fig. 34**.

Fragmentation in esters either takes place by rearrangement reactions or by the chemical bond cleavages. The molecular ion so formed (II) undergoes the McLafferty rearrangement to yield an odd electron ion fragment with even m/z value 200. This is followed by the C-O bond cleavage to form fragment with m/z 129 (III), as the excess energy contained within the molecule is greater than the energy required to rupture the bonds. Finally, ion with m/z 111 (IV) is formed due to loss of water molecule.

VI.B.2. Diethylene Glycol, Adipic Acid, Cyclic Diester

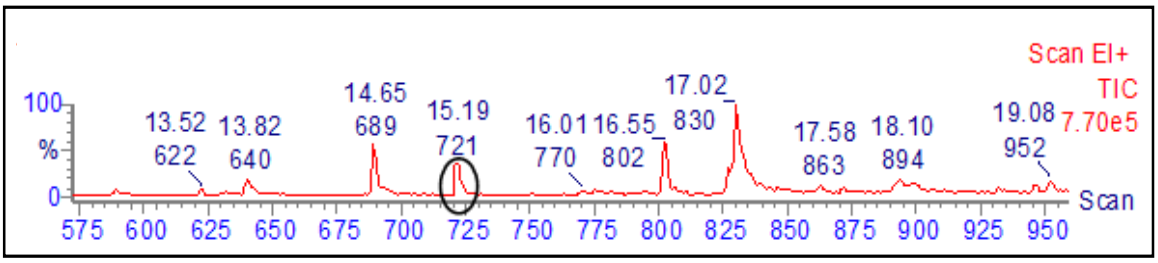


Figure 35: GC-MS-TIC Chromatogram of 10% EtOH Sealant-Side Laminate Extract of Diethylene Glycol, Adipic Acid, Cyclic Diester Peak

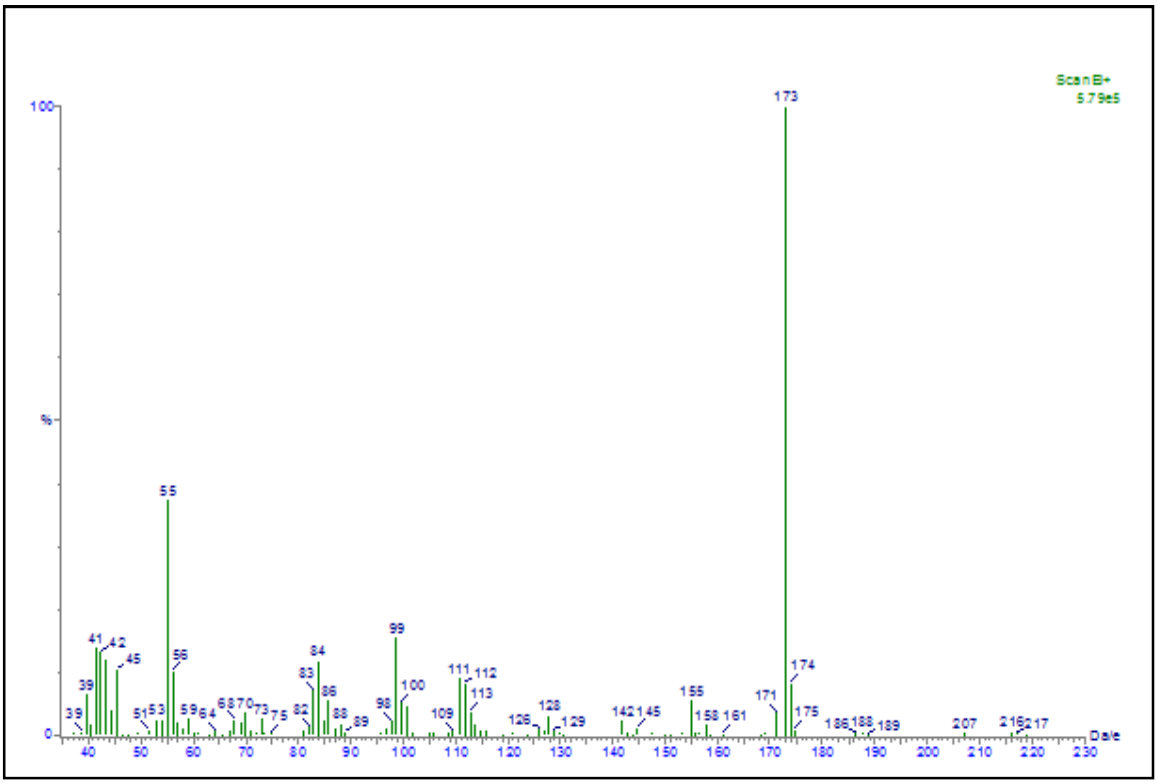


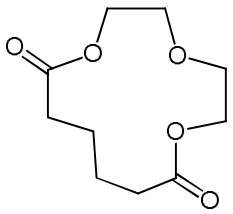
Figure 36: Mass Spectrum (EI) of Diethylene Glycol, Adipic Acid, Cyclic Diester

10 Highest Peaks of EI Spectrum

173 (100) 55 (37.37) 99 (15.27) 41 (13.89) 42 (13.07)

43 (11.87) 84 (11.68) 45 (10.37) 56 (9.98) 111 (9.12)

Table 13: Chemistry and Migratory Properties of Diethylene Glycol, Adipic Acid, Cyclic Diester

Common Name	**IUPAC Name**	**M.W. g/mol**	**Structure**	**Retention Index**	**Concentration**
Diethylene Glycol Adipate Cyclic Diester	1,4,7-trioxacyclotridecane-8,13-dione	216		1618	**Range**: ppb- 120- 130 ng/cm^2- 70.58- 76.57 **Avg** = 124.8 ppb σ = ± 4 ppb N = 4

EI spectrum of the compound, **Fig. 36**, showed the presence of the molecular ion. However, the molecular weight was confirmed by obtaining the CI spectrum of the same compound **(Fig. 37)**. The characteristic fragmentation pattern helped in deducing the chemical structure of the compound. Retention time was normalized with the mixture of closely eluting n-paraffin alkanes to calculate the retention time index, which is a system independent variable. The migratory concentration range was found to be 120 ppb to 130 ppb in the food simulant, equivalent to 70.58 ng/cm^2 - 76.57 ng/cm^2 of surface area exposed to extraction with average concentration of 124.8 ppb.

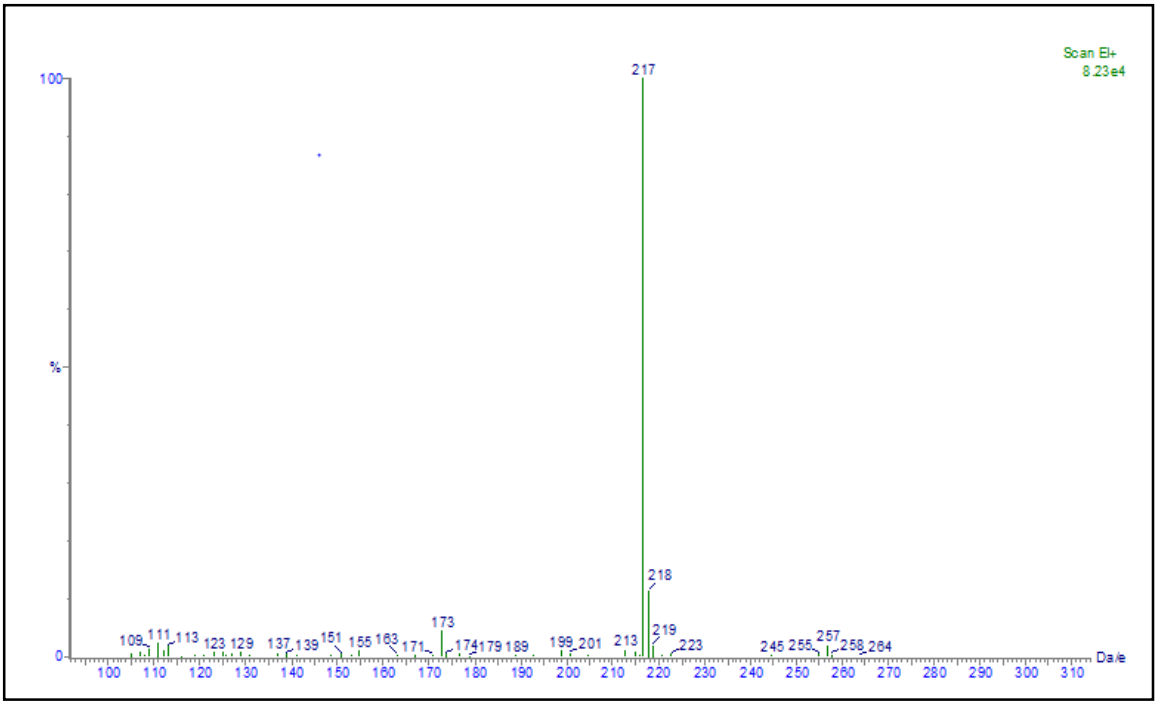


Figure 37: Mass Spectrum (CI) of Diethylene Glycol, Adipic Acid, Cyclic Diester

Formation of Diethylene Glycol, Adipic Acid, Cyclic Diester

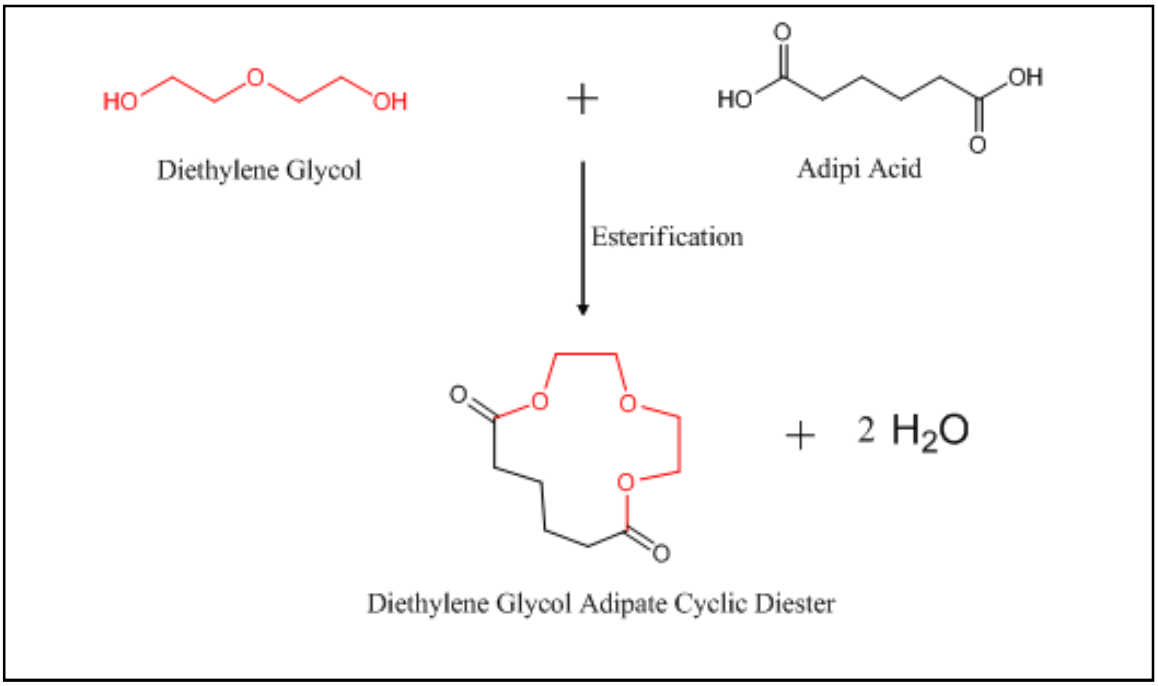


Figure 38: Formation of Diethylene Glycol, Adipic Acid, Cyclic Diester

Fragmentation Analysis

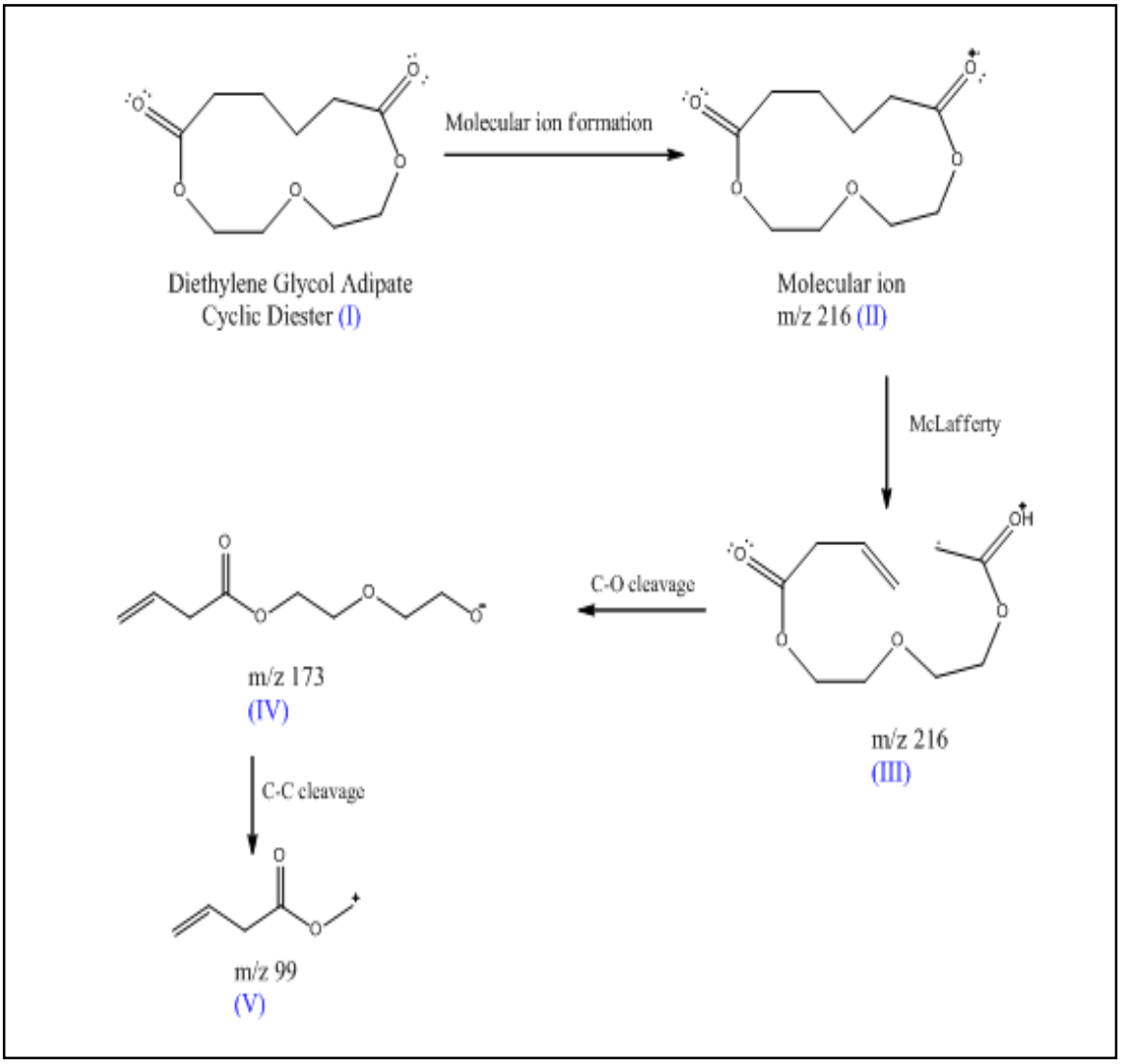


Figure 39: Fragmentation Analysis of Diethylene Glycol, Adipic Acid, Cyclic Diester

The mass spectrum of the molecule contains the distribution of the ion fragments of the possible products. As the vaporized sample passes through the ionization chamber of the mass spectrometer, the high energy electron beam knocks off the electron from the molecule to form a molecular ion (II). The possible fragmentation mechanism has been suggested in **Fig. 39**. In practice, it is not possible to assign the chemical structure to each and every fragment formed; hence, only few fragments have been discussed.

The two chief paths of fragmentation in esters are rearrangement reaction or chemical bond cleavages. The molecular ion so formed (II) undergoes McLafferty rearrangement to form an ion fragment with an m/z value 216 (III) and an odd number of electrons. The double bonded oxygen abstracts one gamma hydrogen atom, thus breaking the bond between alpha and beta carbon atoms. Further, the energy retained by the fragment is greater than the energy required to break C-O bond. The fragment with m/z value 173 (III) is formed by C-O bond cleavage.

The fragmentation pattern aids in deducing the chemical structure of the compound.

VI.B.3. 1,6-Hexanediol,Adipic Acid, Cyclic Diester

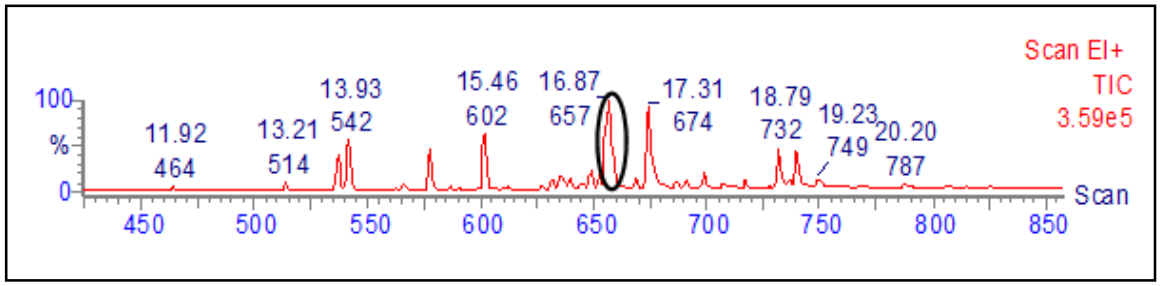


Figure 40: GC-MS-TIC Chromatogram of 10% EtOH Sealant-Side Laminate Extract of 1,6-Hexanediol, Adipic Acid, Cyclic Diester Peak

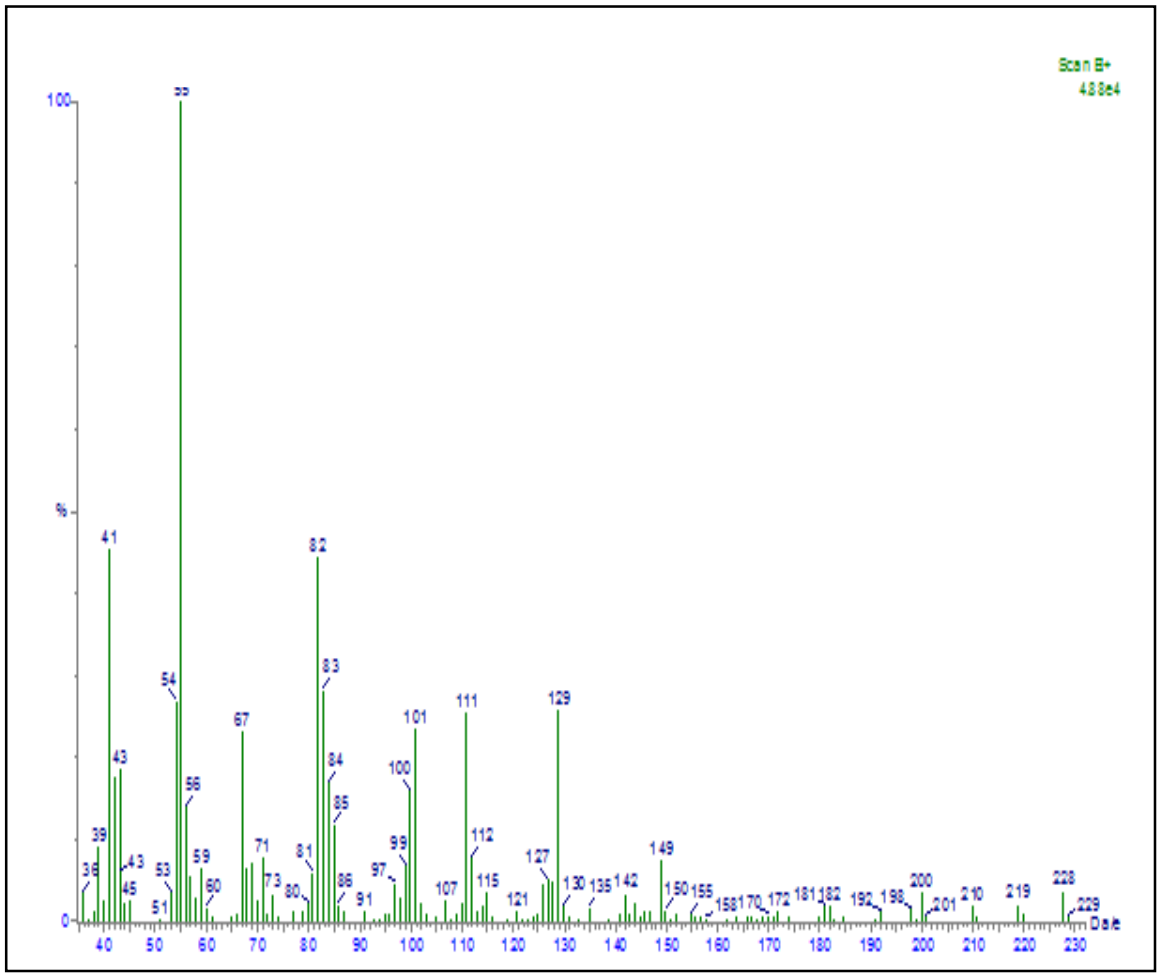


Figure 41: Mass Spectrum (EI) of 1,6-Hexanediol, Adipic Acid, Cyclic Diester

10 Highest Peaks

55 (100)	82 (68.66)	41 (54.73)	67 (34.03)	83 (34.01)
129 (31.52)	111 (25.71)	54 (25.67)	42 (23.77)	101 (23.52)

Table 14: Chemistry and Migratory Properties of 1,6-Hexanediol, Adipic Acid, Cyclic Diester

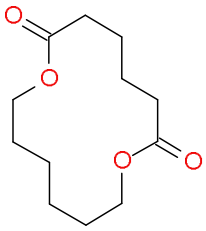
Common Name	**IUPAC Name**	**M.W. g/mol**	**Structure**	**Retention Index**	**Concentration**
1,6-Hexanediol Adipate Cyclic Diester	1,8-dioxacyclotetradecane-2,7-dione	228		1762	**Range**: ppb: 76 – 86 ng/cm^2- 44.7 - 50.59 **Avg**–81 ppb **σ** = ±4.3 ppb **N** = 4

Figure 41 represents the EI spectrum of 1,6-Hexanediol Adipate Cyclic Diester, which shows the presence of molecular ion 228. The molecular weight was confirmed by obtaining the CI spectrum of the same compound **(Fig. 42)**. The chemical structure was deduced by studying the characteristic fragmentation pattern of the compound. The retention time index was calculated by normalizing the elution time with closely eluting n-paraffin mixture**.** The migratory concentration range of the polyester oligomer migrated in the food simulant was found to be 76 ppb – 86 ppb in the food simulant with the standard deviation of ±4.3 ppb. The concentration is also expressed in ng/cm^2 which reflects extractable concentration per unit surface area.

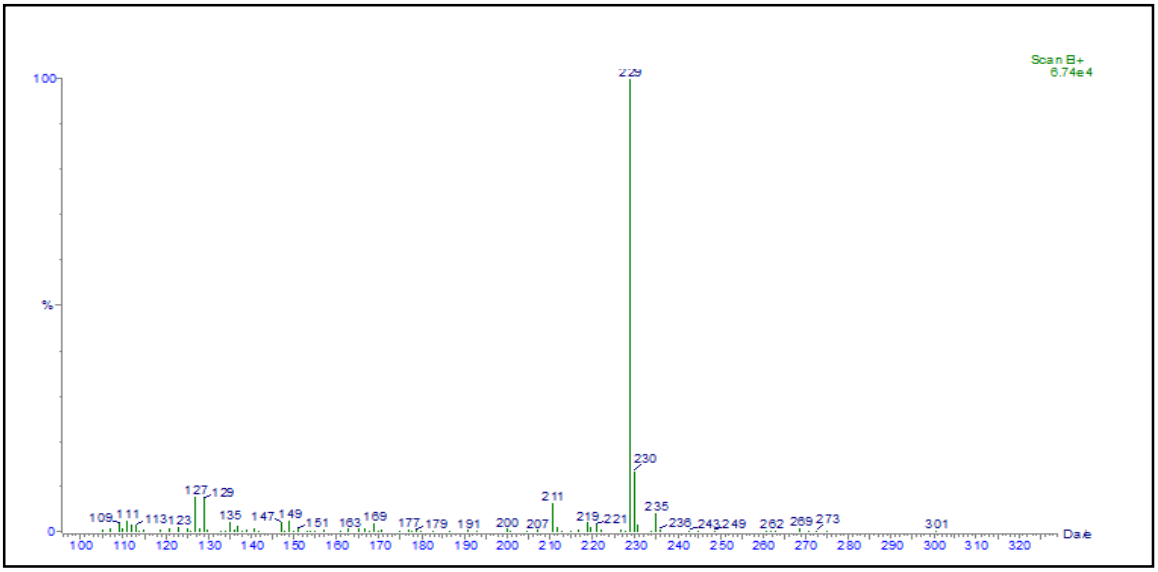


Figure 42: Mass Spectrum (CI) of 1,6-Hexanediol, Adipic Acid, Cyclic Diester

Formation of 1,6-Hexanediol Adipate

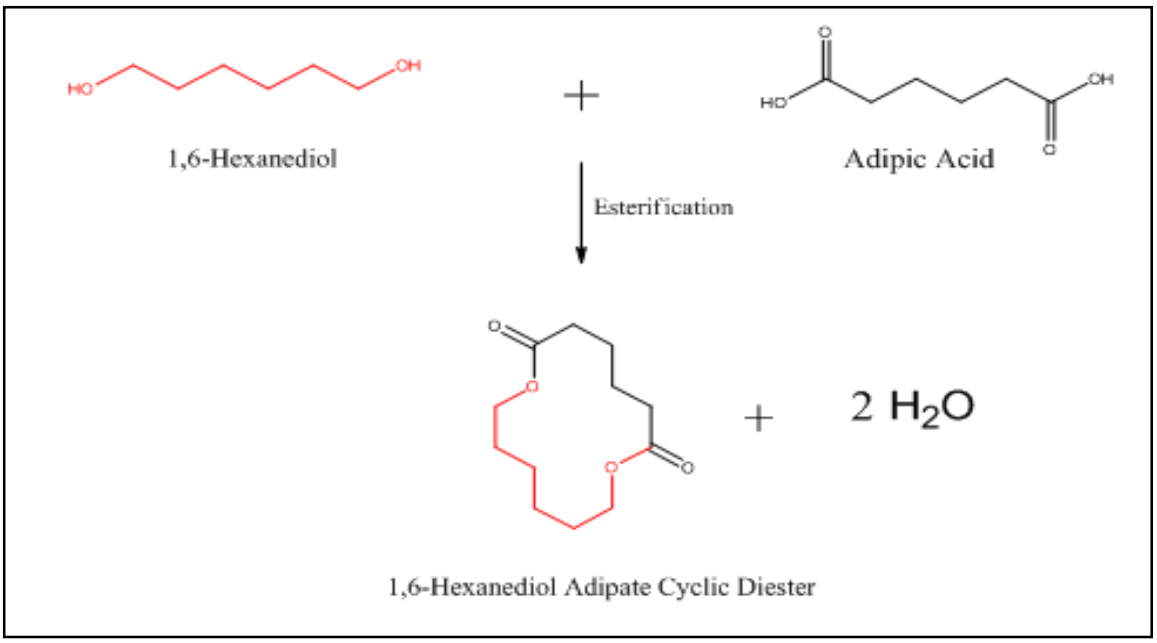


Figure 43: Formation of 1,6-Hexanediol, Adipic Acid, Cyclic Diester

Fragmentation Analysis

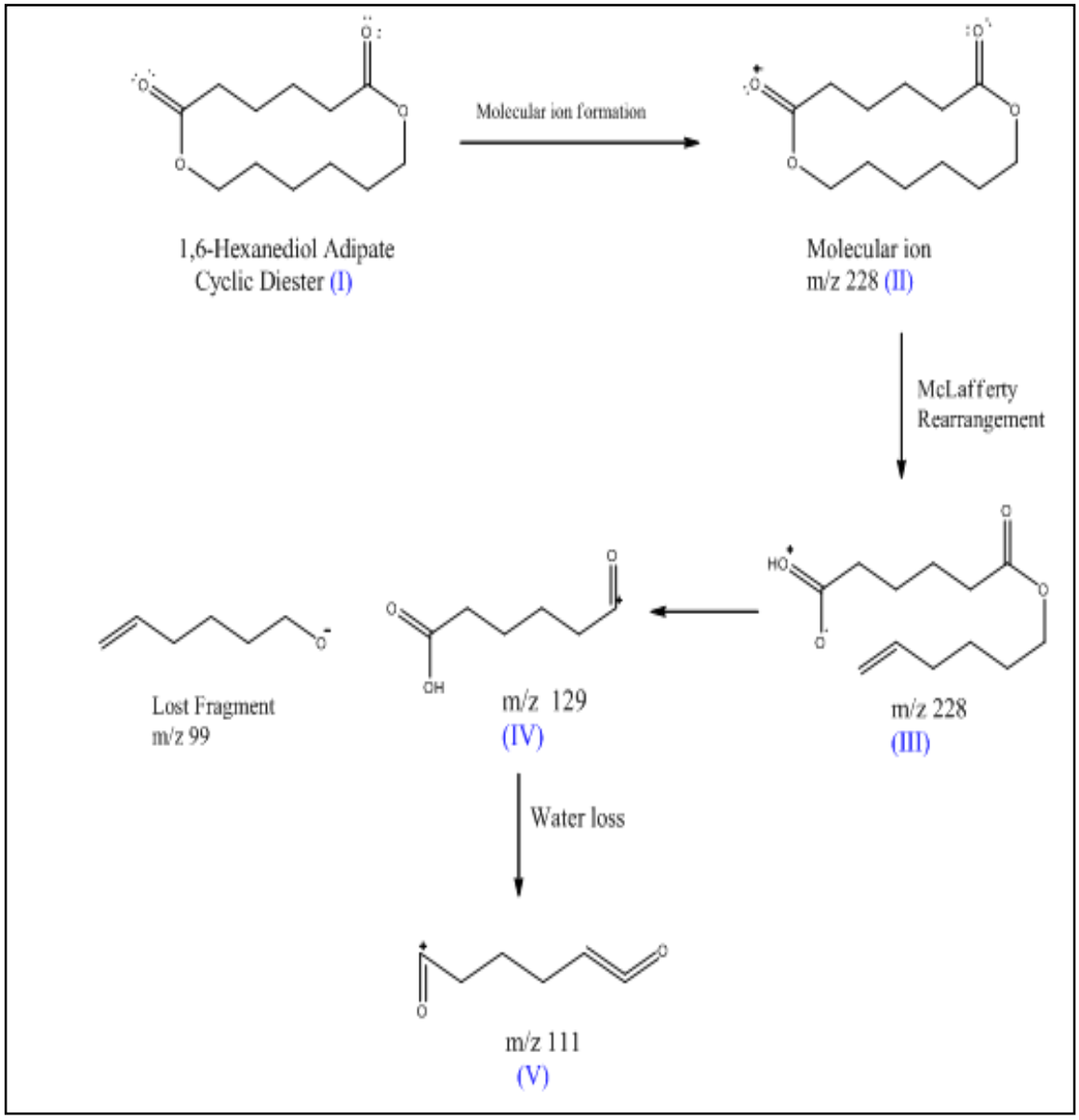


Figure 44: Fragmentation Analysis of 1,6-Hexanediol, Adipic Acid, Cyclic Diester

When gaseous phase sample passes through the ionization chamber of the mass spectrometer, it interacts with the high energy electron beams. This interaction knocks off the non-bonding electron to form molecular ion (II). As the molecular ion retains the excess energy, it is highly unstable and splits into different fragments in order to achieve stability. The mechanism of fragment formation is illustrated in the above **Fig. 44**. However, the mechanisms associated with the fragmentation are complex and hence only few fragment formation paths have been discussed.

Fragmentation in esters is initiated either by chemical bond cleavage or by the McLafferty rearrangement reactions. The molecular ion thus formed (II) undergoes the McLafferty rearrangement to form an odd electron ion with even m/z ratio value 228 (III). The rearrangement causes the cyclic compound to rupture and form a straight chain compound. At this instant, the energy content of the fragment is higher than the energy required to break the C-O bond. This leads to the chemical bond rupture between carbon and oxygen to yield an ion fragment with m/z value 129 (III). Finally, the water molecule is lost to form a fragment with m/z value 111 (IV).

VI.B.4. Diethylene Glycol, Phthalic Acid, Cyclic Diester

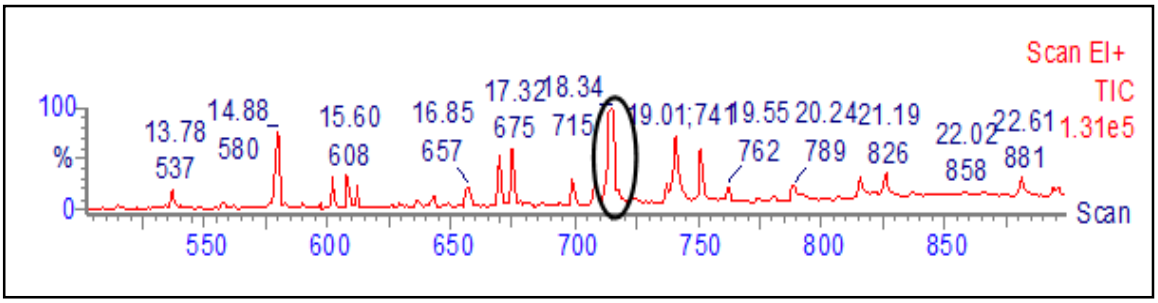


Figure 45: GC-MS-TIC Chromatogram of 95% EtOH Sealant-Side Laminate Extract of Diethylene Glycol, Phthalic Acid, Cyclic Diester Peak

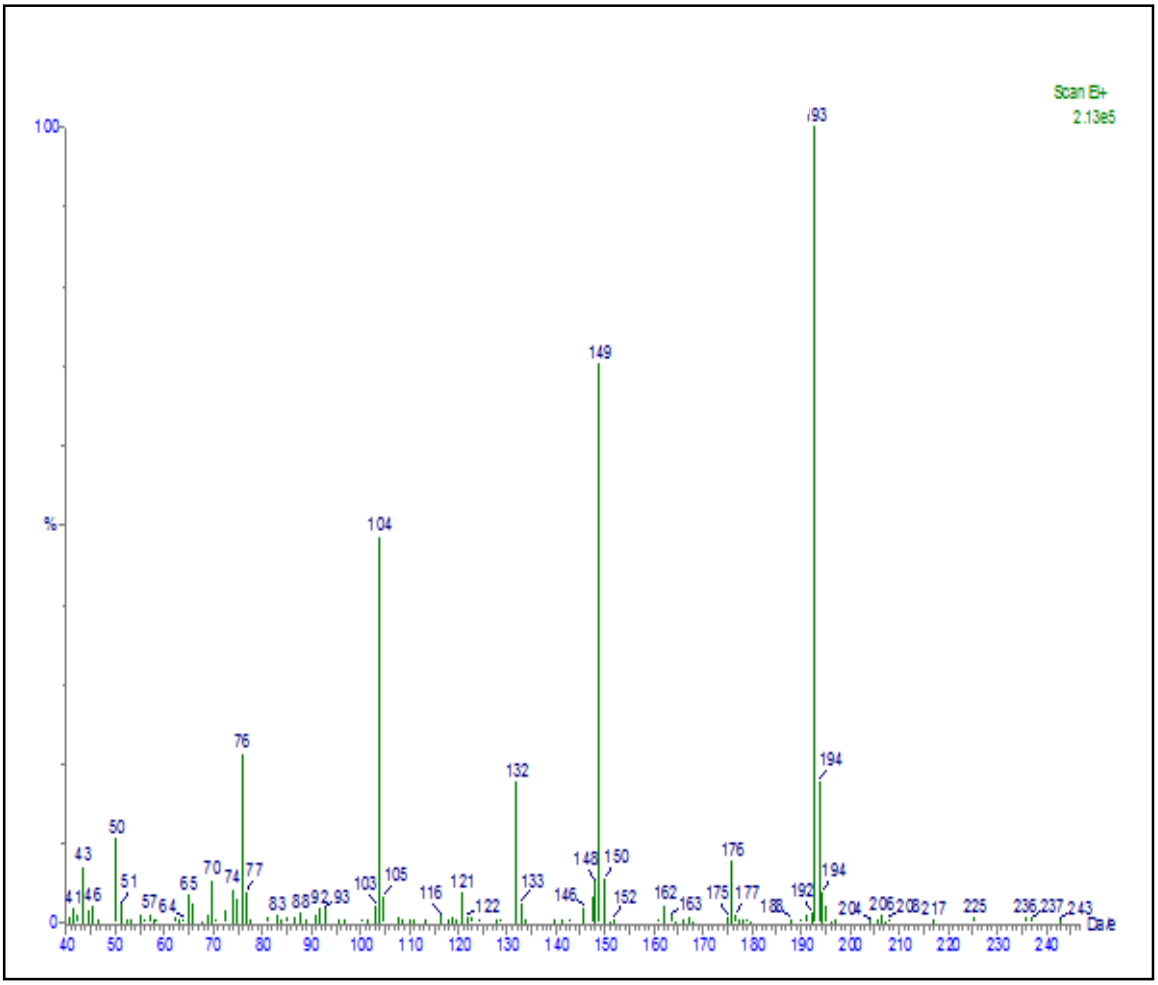
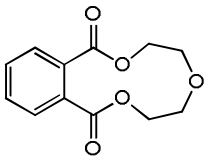


Figure 46: Mass Spectrum (EI) of Diethylene Glycol, Phthalic Acid, Cyclic Diester

10 Highest Peaks

193 (100)	149 (84.21)	104 (46.11)	76 (29.45)	192 (12.24)
148 (10.93)	50 (10.91)	194 (9.03)	176 (8.55)	132 (7.21)

Table 15: Chemistry and Migratory Properties of Diethylene Glycol, Phthalic Acid, Cyclic Diester

Common Name	IUPAC Name	M.W. g/mol	Structure	Retention Index	Concentration
DEG-Phthalate Cyclic Diester	9,10-benzo[1,4,7] trioxacycloundecane-8,11-dione	236		1876	**Range:** ppb: 207 - 220 ng/cm^2: 121.76 – 129. 4 **Avg** = 213.3 ppb **σ** = ±5.4 ppb **N** = 4

In the **Fig. 46** the peak with m/z ratio 236 was tentatively assigned as Diethylene Glycol Phthalate Cyclic Diester. The mass spectrum of phthalic acid isophthalic acid matched very closely with respect to GC retention time and the fragmentation pattern. Since there were no previously synthesized extracts of Diethylene Glycol Phthalate or Diethylene Glycol Isophthalate as a point of reference, the identity of the compound could not be confirmed. **Table 15** summarizes the IUPAC name and the structure of the compound along with other parameters like retention time index and migratory concentration. In the mass spectrum **(Fig. 46)**, the molecular ion with m/z ratio 236 was found to be present. The chemical structure was deduced by studying the characteristic fragmentation pattern of the compound. Some of the fragments formed are discussed in fragment analysis section. The migratory concentration range was found to be 207 – 220 ppb in the food simulant (also expressed in ng/cm^2 of surface area exposed to extraction) with the

standard deviation ±5.4 ppb. The retention time index was calculated by comparing its retention time to the mixture of closely eluting n-paraffin alkanes.

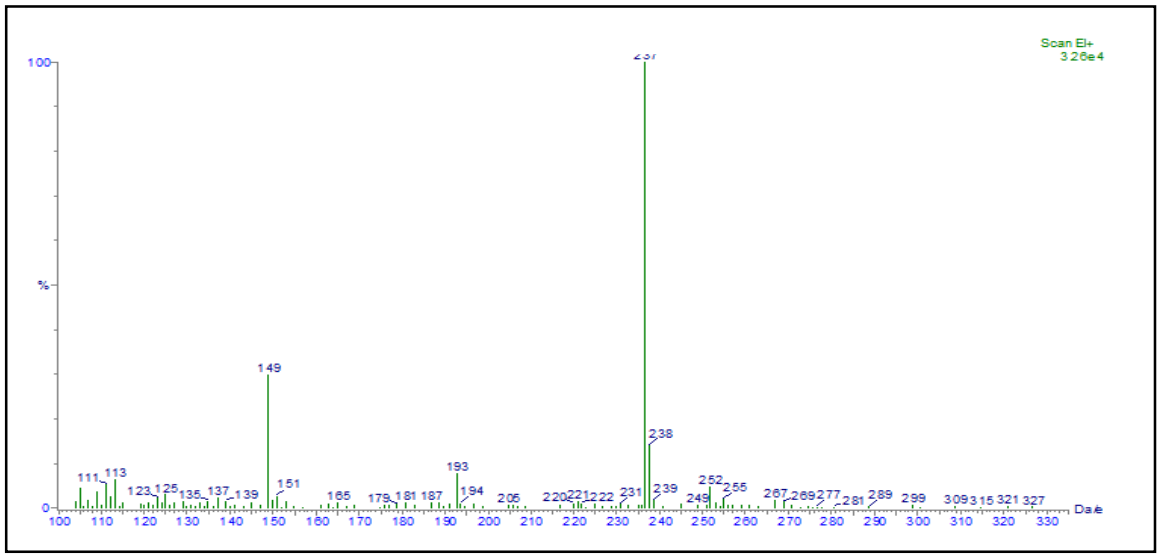


Figure 47: Mass Spectrum (CI) of Diethylene Glycol, Phthalic Acid, Cyclic Diester

Formation of Diethylene Glycol, Phthalic Acid, Cyclic Diester

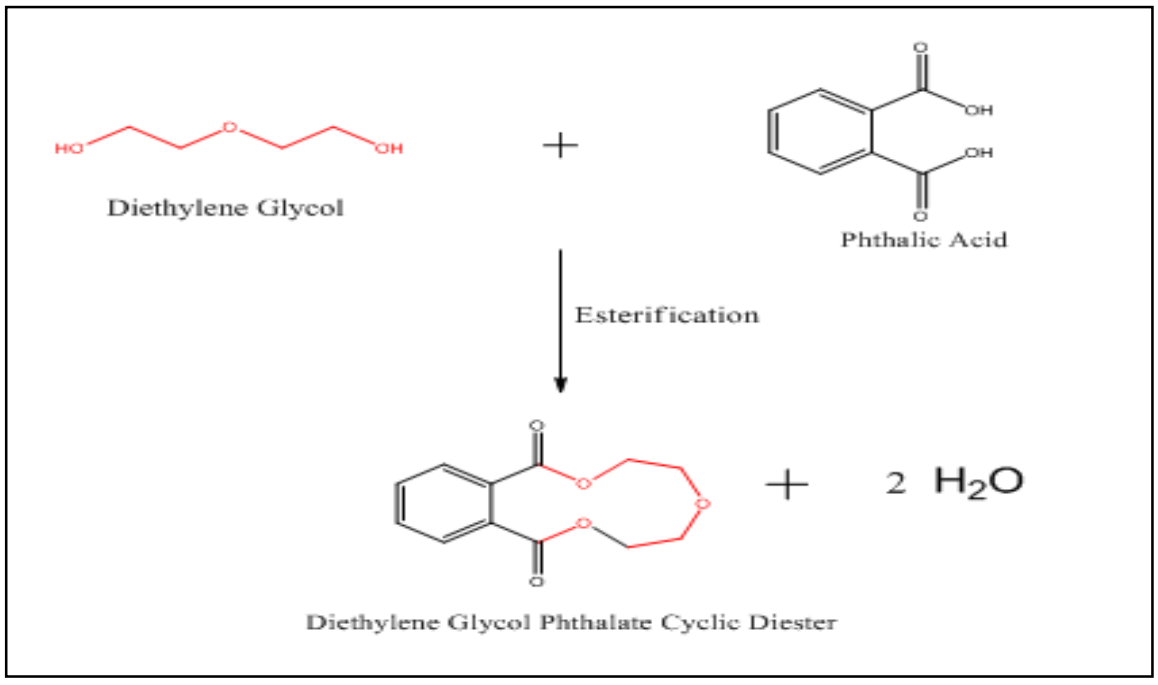


Figure 48: Formation of Diethylene Glycol, Phthalic Acid, Cyclic Diester

Fragmentation Analysis

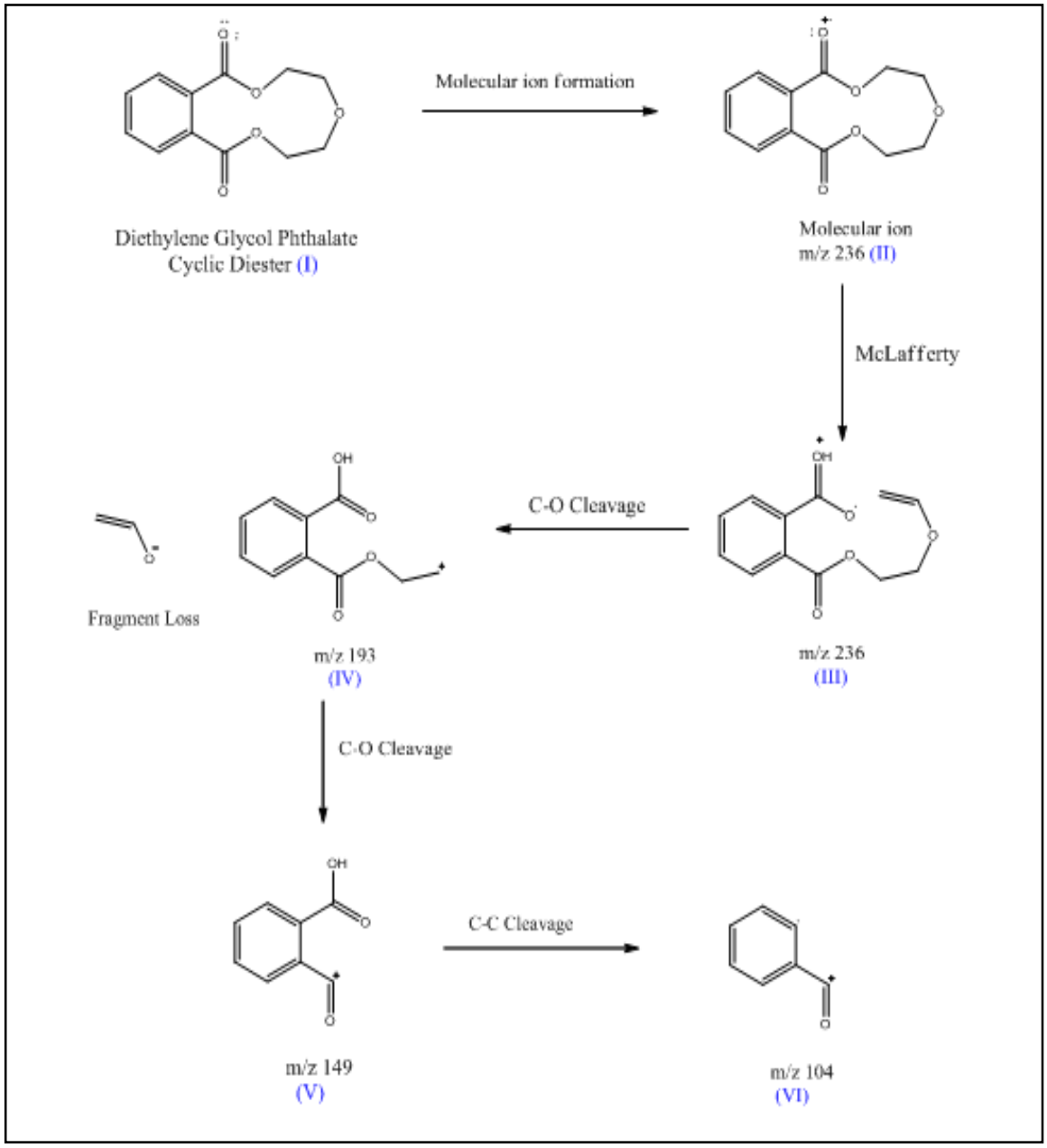


Figure 49: Fragmentation Analysis for Diethylene Glycol, Phthalic Acid, Cyclic Diester

The mass spectrum is produced by ionizing many molecules and hence the spectrum has a distribution of possible ion products. Diethyelene Glycol Phthalate Cyclic Diester in gaseous phase passes through the ionization chamber of the mass spectrometer and interacts with the high energy electron beams. This interaction knocks off the non-bonding electron to form a molecular ion (II). As the molecular ion retains the excess energy, it is highly unstable and splits into different fragments in order to achieve the stability. The mechanism of fragment formation is illustrated in **Fig. 49** above**.** However, the mechanisms associated with the fragmentation are complex and hence only few fragment formation paths have been discussed.

Fragmentation in esters is initiated either by alpha cleavage or by the McLafferty rearrangement reactions. The molecular ion thus formed (II) undergoes the McLafferty rearrangement to form an odd electron ion with even an m/z ratio value of 236 (III). This is followed by carbon-oxygen bond cleavage as the excess energy is greater than the energy required to break the chemical bond, thus forming ion fragment with m/z value 193 (IV). Since the structure contains multiple carbon oxygen bonds, another carbon oxygen bond cleavage takes place to yield a fragment with m/z value 149 (V). Further, the fragment with m/z value 104 is formed (VI) by C-C bond cleavage.

VI.B.5. Neopentyl Glycol, Adipic Acid, Cyclic Diester

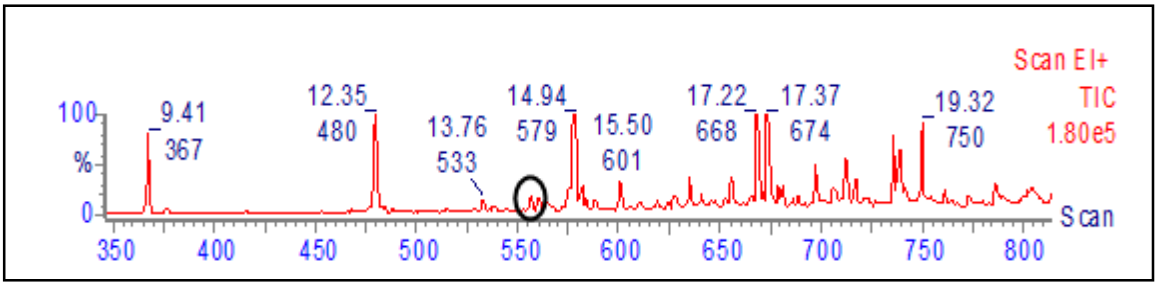


Figure 50: GC-MS-TIC Chromatogram of 10% EtOH Sealant-Side Laminate Extract of Neopentyl Glycol, Adipic Acid, Cyclic Diester Peak

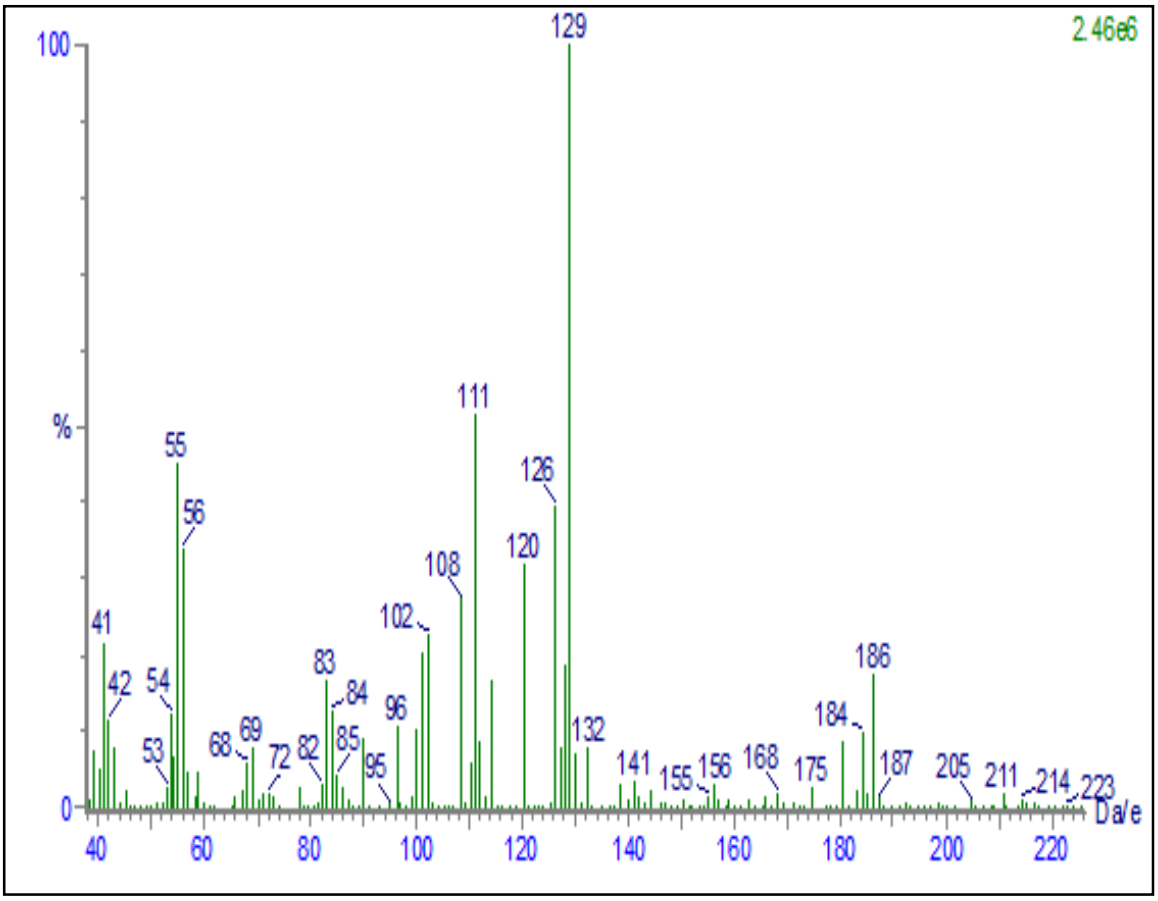
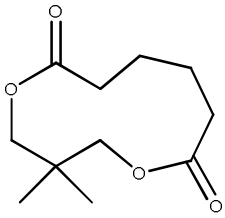


Figure 51: Mass Spectrum (EI) of Neopentyl Glycol, Adipic Acid, Cyclic Diester

10 Highest Peaks

57 (100)	55 (61.58)	129 (44.70)	56 (39.07)	41 (29.80)
111 (28.47)	40 (27.15)	69 (26.82)	179 (25.16)	39 (21.52)

Table 16: Chemistry and Migratory Properties of Neopentyl Glycol, Adipic Acid, Cyclic Diester

Common Name	IUPAC Name	M.W. g/mol	Structure	Retention Index	Concentration
Neopentyl Glycol Adipate Cyclic Diester	3,3-dimethyl-1,5-dioxacyclounde cane-6,11-dione	214		1548	**Range**: ppb: 157 – 167 ng/cm^2: 92.34 – 98.22 **Avg** = 161.2 ppb **σ** = ±3.6 ppb **N** =4

The chromatogram and the mass spectrum of the compound have been displayed in **Fig. 50** and **Fig. 51**, respectively**.** A mass spectrum is essentially a plot of m/z ratio vs. the intensity of the ion fragment. The molecular ion, formed by knocking off the lone electron, with m/z ratio 214 was found to be present with a weak intensity. The chemical structure was deduced by analyzing the fragmentation pattern. The migratory concentration and the retention time index were calculated as discussed before. The concentration range was found to be 157 – 167 ppb in the food simulant with the standard deviation of ±3.6 ppb (**Table 16**) for the four different films analyzed. The concentration was also expressed in ng/cm^2 which reflects the extractable concentration per unit surface are

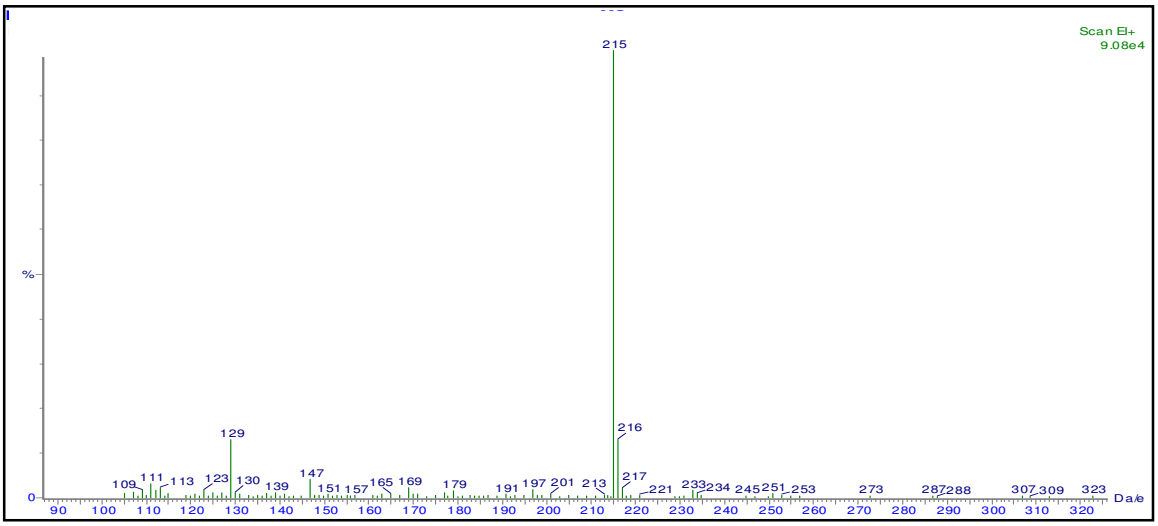


Figure 52: Mass Spectrum (CI) of Neopentyl Glycol, Adipic Acid, Cyclic Diester

Formation of Neopentyl Glycol, Adipic Acid, Cyclic Diester

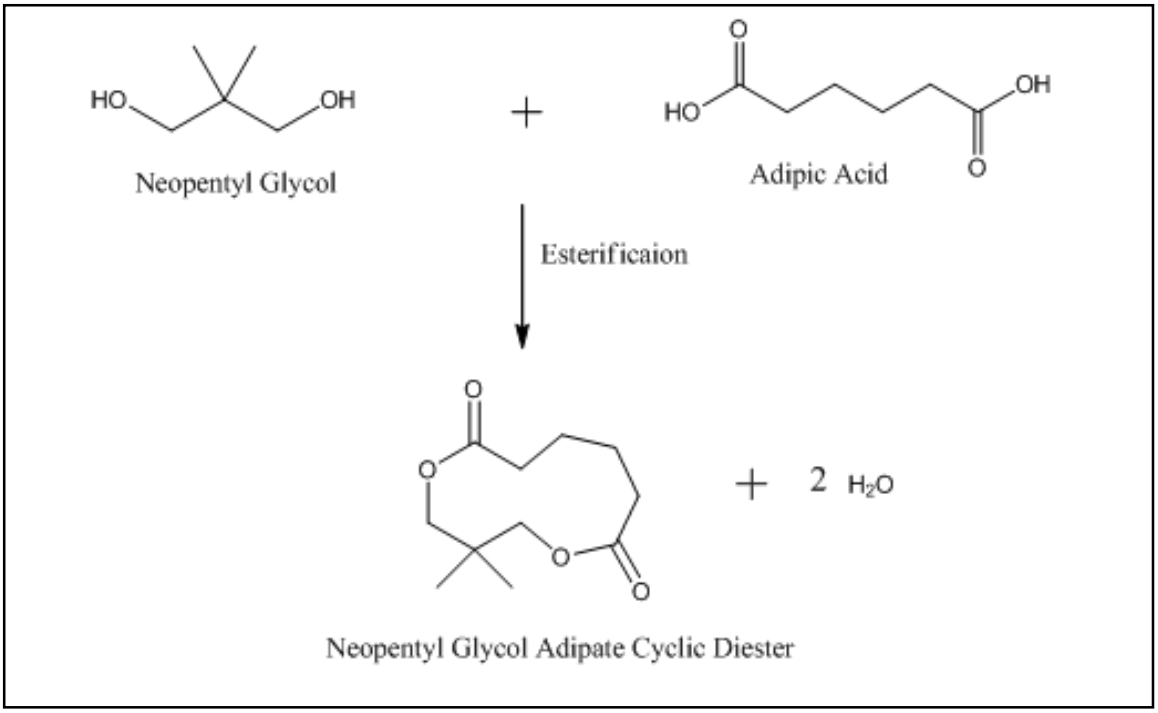


Figure 53: Formation of Neopentyl Glycol, Adipic Acid, Cyclic Diester

Fragmentation Analysis

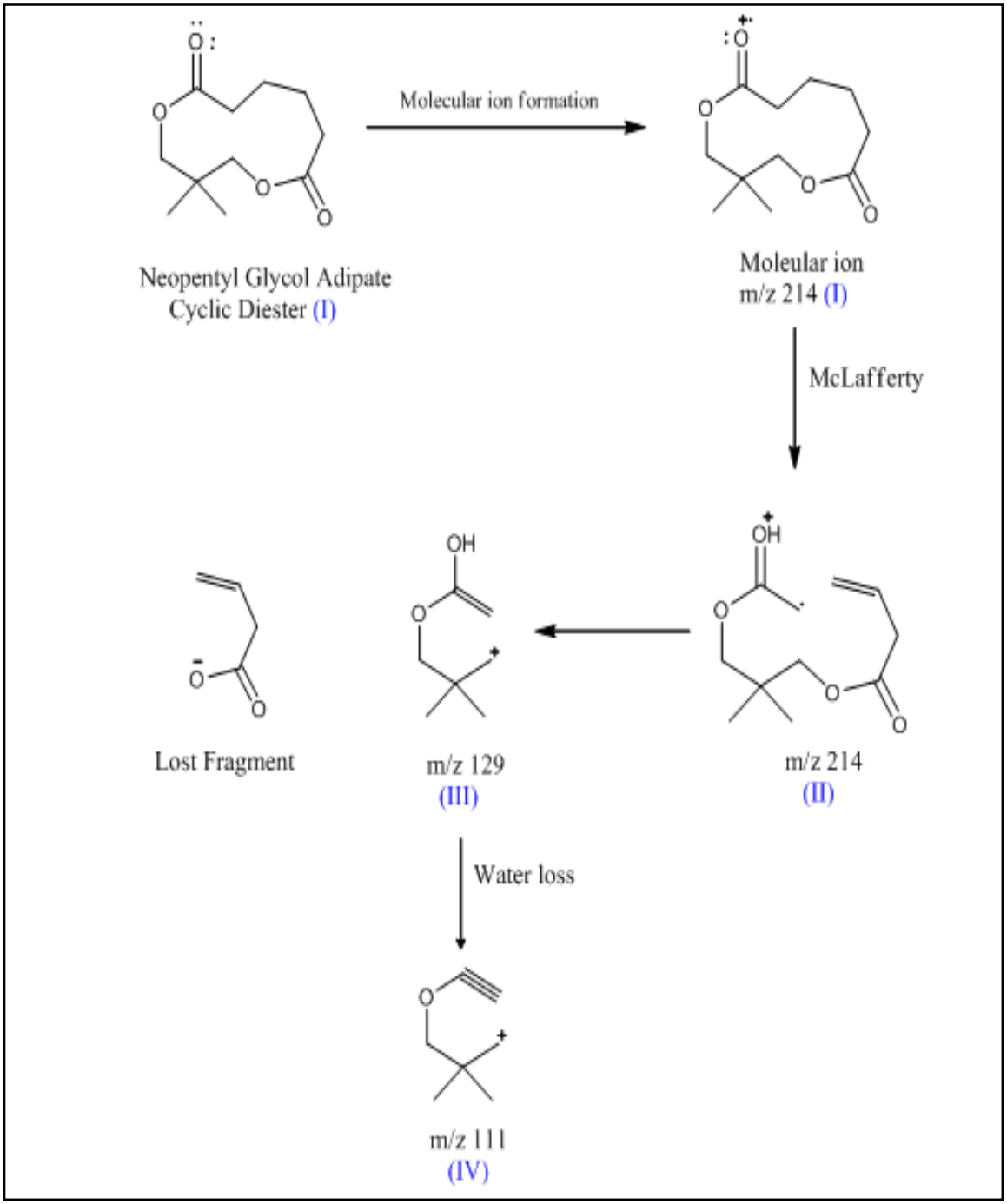


Figure 54: Fragmentation Analysis of Neopentyl Glycol, Adipic Acid, Cyclic Diester

The mass spectrum is produced by ionizing many molecules and hence the spectrum has a distribution of possible ion products. As Neopentyl Glycol Adipate Cyclic Diester in the gaseous phase passes through the ionization chamber of the mass spectrometer; it is bombarded with the high energy electron beams. This interaction with high energy electrons knocks off the non-bonding electron to form molecular ion (II). As the molecular ion retains the excess energy, it is highly unstable and undergoes fragmentation in order to gain the stability. The mechanism of fragment formation is illustrated in the above **Fig. 54.** However, the mechanisms associated with the fragmentation are complex and hence only few fragment formation paths have been discussed.

Fragmentation in esters is initiated by either alpha cleavage or the McLafferty rearrangement reactions. The molecular ion thus formed (II) undergoes the McLafferty rearrangement to form an odd electron ion with even m/z ratio value 214 (III). This is followed by carbon-oxygen bond cleavage as the excess energy is greater than the energy required to break the chemical bond, thus forming an ion fragment with m/z value 129 (IV). Further, the fragment with m/z value 111 is formed (VI) by loss of water molecule.

VI.B.6. Di-(1,4-Butanediol, Adipic Acid, Cyclic Diester)

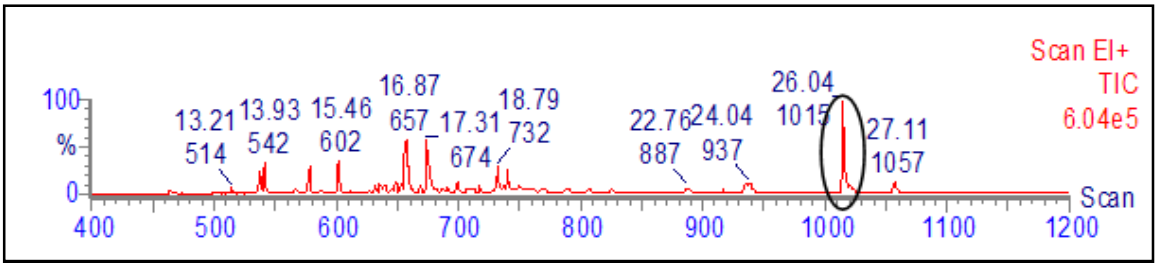


Figure 55: GC-MS-TIC Chromatogram of 10% EtOH Sealant-Side Laminate Extract of Di-(1,4-Butanediol, Adipic Acid, Cyclic Diester) Peak

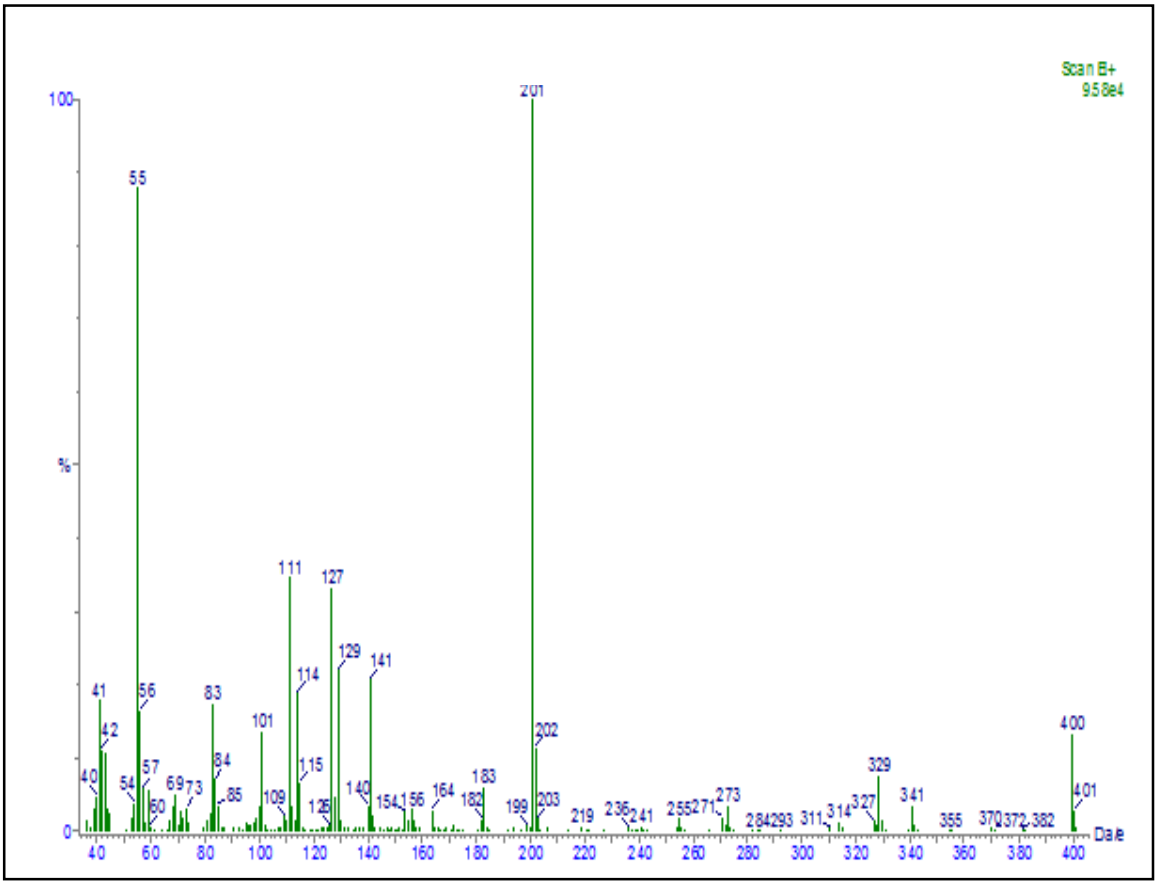


Figure 56: Mass Spectrum (EI) of Di-(1,4-Butanediol, Adipic Acid, Cyclic Diester)

10 Highest Peaks

201 (100) 55 (87.98) 111 (34.70) 127 (33.24) 129 (21.98)

141 (20.75) 114 (18.92) 41 (17.88) 83 (17.34) 56 (16.50)

Table 17: Chemistry and Migratory Properties of Di-(1,4-Butanediol Adipic Acid Cyclic Diester)

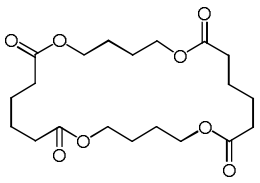
Common Name	IUPAC Name	M.W. g/mol	Structure	Retention Index	Concentration
Di-(1,4-Butanediol Adipate Cyclic Diester)	1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetrone	400		2690	**Range:** ppb: 170-183 ng/cm^2: 100-107.64 **Avg** = 176.3 ppb **σ** = ±5.5 ppb **N** = 4

Figure 55 and **Fig. 56** represent the chromatogram and the mass spectrum of Di-(1,4-Butanediol Adipate Cyclic Diester), respectively. It is a dimer of 1,4-Butanediol Adipate Cyclic Diester discussed previously. The molecular ion peak with m/z ratio 400 was found to be present in the mass spectrum. The molecular weight was confirmed by analyzing the CI spectrum **(Fig. 57**). Further, the chemical structure was deduced by studying the characteristic fragmentation pattern. It was found that the fragmentation pattern followed closely to that of 1,4-Butanediol Adipate. Some of the fragment formations are discussed in the fragmentation analysis section. The migratory concentration range was found to be 170-180 ppb in the food simulant (also expressed in ng/cm^2 of surface area exposed to extraction). As discussed previously, the retention time index was calculated by normalizing its retention time to that of closely eluting n-paraffin alkanes.

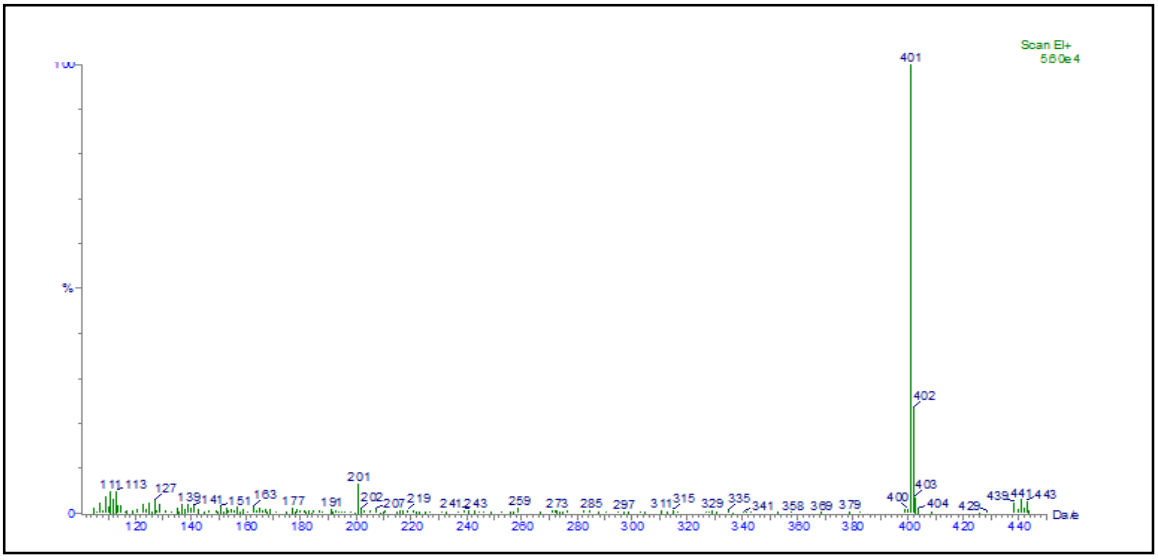


Figure 57: Mass Spectrum (CI) of Di-(1,4-Butanediol, Adipic Acid, Cyclic Diester)

Formation of Di-(1,4-Butanediol, adipic acid, cyclic diester)

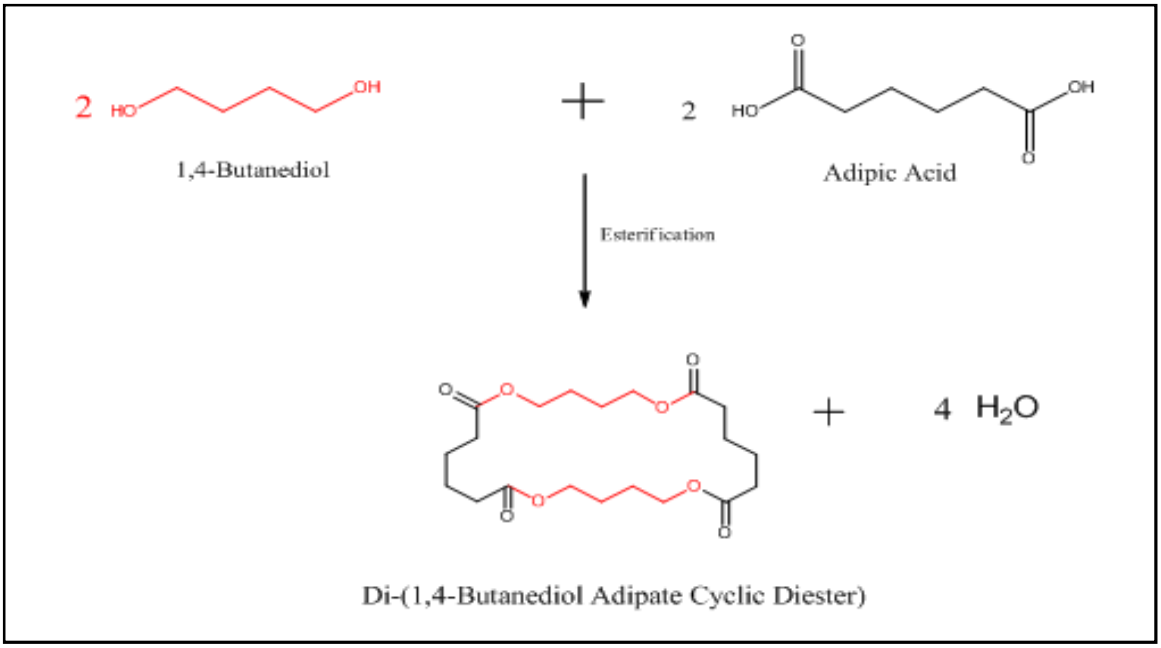


Figure 58: Formation of Di-(1,4-Butanediol, Adipic Acid, Cyclic Diester)

Fragmentation Analysis

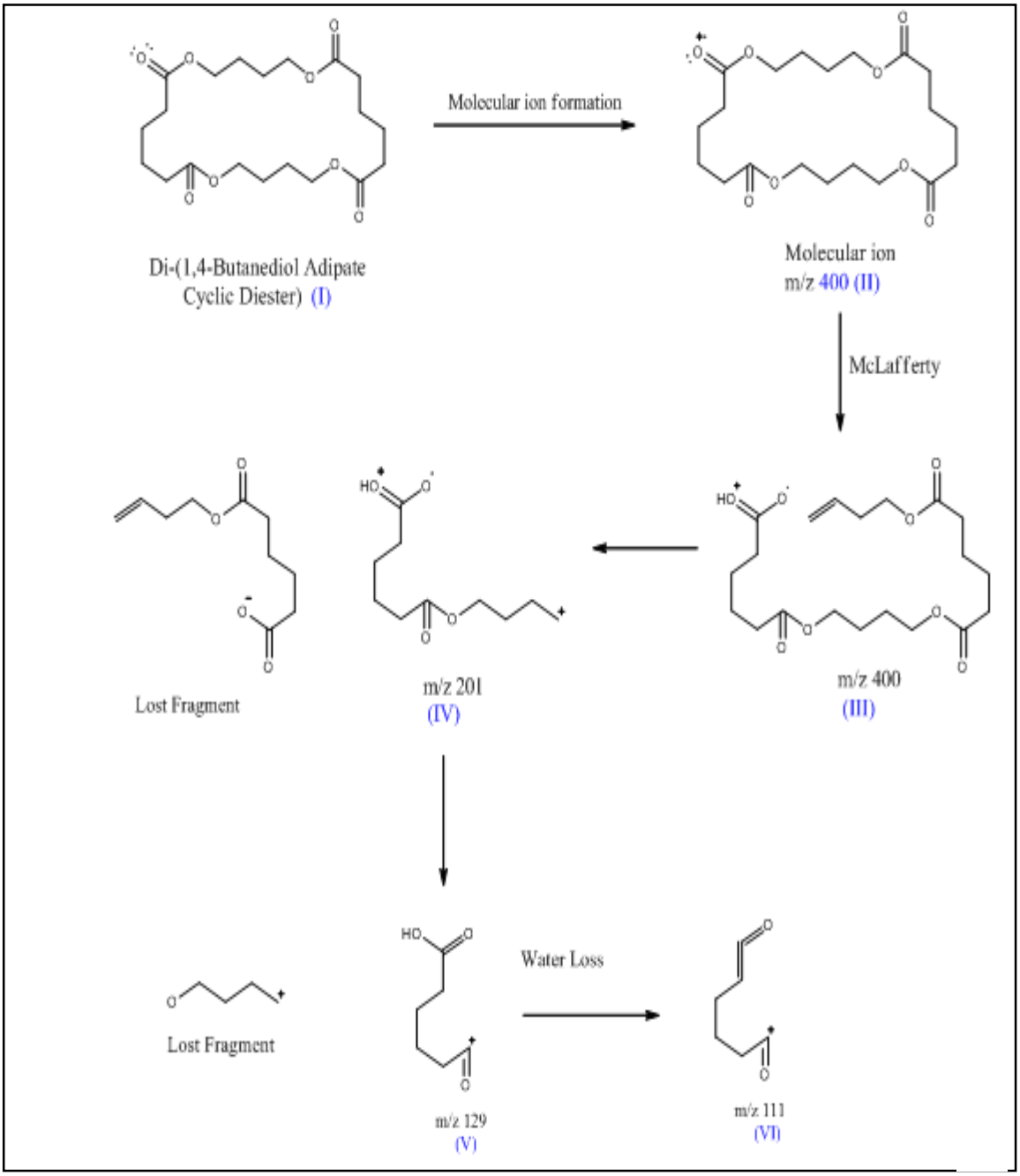


Figure 59: Fragmentation Analysis of Di-(1,4-Butanediol, Adipic Acid, Cyclic Diester)

The mass spectrum is produced by ionizing many molecules and hence the spectrum has a distribution of possible ion products. Di-(1,4-Butanediol Adipate Cyclic Diester) in the gaseous phase passes through the ionization chamber of the mass spectrometer; it interacts with the high energy electron beams. This interaction knocks off the non-bonding electron to form molecular ion (II). As the molecular ion retains the excess energy, it is highly unstable and splits into different fragments in order to achieve the stability. The mechanism of fragment formation is illustrated in the above **Fig. 59.** However, the mechanisms associated with the fragmentation are complex; hence, only few fragment formation paths have been discussed.

Fragmentation in esters is initiated by either alpha cleavage or the McLafferty rearrangement reactions. The molecular ion thus formed (II) undergoes the McLafferty rearrangement to form an odd electron ion with an even m/z ratio value of 400 (III). This is followed by carbon-oxygen bond cleavage as the excess energy is greater than the energy required to break the chemical bond, thus forming an ion fragment with m/z value 201 (IV). Since the structure contains multiple carbon oxygen bonds, another carbon oxygen bond cleavage takes place to yield a fragment with m/z value 129 (V). Further, the fragment with m/z value 111 is formed (VI) by loss of water molecule.

Di-(1,4-Butanediol Adipate Cyclic Diester) is a dimer of 1,4-Butanediol Adipate Cyclic Diester. It was observed that the fragmentation pattern of this dimer matched closely with the fragmentation pattern of 1,4-Butanediol Adipate Cyclic Diester.

VI.B.7. Di-(Diethylene Glycol Adipic Acid, Cyclic Diester)

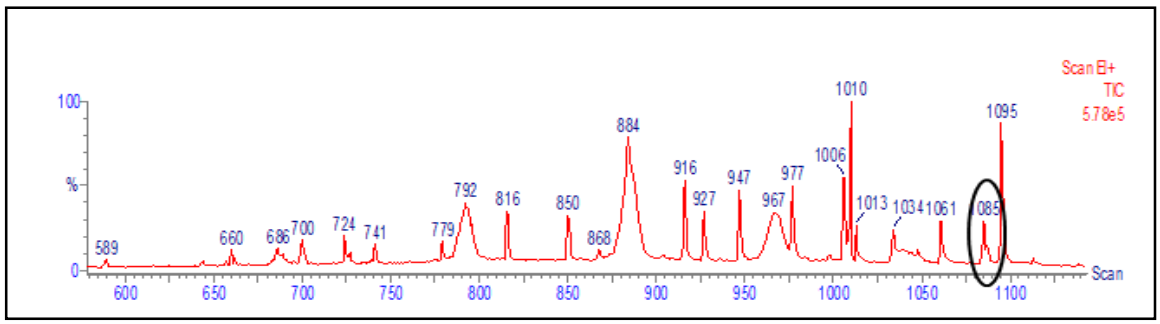


Figure 60: GC-MS-TIC Chromatogram of 10% EtOH Sealant-Side Laminate Extract of Di-(Diethylene Glycol, Adipic Acid, Cyclic Diester) Peak

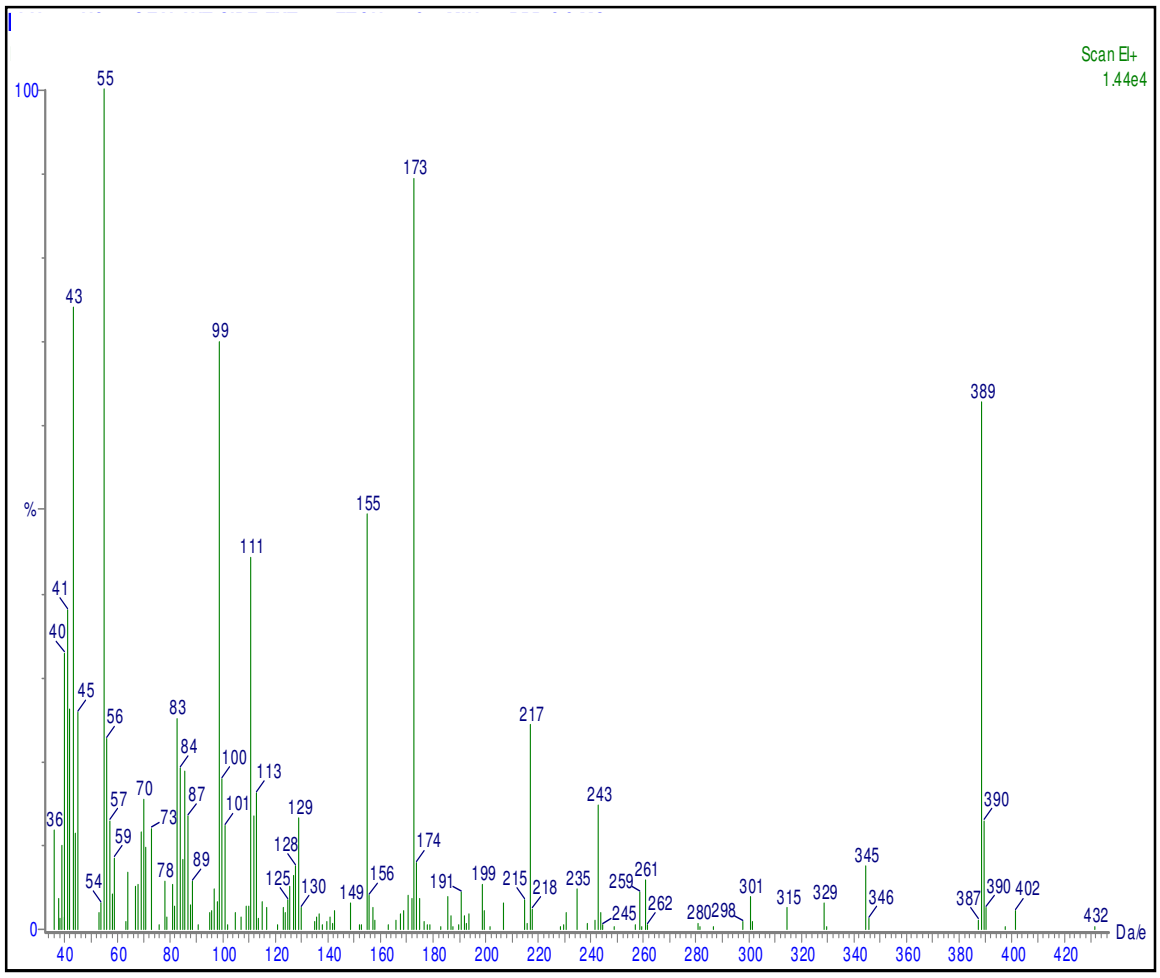
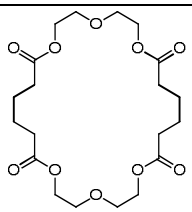


Figure 61: Mass Spectrum (EI) of Di-(Diethylene Glycol, Adipic Acid, Cyclic Diester)

10 Highest Peaks

55 (100)	173 (90.12)	43 (76.17)	99 (70.07)	389 (62.4)
155 (50.18)	111 (46.34)	41(38.83)	40 (34.89)	83 (26.29)

Table 18: Chemistry and Migratory Properties of Di-(Diethylene Glycol, Adipic Acid, Cyclic Diester)

Common Name	IUPAC Name	M.W. g/mol	Structure	Retention Index	Concentration
Di-(Diethylene Glycol Adipate Cyclic Diester)	1,4,7,14,17,20-hexaoxacyclohexacosane-8,13,21,26-tetraone	432		2975	**Range**: ppb: 130-140 ng/cm2: 76.47-82.35 **Avg** = 134.2 ppb **σ** = ±4 ppb **N** = 4

The chromatogram and the mass spectrum of the compound have been displayed in **Fig. 60** and **Fig. 61**, respectively. A mass spectrum is essentially a plot of m/z ratio vs. the intensity of the ion fragment. The molecular ion, formed by knocking off the lone electron, with m/z ratio 432 was found to be present with a weak intensity. The chemical structure was deduced by analyzing the fragmentation pattern. The migratory concentration and the retention time index were calculated as discussed before. The concentration range of the migrated Di-(Diethylene Glycol Adipate Cyclic Diester) was found to be 130-140 ppb in the food simulant, equivalent to 76.47-82.35 ng/cm^2 of surface area exposed to extraction.

Formation Di-(Diethylene Glycol, Adipic Acid, Cyclic Diester)

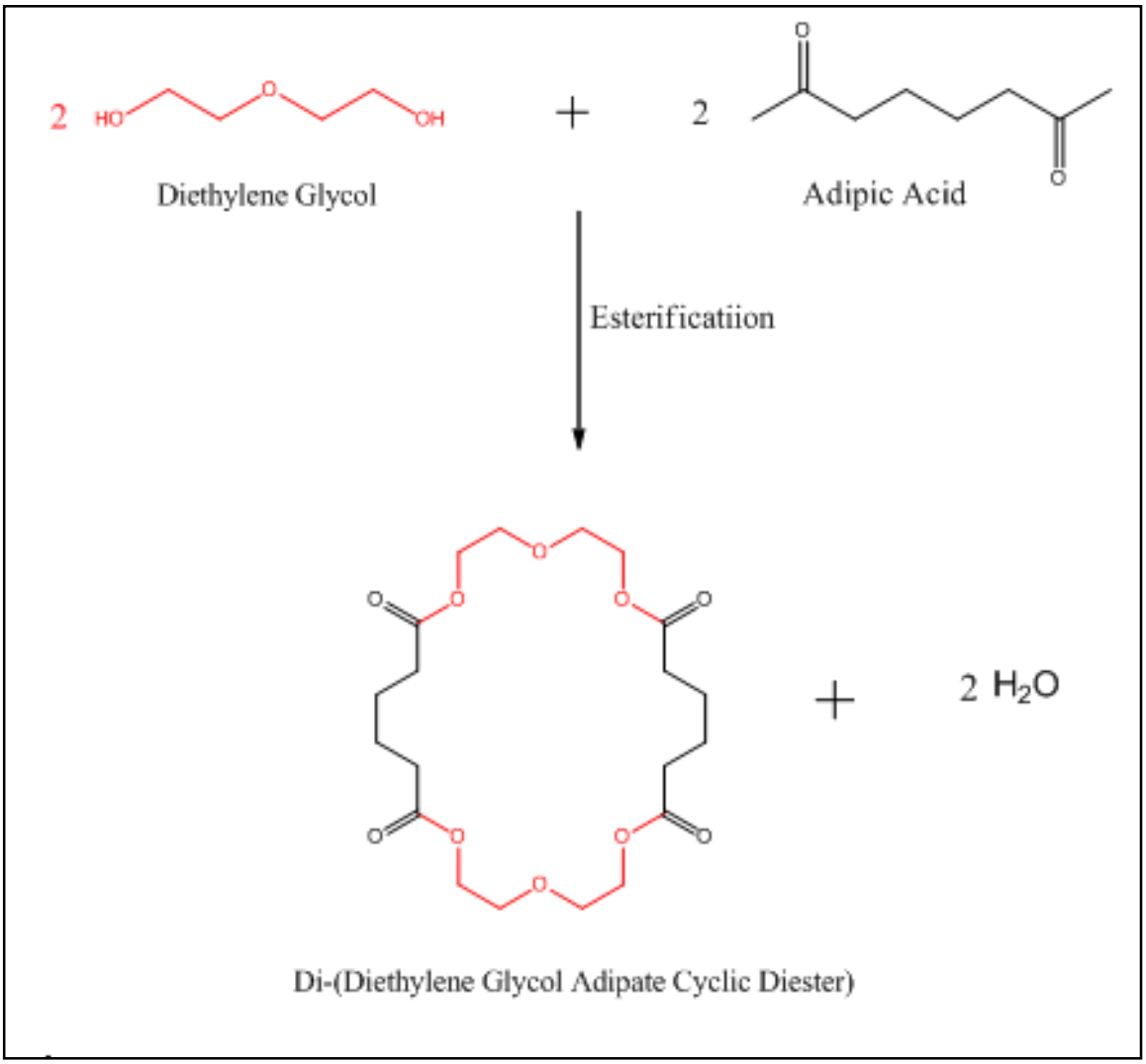


Figure 62: Formation of Di-(Diethylene Glycol, Adipic Acid, Cyclic Diester)

Fragmentation Analysis

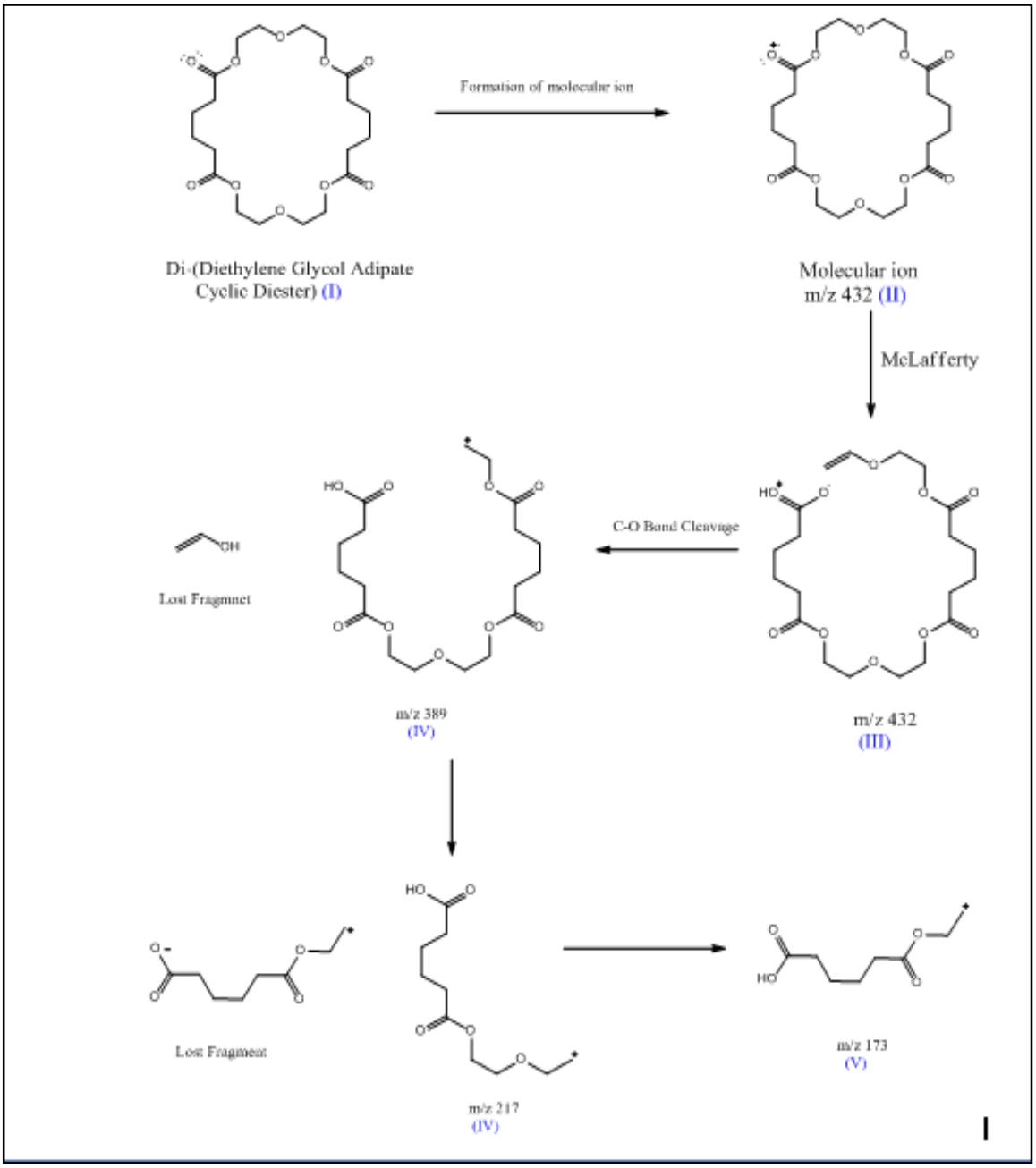


Figure 63: Fragmentation Analysis of Di-(Diethylene Glycol, Adipic Acid, Cyclic Diester)

The mass spectrum is produced by ionizing many molecules and hence the spectrum has a distribution of possible ion products. As the dimer, Di-(Diethylene Glycol Adipate Cyclic Diester), in the gaseous phase passes through the ionization chamber of the mass spectrometer it is bombarded with the high energy electron beams. This interaction with high energy electrons knocks off the non-bonding electron to form molecular ion (II). As the molecular ion retains the excess energy, it is highly unstable and undergoes fragmentation in order to gain the stability. The mechanism of fragment formation is illustrated in the above **Fig. 63.** However, the mechanisms associated with the fragmentation are complex and hence only few fragment formation paths have been discussed.

Fragmentation in esters is initiated by either alpha cleavage or the McLafferty rearrangement reactions. The molecular ion thus formed (II) undergoes the McLafferty rearrangement to form an odd electron ion with an even m/z ratio value of 432 (III). This is followed by carbon-oxygen bond cleavage as the excess energy is greater than the energy required to break the chemical bond, thus forming the ion fragment with m/z value 389 (IV). As the molecule contains multiple C-O bonds, the C-O bonds rupture to yield the fragment with m/z 217 (V) and the fragment with m/z value 117 (VI), subsequently.

It has been observed that the fragmentation pattern of Di-(Diethylene Glycol Adipate Cyclic Diester) matched closely with that of its monomer, Diethylene Glycol Adipate Cyclic Diester.

VI.B.8. Di-(Diethylene Glycol, Phthalic Acid, Cyclic Diester)

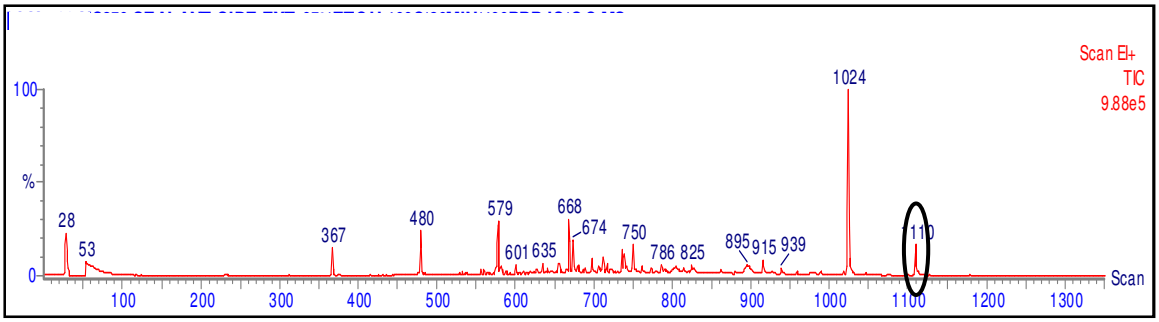


Figure 64: GC-MS-TIC Chromatogram of 95% EtOH Sealant-Side Laminate Extract of Di-(Diethylene Glycol, Phthalic Acid, Cyclic Diester) Peak

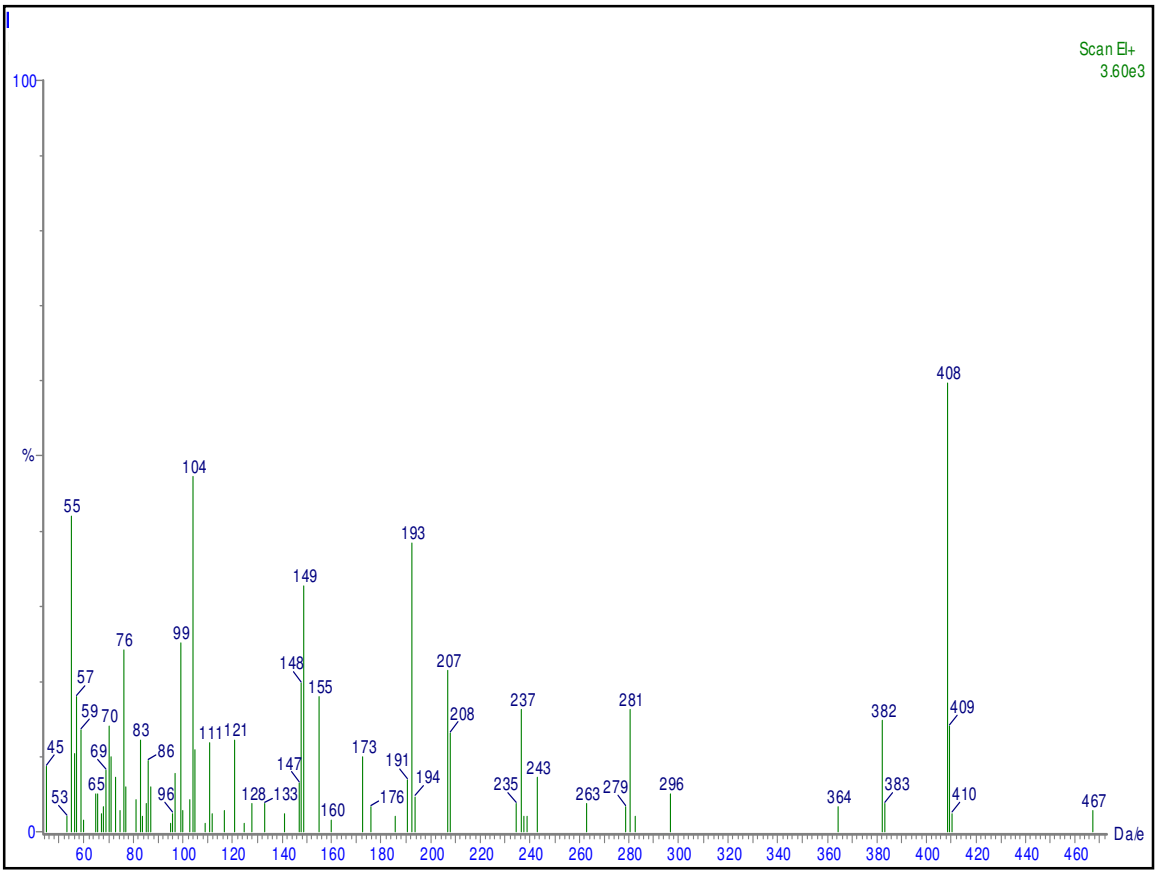


Figure 65: Mass Spectrum (EI) of Di-(Diethylene Glycol, Phthalic Acid, Cyclic Diester)

10 Highest Peaks

408 (100)	104 (81.33)	55 (76.85)	193 (71.33)	149 (63.33)
99 (46.63)	76 (41.27)	148 (33.34)	207 (31.66)	57 (28.13)

Table 19: Chemistry and Migratory Properties of Di-(Diethylene Glycol Phthalic Acid, Cyclic Diester)

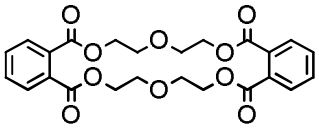
Common Name	M.W. g/mol	Structure	Retention Index	Concentration
Di –(Diethylene Glycol Phthalate Cyclic Diester)	472		3012	**Range**: ppb: 80-95 ng/cm^2: 47.06-55.88 **Avg** = 89.5 ppb **σ** = ±5.7 ppb **N** = 4

Figure 65 shows the molecular ion fragment with m/z ration 472, tentatively assigned as Di-(Diethylene Glycol Phthalate Cyclic Diester). Since the mass spectrum of phthalic acid and isophthalic acid matched closely with respect to retention time and the fragmentation pattern and no previously synthesized extracts were available as reference, the identity of the compound could not be confirmed. The molecular ion is not seen in the mass spectrum due to the extensive fragmentation. The complex chemical structure was obtained by studying the fragmentation pattern. The Retention time index was calculated by normalizing its retention time to the mixture closely eluting n-paraffin alkanes.

The concentration of the analyte was calculated using the correlation of direct dependence of the concentration of the analyte peak to its integrated area. By

integrating the mass spectrum, the peak areas of the analyte and the internal standard were determined. The concentration range was found to be 80 – 95 ppb in the food simulant with a standard deviation of ±5.7 ppb (**Table 19**).

Formation of Di-(Diethylene Glycol, Phthalic Acid, Cyclic Diester)

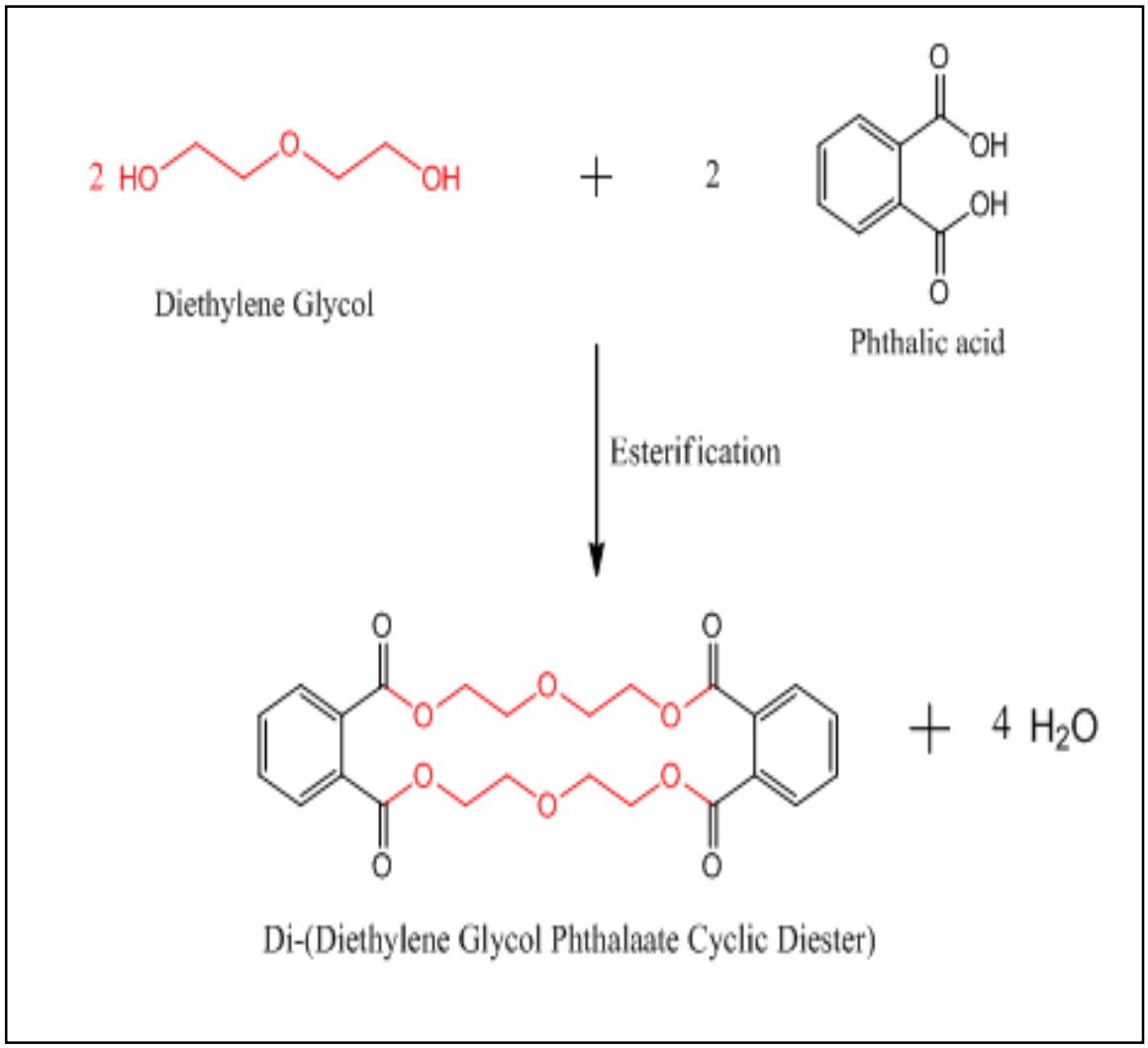


Figure 66: Formation of Di-(Diethylene Glycol, Phthalic Acid, Cyclic Diester)

Fragmentation Analysis

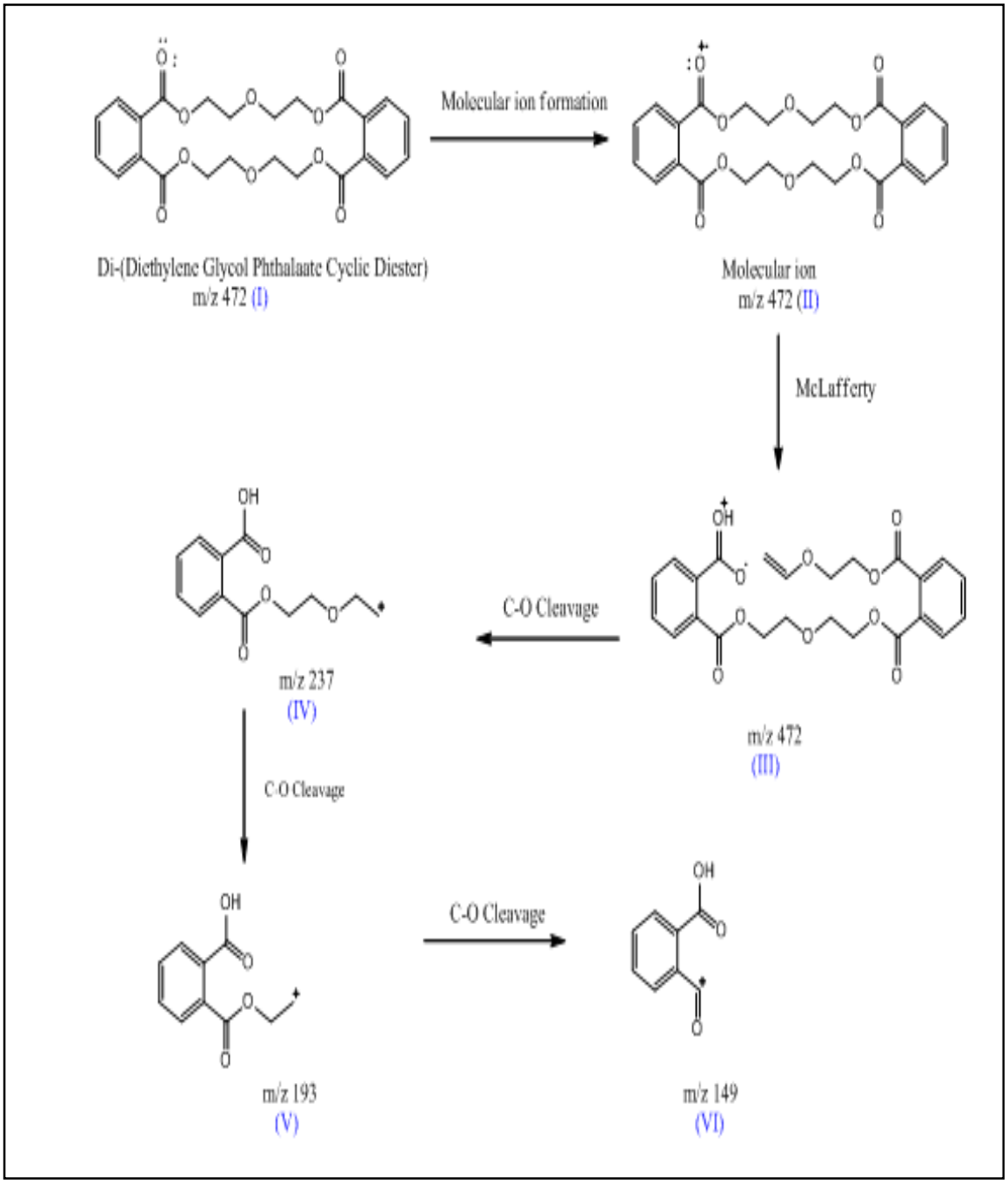


Figure 67: Fragmentation Analysis of Di-(Diethylene Glycol, Phthalic Acid, Cyclic Diester)

The mass spectrum is formed by ionizing the molecule with high energy electron beams. It is a distribution of possible product ions. As the dimer, Di-(Diethylene Glycol Isophthalate), passes through the ionization chamber of the mass spectrometer, it ionizes to form the molecular ion. Due to the retention of excess energy, sometimes the molecular ion instantaneously fragments into ion fragments and hence it is not seen in the mass spectrum. The possible mechanism for fragmentation is suggested in **Fig. 67**. It is not practically possible to deduce the structure of every fragment formed; and hence, only few of the fragments have been discussed.

The fragmentation pattern in esters follows either the rearrangement reaction or the chemical bond cleavages. The molecular ion (II) undergoes McLafferty rearrangement to form an ionizing fragment with m/z value 237. Since the dimer compound contains multiple C-O bonds; their cleavages lead to formation of fragments with m/z value 193 followed by fragments with m/z value 149.

Also, it has been observed that the fragmentation pattern of Di-(Diethylene Glycol Isophthalate) matched closely with that of its monomer Diethylene Glycol Adipate.

VI.B.9. Di-(Neopentyl Glycol, Adipic Acid, Cyclic Diester)

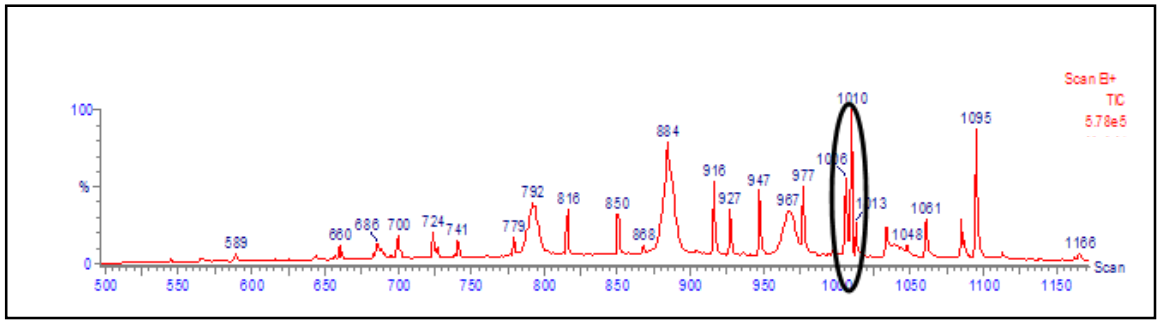


Figure 68: GC-MS-TIC Chromatogram of 95% EtOH Sealant-Side Laminate Extract of Di-(Neopentyl Glycol, Adipic Acid, Cyclic Diester) Peak

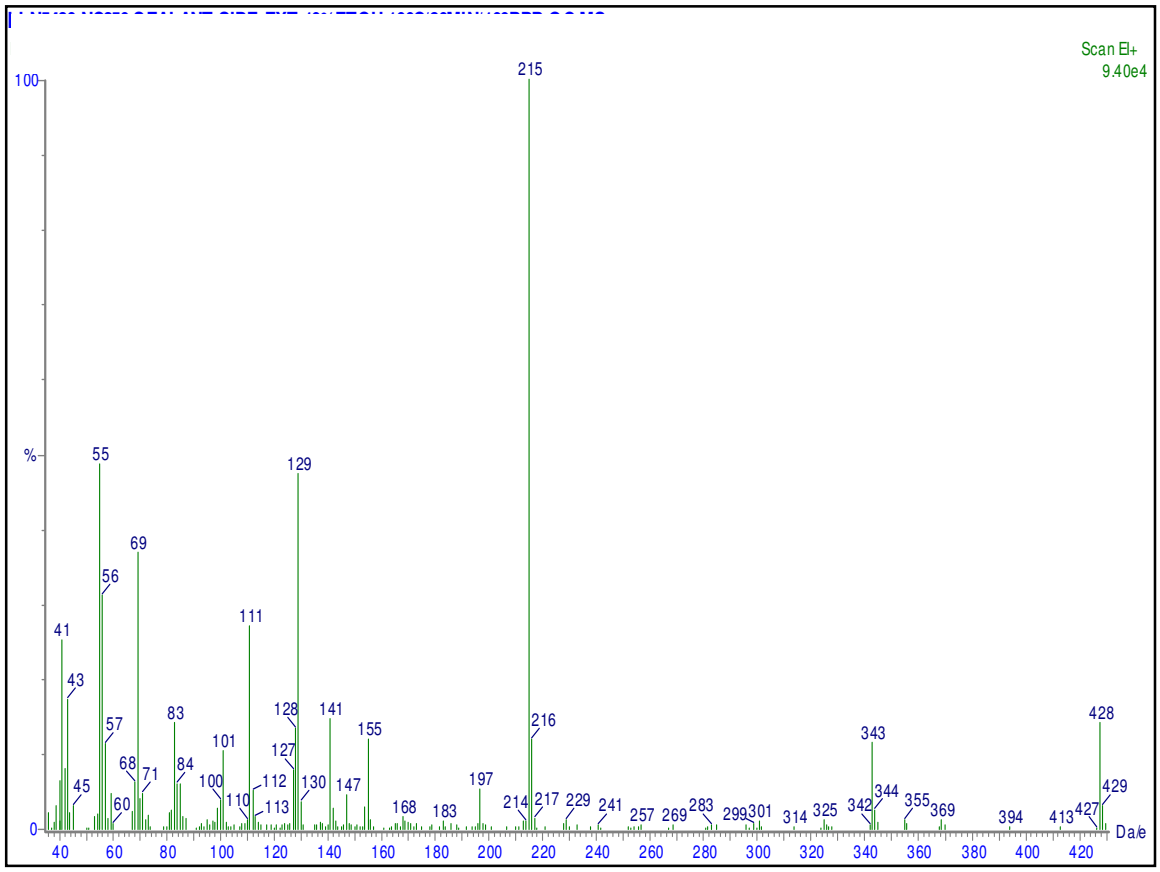
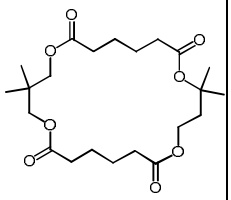


Figure 69: Mass Spectrum (EI) of Di-(Neopentyl Glycol, Adipic acid, Cyclic Diester)

10 Highest Peaks

215 (100)	55 (47.39)	129 (48.53)	69 (36.69)	56 (31.12)
111 (26.99)	41 (25.13)	141 (14.66)	83 (13.99)	428 (13.99)

Table 20: Chemistry and Migratory Properties of Di-(Neopentyl Glycol, Adipic Acid, Cyclic Diester)

Common Name	IUPAC Name	M.W. g/mol	Structure	Retention Index	Concentration
Di-(Neopentyl Glycol Adipate Cyclic Diester)	2,2,14,14-tetramethyl-1,5,12,16-tetraoxacyclodocosane-6,11,17,22-tetrone	428		2819	**Range:** ppb: 310-320 ng/cm2: 182.34-188.22 Avg = 314 ppb σ = ± 3.7 ppb N = 4

The chromatogram and the mass spectrum of the compound have been displayed in **Fig. 68** and **Fig. 69,** respectively. A mass spectrum is essentially a plot of m/z ratio vs. the intensity of the ion fragment. The molecular ion, formed by knocking off the lone electron, with m/z ratio 214 was found to be present with a weak intensity. The chemical structure was deduced by analyzing the fragmentation pattern. The migratory concentration and the retention time index were calculated as discussed before. The concentration range of the migrated Di-(Neopentyl Glycol Adipate Cyclic Diester) was found to 310 – 320 ppb in the food simulant with the standard deviation ±3.7 ppb. Concentration was also expressed in ng/cm^2 of surface area exposed to extraction (**Table 20**).

Formation of Di-(Neopentyl Glycol, Adipic Acid, Cyclic Diester)

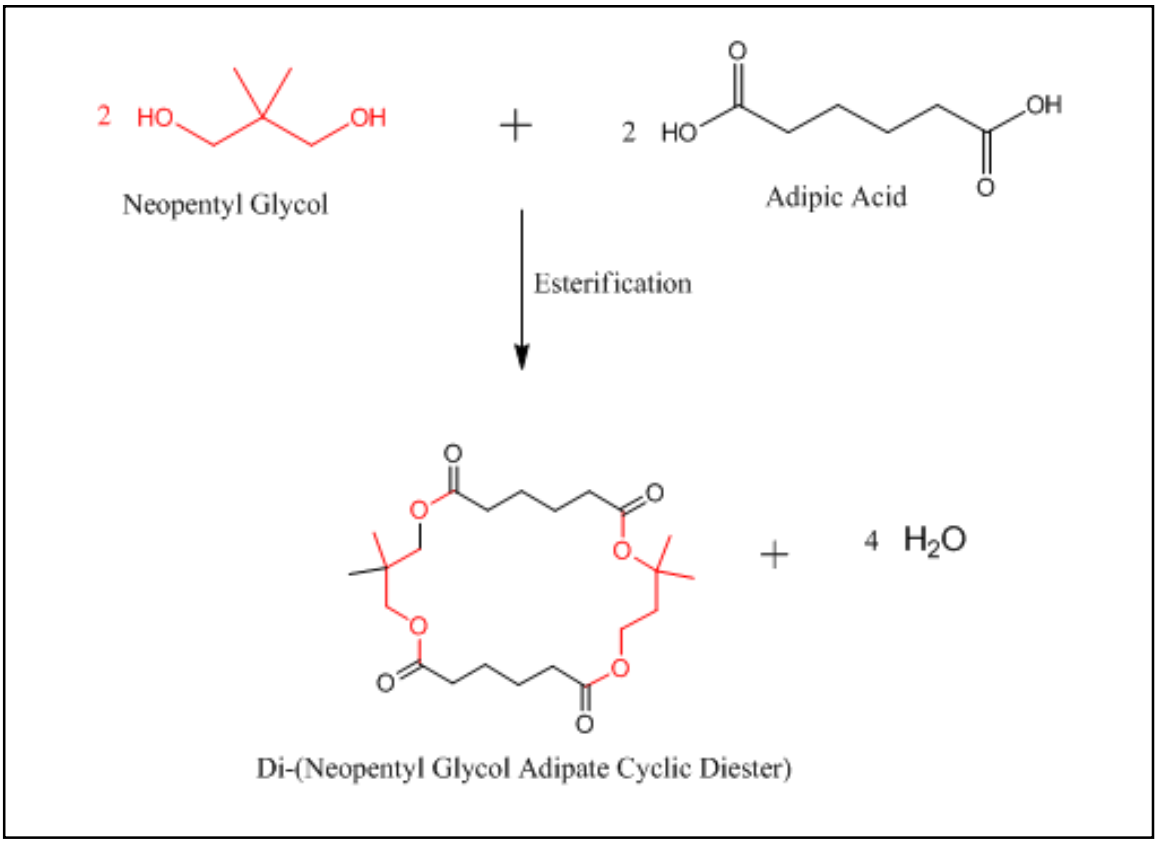


Figure 70: Formation of Di-(Neopentyl Glycol, Adipic Acid, Cyclic Diester)

Fragmentation Analysis

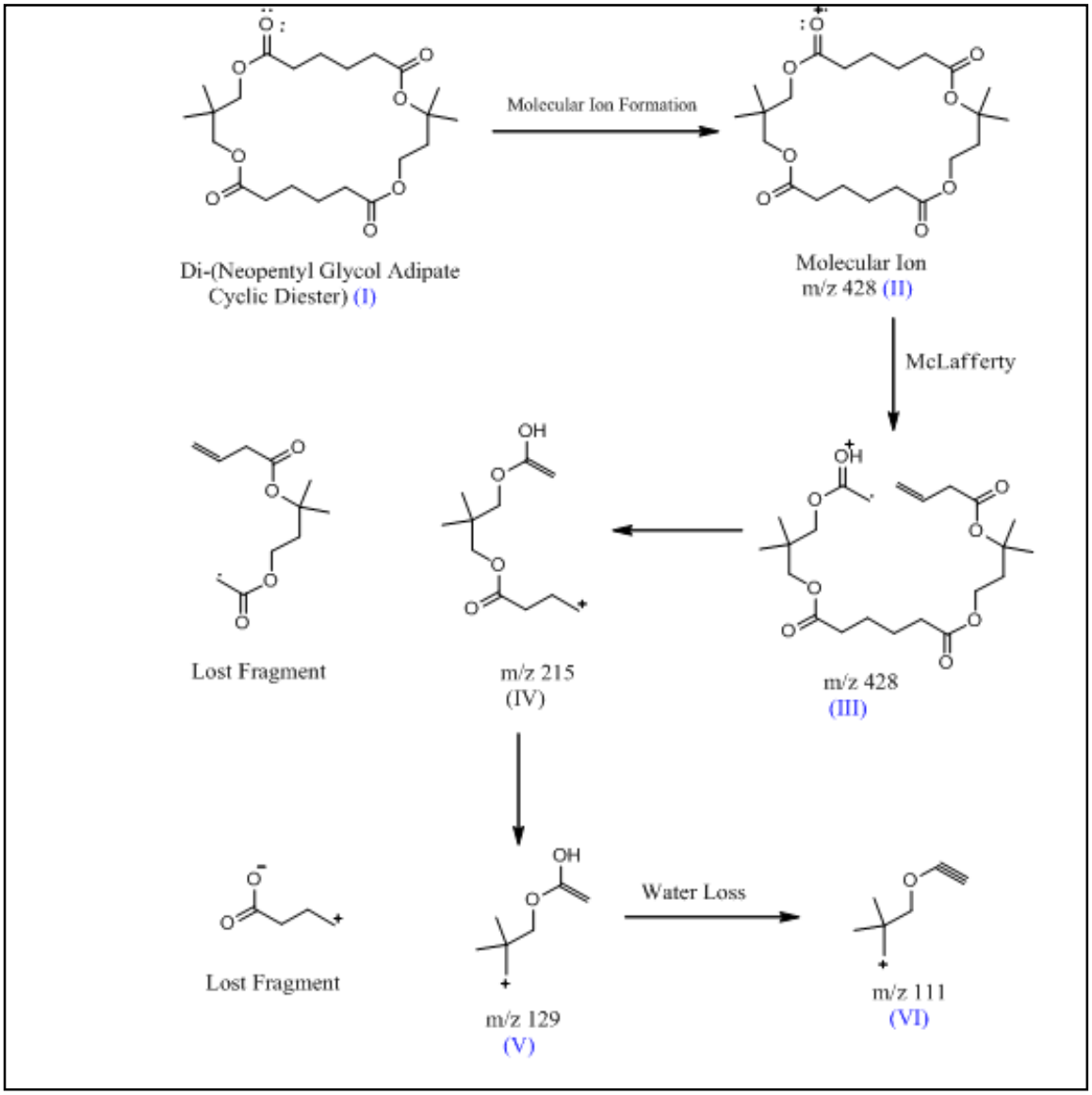


Figure 71: Fragmentation Analysis of Di-(Neopentyl Glycol, Adipic Acid, Cyclic Diester)

A mass spectrum is the plot of m/z ratio vs. the intensity of the fragment ion. Hence, mass spectrum essentially consists of a distribution of possible product ions. When Di-(Neopentyl Glycol Adipate Cyclic Diester), in the gaseous, form passes through the ionization chamber of the mass spectrometer, it is collided with high intensity electron beams, which can withdraw the non-bonding electron. This results in the formation of molecular ion (II) as displayed in **Fig. 71**. Due to excess energy contained within the molecular ion, the instability leads to fragmentation of the molecular ion. This fragmentation pattern provides information about the structure of the molecule. However, it is extremely complex to assign structure to each and every fragment formed in the spectrum. Hence, for the practical purposes, only some of the fragmentation paths have been illustrated in detail.

Essentially two main fragmentation patterns are associated with esters viz., cleavage reactions and McLafferty rearrangement reactions. The McLafferty rearrangement also leads to chain opening in case of cyclic esters. The molecular ion thus formed (II) undergoes the McLafferty rearrangement to form an odd electron ion with even m/z ratio value 428 (III). This is followed by C-C bond cleavage as the excess energy is greater than the energy required to break the chemical bond, thus forming an ion fragment with m/z value 215 (IV). The carbon-oxygen bond, having low bond energy, ruptures to form a fragment with m/z value 129 (V). Further, the fragment with m/z value 111 is formed (VI) by loss of water molecule.

VI.B.10. Ethylene Glycol, Adipic acid, Cyclic Diester

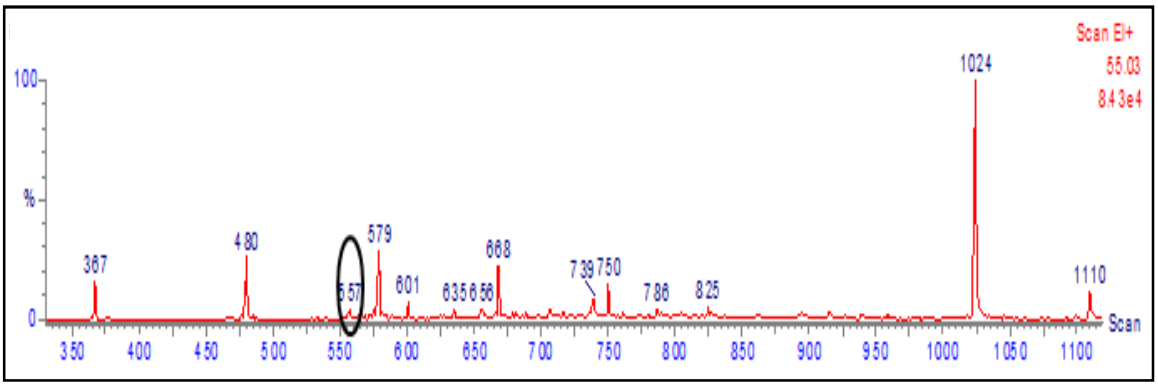


Figure 72: GC-MS-TIC Chromatogram of 95% EtOH Sealant-Side Laminate Extract Depicting Ethylene Glycol, Adipic Acid, Cyclic Diester Peak

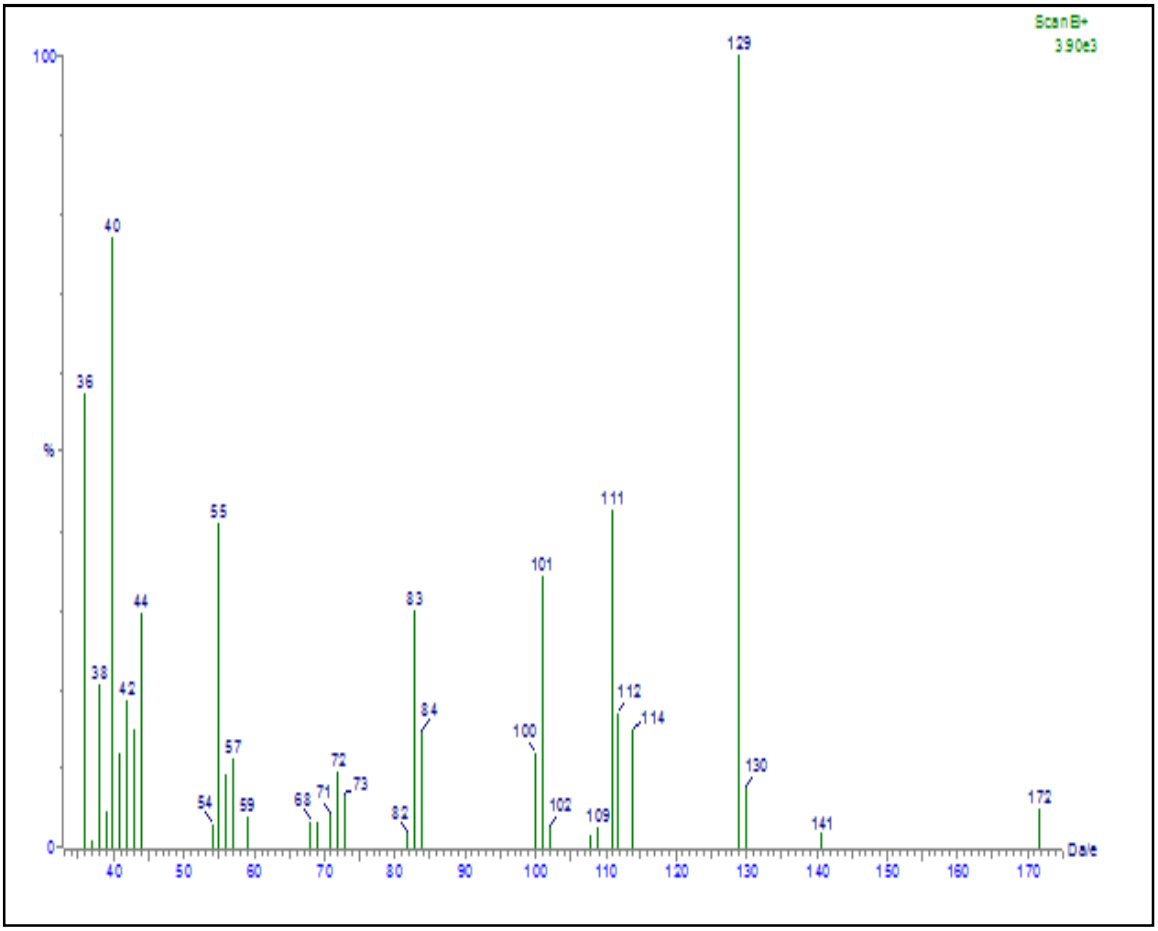


Figure 73: Mass spectrum (EI) of Ethylene Glycol, Adipic Acid, Cyclic Diester

10 Highest peaks

193 (100)	149 (77.04)	104 (57.37)	76 (42.62)	192 (40.98)
148 (34.42)	50 (29.91)	194 (29.50)	176 (20.49)	132 (18.44

Table 21: Chemistry and Migratory Properties of Ethylene Glycol, Adipic Acid, Cyclic Diester

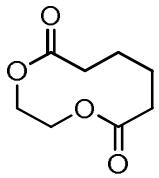
Common Name	IUPAC Name	M.W. g/mol	Structure	Retention Index	Concentration
Ethylene Glycol Adipate Cyclic Diester	1,4-dioxecane-5,10-dione	172		1510	**Range**: ppb: 138-144 ng/cm2: 81.17-84.7 Avg – 141 ppb σ = ± 2.12 N = 4

Figure 72 and **Fig**. **73** represent the chromatogram and the mass spectrum of Ethylene Glycol Adipate Cyclic Diester, respectively. The molecular ion peak with m/z ratio 72 was found to be present in the mass spectrum. Further, the chemical structure was deduced by studying the characteristic fragmentation pattern. Some of the fragment formations are discussed in the fragmentation analysis section. The migratory concentration range was found to be 138 – 144 ppb in the food simulant. As discussed previously, the retention time index was calculated by normalizing its retention time to that of closely eluting n-paraffin alkanes (**Table 21)**.

Formation of Ethylene Glycol, Adipic Acid, Cyclic Diester

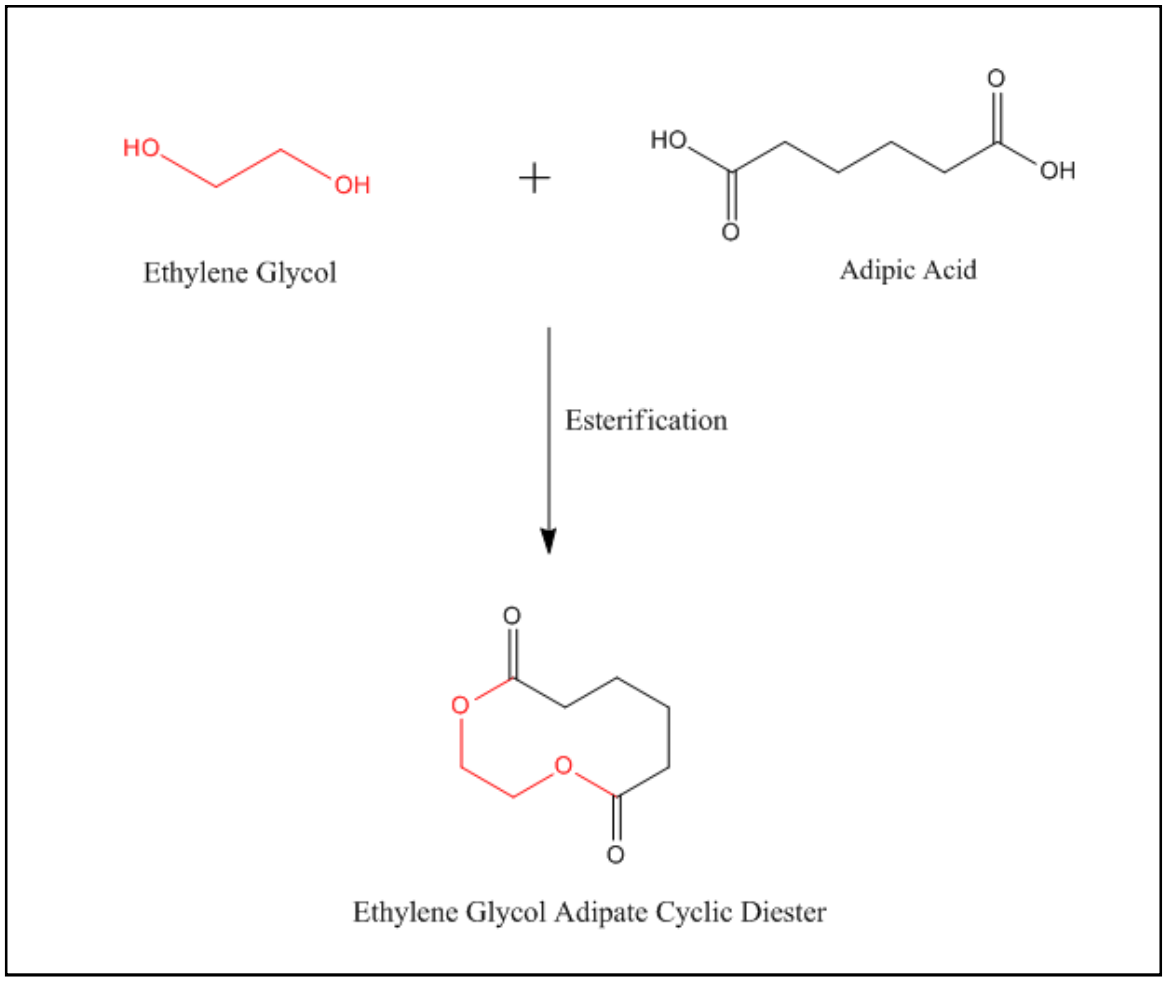


Figure 74: Formation of Ethylene Glycol, Adipic Acid, Cyclic Diester

Fragmentation Analysis

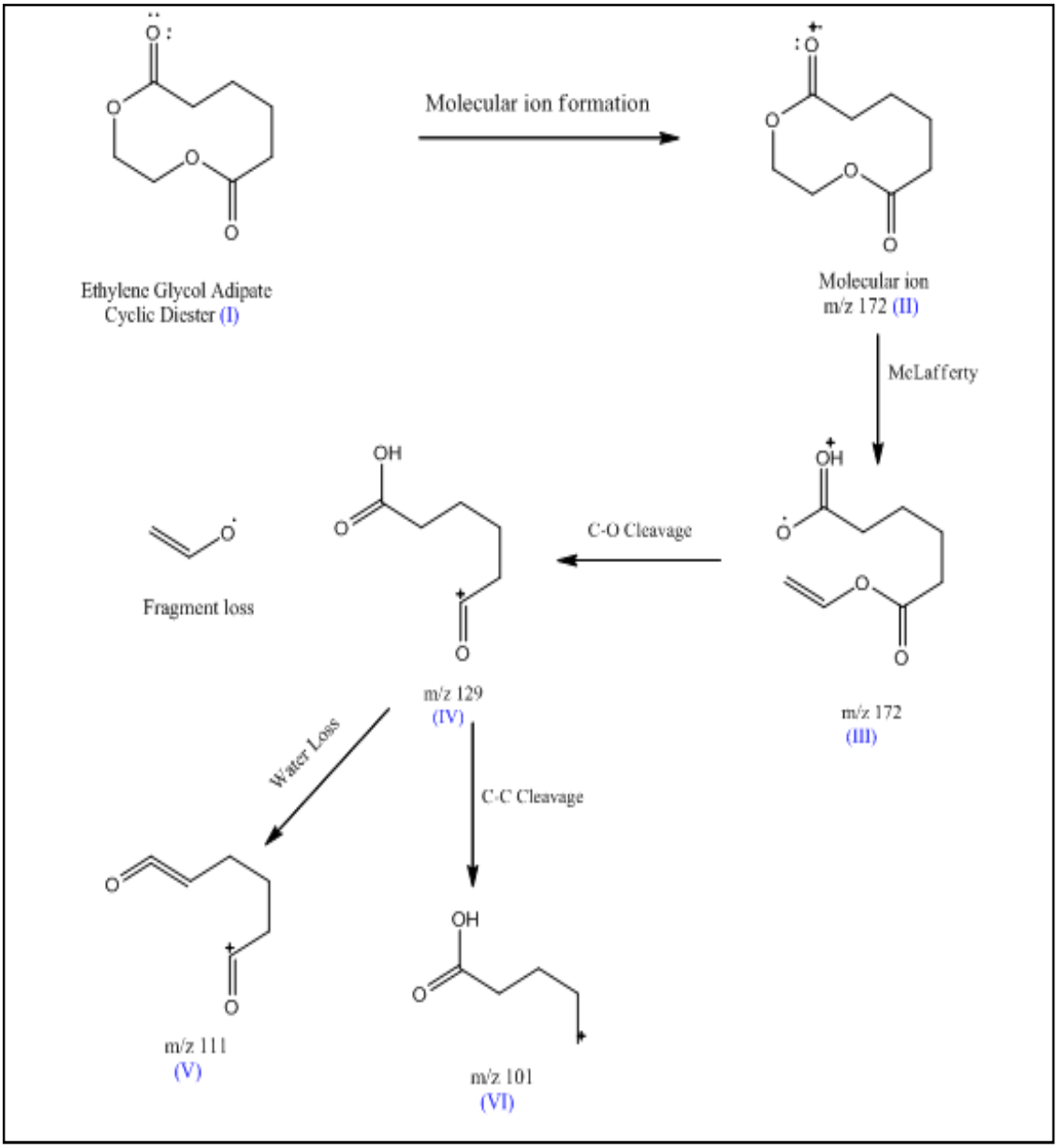


Figure 75: Fragmentation Analysis of Ethylene Glycol, Adipic Acid, Cyclic Diester

The mass spectrum is produced by ionizing many molecules and hence the spectrum has a distribution of possible ion products. Ethylene Glycol Adipate Cyclic Diester in the gaseous phase passes through the ionization chamber of the mass spectrometer; it interacts with the high energy electron beams. This interaction knocks off the non-bonding electron to form molecular ion (II). As the molecular ion retains the excess energy, it is highly unstable and splits into different fragments in order to achieve the stability. The possible mechanism of fragment formation is illustrated in the above **Fig. 75.** However, the mechanisms associated with the fragmentation are complex; hence, only few fragment formation paths have been discussed.

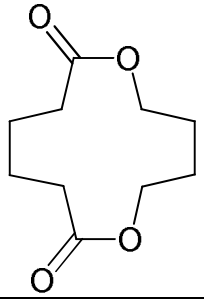
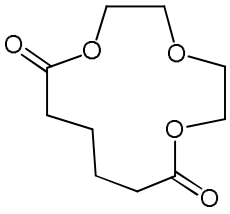
Fragmentation in esters is initiated by either alpha cleavage or the McLafferty rearrangement reactions. The molecular ion thus formed (II) undergoes the McLafferty rearrangement to form an odd electron ion with an even m/z ratio value of 172 (III). This is followed by carbon-oxygen bond cleavage as the excess energy is greater than the energy required to break the chemical bond, thus forming an ion fragment with m/z value 129 (IV). The fragment with m/z value 111 is formed (V) by loss of water molecule. At this stage, carbon-carbon bond cleavage takes place to form fragment with m/z value 101 (VI).

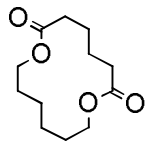
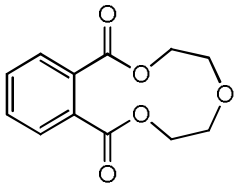
The following **Table 22** summarizes the compounds identified along with their chemical structures, retention time indices, 10 highest peak fragments and the migratory concentrations expressed in 3 different units.

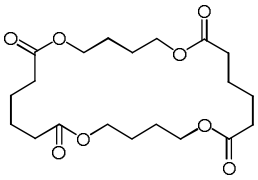
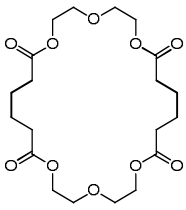
Extraction Method: 51 cm^2, 30 ml 10% and 95% EtOH

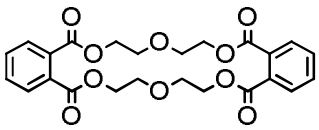
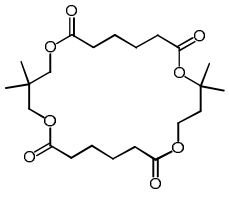
Extractant Volume to Surface Area Ratio = 0.59 ml/cm^2

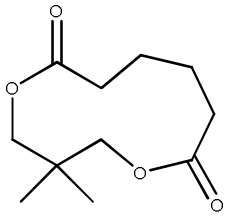
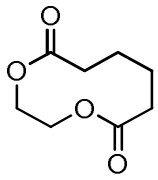
Table 22: Summary of Short Chain Cyclic Polyester Oligomers

Compound	**10 Highest Peaks**	**Conc. * ppb (w/w)**	**Conc. ** ng/cm^2**	**Conc.*** $\mu g/dm^2$**
Common Name: 1,4-Butanediol, adipic acid, cyclic diester **IUPAC Name**: 1,6-dioxacyclododecane-7,12-dione **M.W**. 200 g/mol **RI** – 1514 	129 (100), 55 (73.29) 111 (46.94) 101 (39.17) 41 (36.15) 83 (33.57) 42 (32.28) 56 (19.76), 43 (14.63) 39 (13.19)	Range: 105 ppb to 116 ppb	Range: 61.76 ng/cm^2 to 68.23 ng/cm^2	Range: 6.18 $\mu g/dm^2$ To 6.82 $\mu g/dm^2$
Common Name: Diethylene glycol, adipic acid, cyclic diester **IUPAC Name**: 1,4,7-trioxacyclotridecane-8,13-dione **M.W.** 216 g/mol **RI** - 1618 	173 (100 55 (37.37) 99 (15.27) 41 (13.89) 42 (13.07) 43 (11.87) 84 (11.68) 45 (10.37) 56 (9.98) 111 (9.12)	Range: 120 ppb to 130 ppb	Range: 70.58 ng/cm^2- to 76.47 ng/cm^2	Range: 7.05 $\mu g/dm^2$ to 7.65 $\mu g/dm^2$

Common Name: 1,6-Hexanediol, adipic acid, cyclic diester **IUPAC Name**: 1,8-dioxacyclotetradecane-2,7-dione **M.W**. 228 g/mol **RI** – 1762 	55 (100) 82 (68.66) 41 (54.73) 67 (34.03) 83 (34.01) 129 (31.52 111 (25.71) 54 (25.67) 42(23.77) 101 (23.52)	Range: 76 ppb to 86 ppb	Range: 44.70 ng/cm^2 to 50.59 ng/cm^2	Range 4.47 µg/dm^2 to 5.06 µg/dm^2
Common Name: Diethylene glycol, phthalic acid, cyclic diester **IUPAC Name**: 9,10-benzo[1,4,7] trioxacycloundecane-8,11-dione **M.W**. 236 g.mol **RI** – 1876 	193 (100) 149(84.21) 104 (46.11) 76(29.45) 192 (12.24) 148(10.93) 50 (10.91) 194 (9.03) 176 (8.55) 132 (7.21)	Range: 207 ppb to 220 ppb	Range: 121.76 ng/cm^2 to 129 .4 ng/cm^2	Range 12.18 µg/dm^2 to 12.94 µg/dm^2

Common Name: Di-(1,4-Butanediol, adipic acid, cyclic diester) IUPAC Name: 1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetrone **M.W**. 400 g/mol **RI** – 2690 	201 (100) 55 (87.98) 111 (34.70) 127 (33.24) 129 (21.98) 41(20.75) 114 (18.92) 41(17.88) 83 (17.34) 56 (16.50)	Range: 170 ppb To 183 ppb	Range: 100 ng/cm^2 to 107.64 ng/cm^2	Range: 10.00 µg/dm^2 to 10.76 µg/dm^2
Common Name: Di-(Diethylene glycol, adipic acid, cyclic diester) **IUPAC Name**: 1,4,7,14,17,20-hexaoxacyclohexacosane-8,13,21,26-tetraone **M.W**. 432 g/mol **RI** – 2975 	55(100) 173(90.12) 43 (76.17) 99 (70.07) 389 (62.4) 55 (50.18) 111(46.34) 41(38.83) 40 (34.89) 83 (26.29)	Range: 130 ppb to 140 ppb	Range: 76.47 ng/cm^2 to 82.35 ng/cm^2	Range: 7.65 µg/dm^2 to 8.24 µg/dm^2

Common Name: Di-(Diethylene glycol, phthalic acid, cyclic diester) **IUPAC Name**: 7,8,10,11,20,21,23,24-octahydrodibenzo [i,t][1,4,7,12,15,18] hexaoxacyclodocosine-5,13,18,26-tetraone **M.W**. 472 g/mol **RI –** 3012 	408(100) 104 (81.33) 55(76.85) 193 (71.33) 149 (63.33) 99 (46.63) 76 (41.27) 148 (33.34) 207 (31.66) 57 (28.13)	Range: 80 ppb to 95 ppb	Range 47.06 ng/cm^2 to 55.88 ng/cm^2	Range: 4.71 µg/dm^2 to 5.59 µg/dm^2
Common Name: Di-(Neopentyl glycol, adipic acid, cyclic diester) **IUPAC Name**: 2,2,14,14-tetramethyl-1,5,12,16-tetraoxacyclodocosane-6,11,17,22-tetrone **M.W.** 428 g/mol **RI** – 2819 	215 (100) 55 (47.39) 129 (48.53) 69 (36.69) 56 (31.12) 111 (26.99) 41 (25.13) 141(14.66) 83 (13.99) 428 (13.99)	Range: 310 ppb to 320 ppb	Range: 182.34 ng/cm^2 to 188.22 ng/cm^2	Range: 18.23 µg/dm^2 to 18.82 µg/dm^2

Common Name: Neopentyl glycol, adipic acid, cyclic diester **IUPAC Name:**3,3-dimethyl-1,5-dioxacycloundecane-6,11-dione **M.W**. 214 g/mol **RI** 1548 	57(100) 55 (61.58) 129(44.70) 56 (39.07) 41 (29.80) 111(28.47) 40(27.15) 69 (26.82) 179 (25.16) 39 (21.52)	Range: 157 ppb to 167 ppb	Range: 92.34 ng/cm^2 to 98.22 ng/cm^2	Range: 9.23 µg/dm^2 to 9.82 µg/dm^2
Common Name: Ethylene glycol, adipic acid, cyclic diester **IUPAC Name**: 1,4-dioxecane-5,10-dione **M.W**. 172 g/mol **RI-** 1510 	193 (100) 149 (77.04) 104 (57.37) 76 (42.62) 192 (40.98) 148 (34.42) 50 (29.91) 194 (29.50) 176 (20.49) 132 (18.44	Range: 138 ppb to 144 ppb	Range: 81.17 ng/cm^2 to 84.70 ng/cm^2	Range: 8.11 µg/dm^2 to 8.47 µg/dm^2

*Concentration in parts per billion (ppb w /v) in EtOH food simulating extraction solvent.

**Concentration in nanograms per square centimeter (ng/cm^2) of surface area exposed to extraction

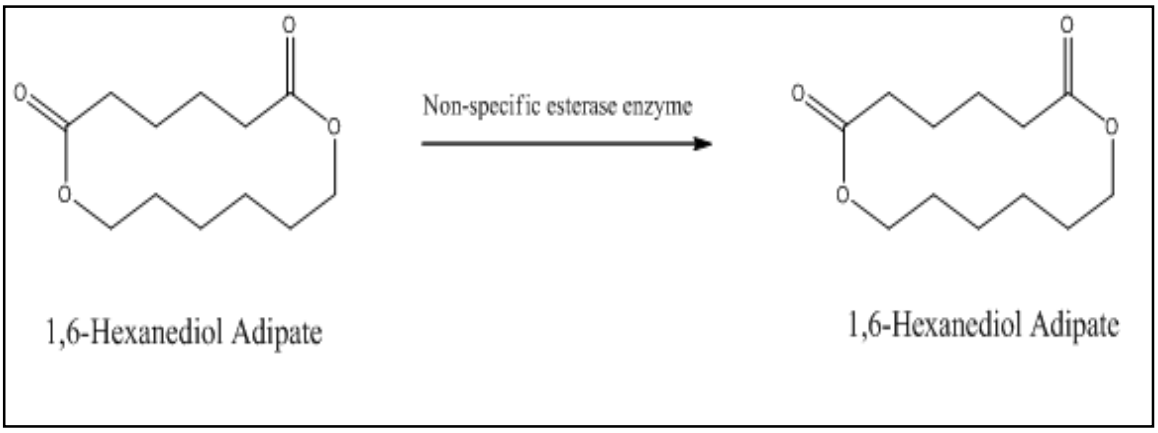
***Concentration in microgram per square decimeter (µg/dm^2) of surface area exposed to extraction

VI.C. In Vitro Metabolism Studies of Short Chain Cyclic Polyester Oligomers

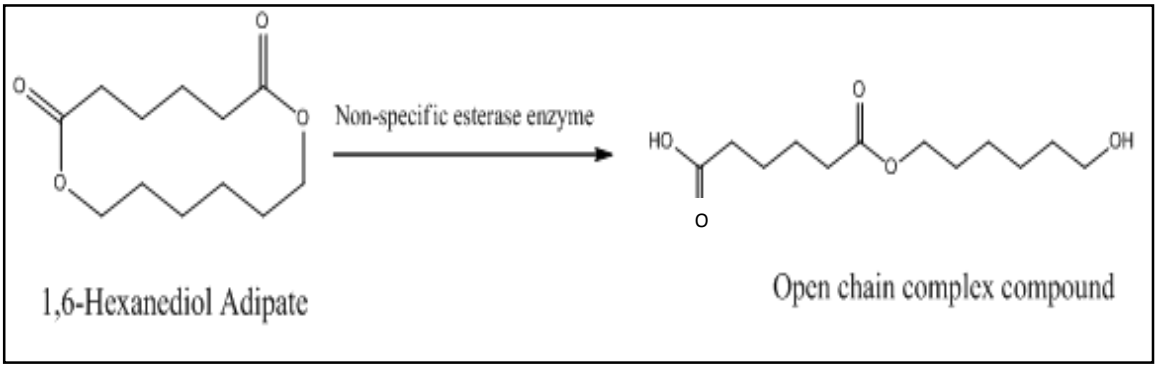
As discussed previously, short chain cyclic polyesters oligomers belong to the class of novel compounds whose toxicological properties have not been assessed as yet. However, the toxicological parameters of their constitutional compounds, dicarboxylic acid and diols, have been well studied. If these short chain cyclic polyester oligomers were to be ingested through dietary intake and absorbed into the bloodstream, they would likely be partially or completely metabolized by the ubiquitous non-specific esterase enzyme present in the human body. Under this section, the results of the porcine enzymatic treatment and the in vivo metabolic fate of these compounds are discussed in detail.

When these polyester oligomers, e.g. 1,6-Hexanediol Adipate, were exposed to the porcine esterase enzyme, there could be three possibilities as shown below:

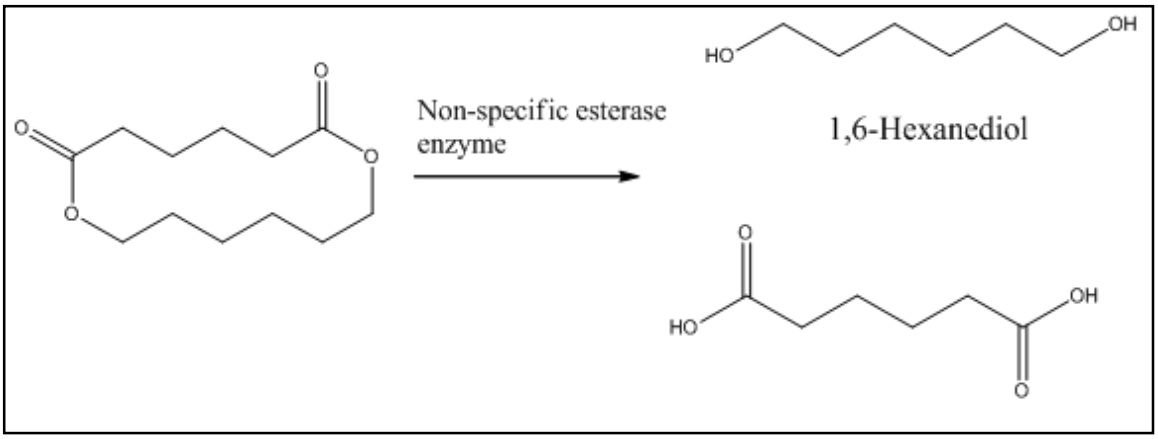
Case I



Case II



Case III



Case I above illustrates that if the ester linkages are extremely strong and resistant, polyester oligomers resist the hydrolysis and remain intact without leading to the cleavage of the ester bonds.

If the ester linkages are partially resistant, exposing to the esterase enzyme leads to the partial hydrolysis, where there is a cleavage of an ester bond. Partial hydrolysis leads to the formation of open chain complex compound, which is shown in **Case II**, above.

However, if the ester linkages are not strong, when exposed to the esterase enzyme, polyester oligomer undergoes complete hydrolysis giving its constitutional diols (1,6-Hexanediol) and dicarboxylic acid (Adipic acid). This is illustrated in **Case III**.

To verify the presence of the constitutional dicarboxylic acids and diols, standard solution of the monomers, which were used as starting materials in the preparation of polyesters, was prepared and derivatized. The reference spectra of derivatized the monomers were obtained by running the GC-MS analysis. The metabolic fate to the porcine enzyme treatment has been discussed individually for the polyester oligomers.

Since, there were no pure standards of short chain cyclic polyester oligomers available, the enzyme treatment was conducted on the trace amounts of oligomers present in the sealant-side laminate extracts.

VI.C.1. 1,4-Butandiol Adipate Cyclic Diester

When 1,4-Butanediol Adipate Cyclic Diester was treated with Porcine esterase enzyme at 37 ℃ for 1 h, it was found that 1,4-Butanediol Adipate was hydrolyzed to its constitutional monomers, 1,4-Butanediol and Adipic Acid, by porcine esterase enzyme. This was confirmed by comparing the mass spectrum of enzyme treated, derivatized sample extract with individual reference mass spectrum of derivatized 1,4-Butanediol and Adipic Acid (**Fig. 77** was compared to **Fig. 76** and **Fig. 79** was compared to **Fig. 78**). No intact peak of 1,4-Butanediol Adipate was found to be present in the enzyme treated laminate extract.

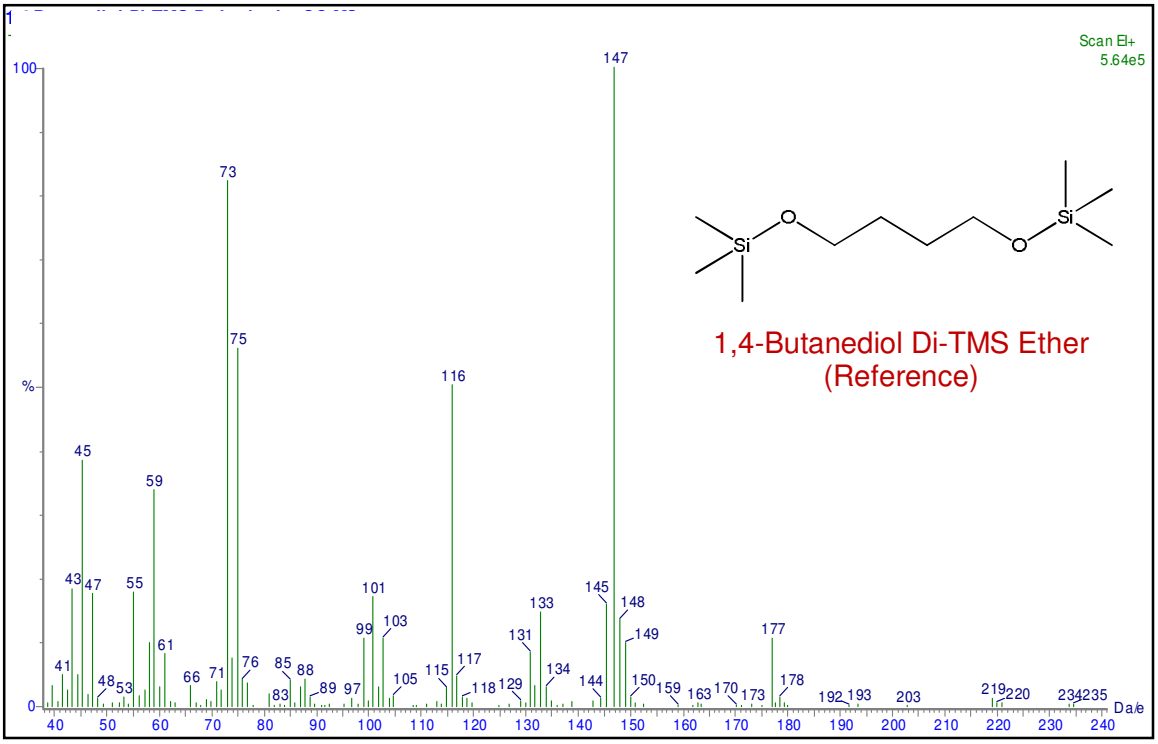


Figure 76: Reference Mass Spectrum (EI) of 1,4-Butanediol- Di-TMS Ether

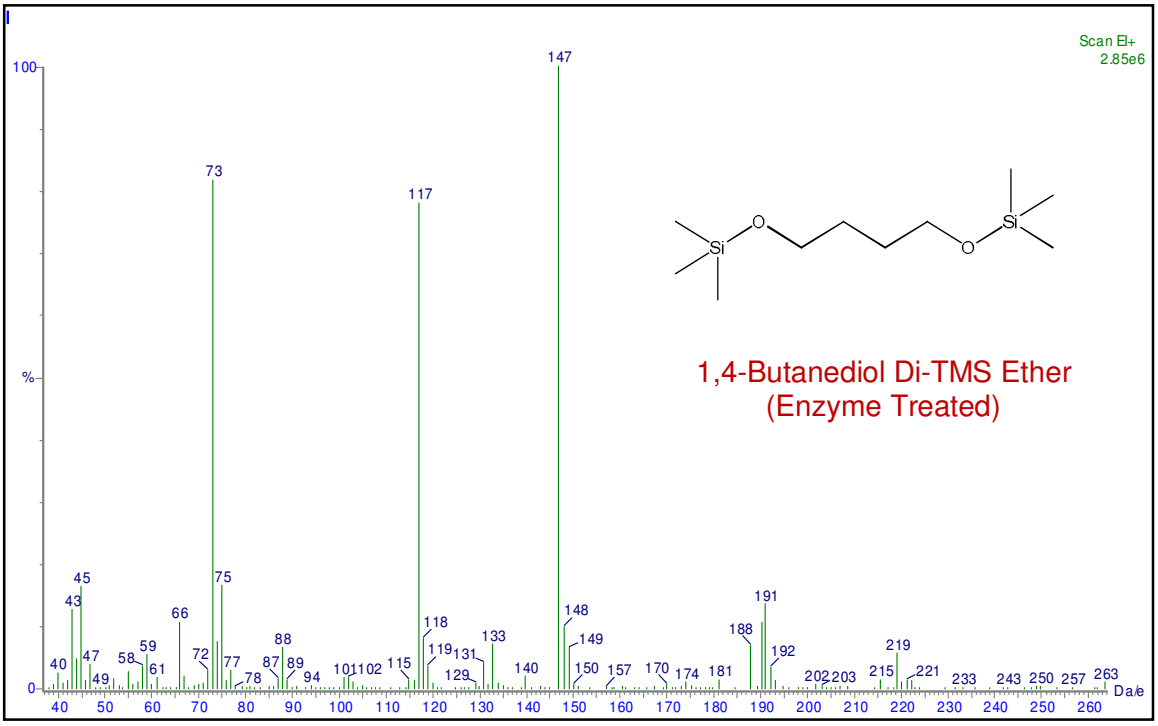


Figure 77: Mass Spectrum (EI) of 1,4-Butanedio- Di-TMS Ether After the Enzyme Treatment

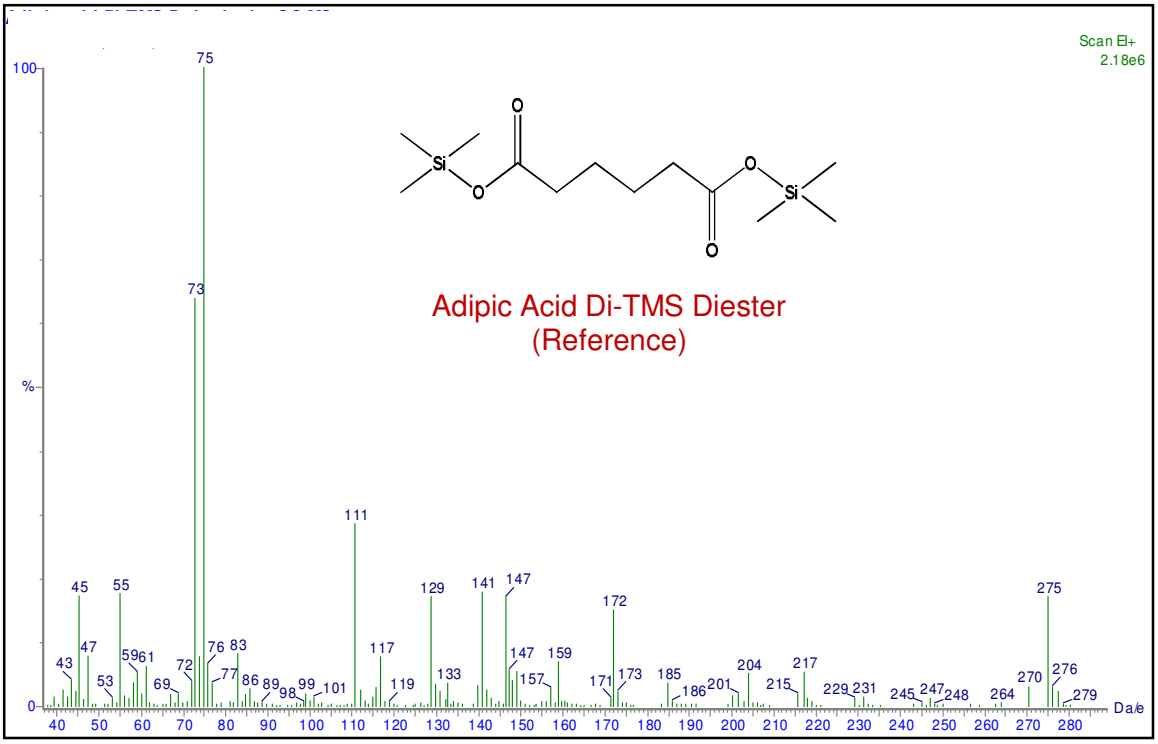


Figure 78: Reference Mass Spectrum (EI) of Adipic Acid-Di-TMS Diester

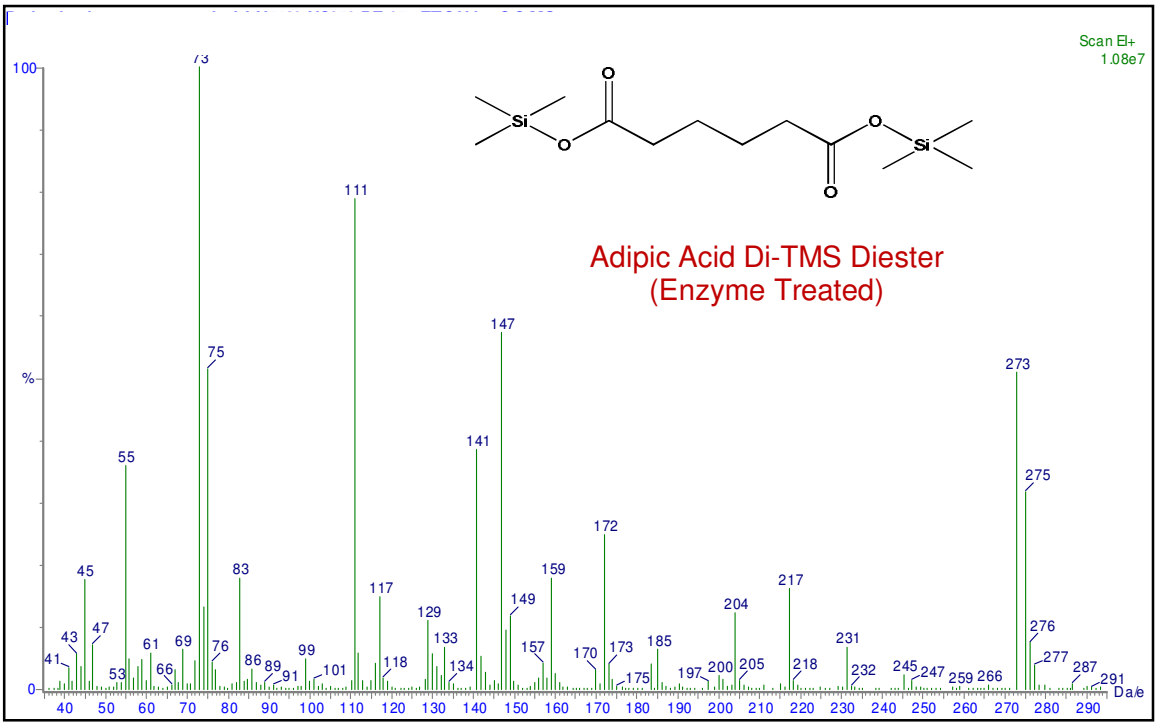


Figure 79: Mass Spectrum (EI) of Adipic Acid-Di-TMS Diester After the Enzyme Treatment

VI.C.2 Diethylene Glycol Adipate Cyclic Diester

The experimental data showed that the exposure to the porcine esterase enzyme at 37 ℃ for 1 h lead to the breakdown of ester bonds in Diethylene Glycol Adipate Cyclic Diester. The parent ion was not found to be present in the mass spectrum after the enzyme treatment. The presence of Adipic acid and Diethylene glycol was identified by comparing their enzyme treated mass spectrum with the individual reference mass spectrum. However, it was observed that, though the intensities of the fragments varied in the reference spectrum and the enzyme treated spectrum, their fragmentation patterns matched distinctly (**Fig. 81** was compared to **Fig. 80** and **Fig 83** was compared to **Fig. 82**). This confirmed that Diethylene Glycol Adipate Cyclic Diester was metabolized completely into its constitutional monomers, Diethylene Glycol and Adipic Acid.

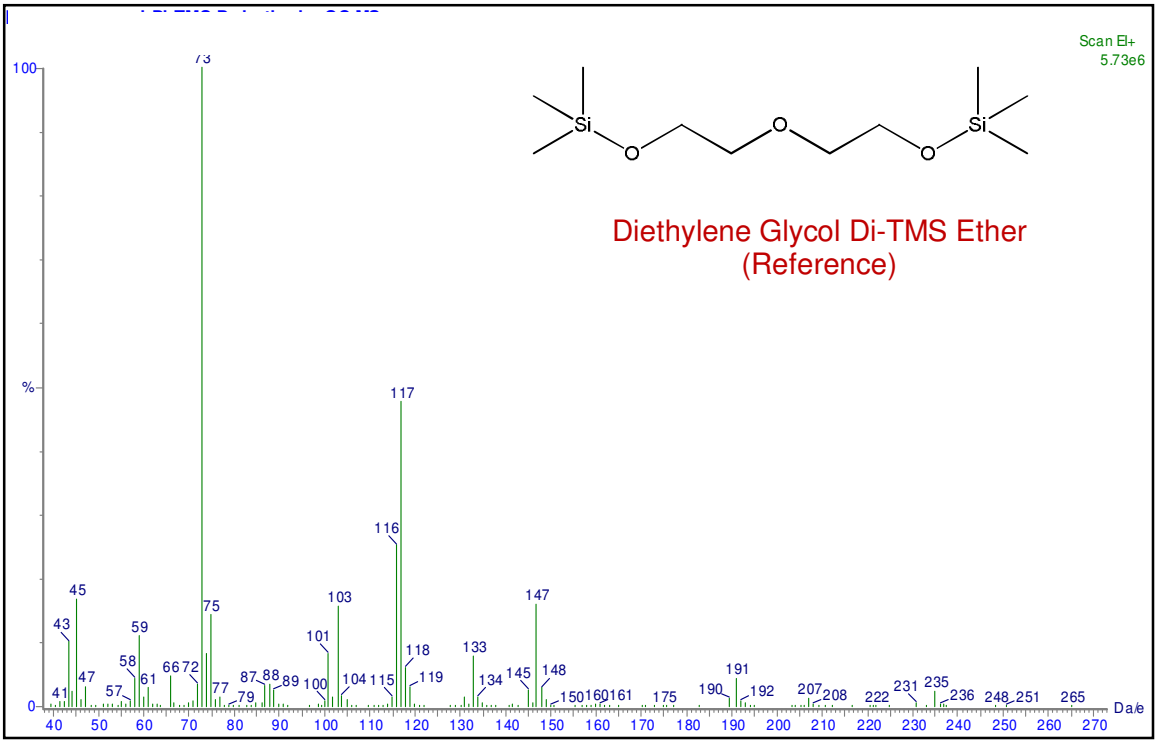


Figure 80: Reference Mass Spectrum (EI) of Diethylene Glycol-Di-TMS Ether

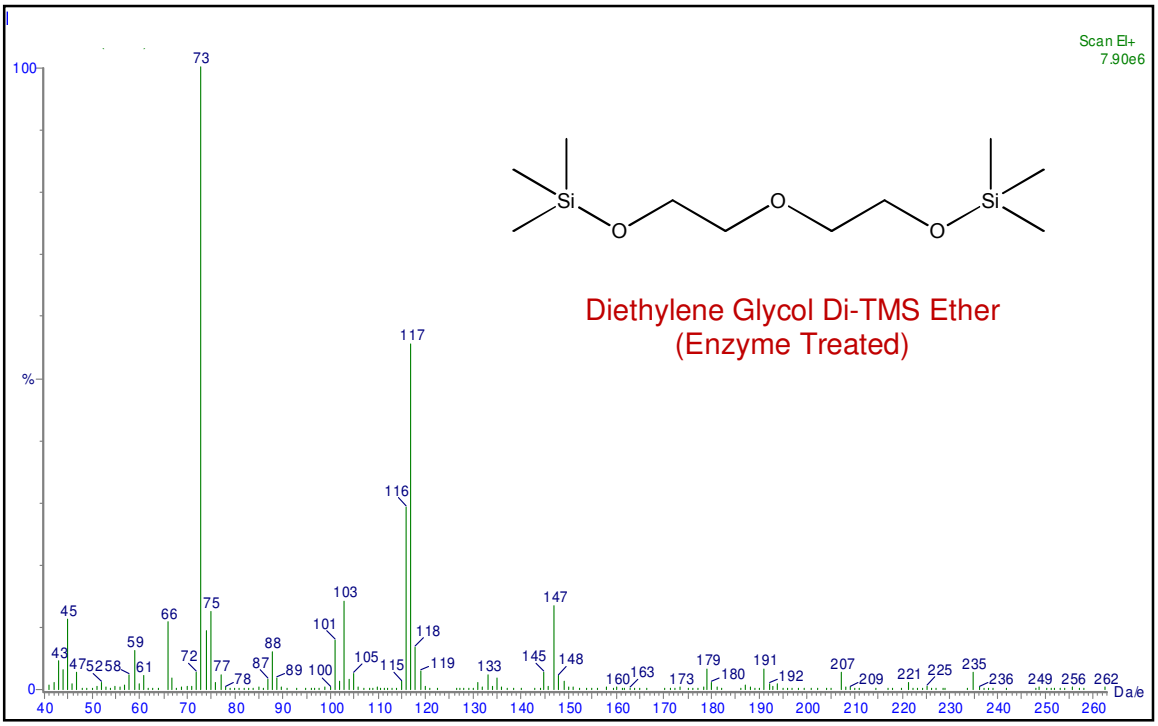


Figure 81: Mass Spectrum (EI) of Diethylene Glycol-Di-TMS Ether After the Enzyme Treatment

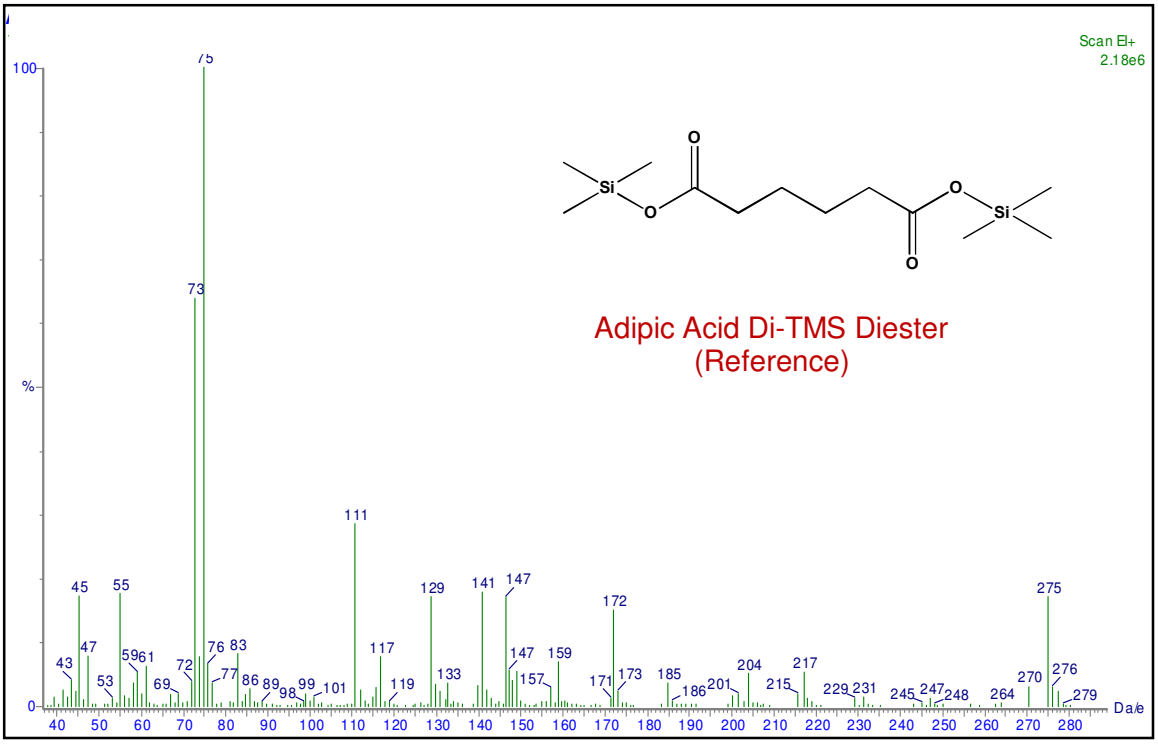


Figure 82: Reference Mass Spectrum (EI) of Adipic Acid- Di-TMS Diester

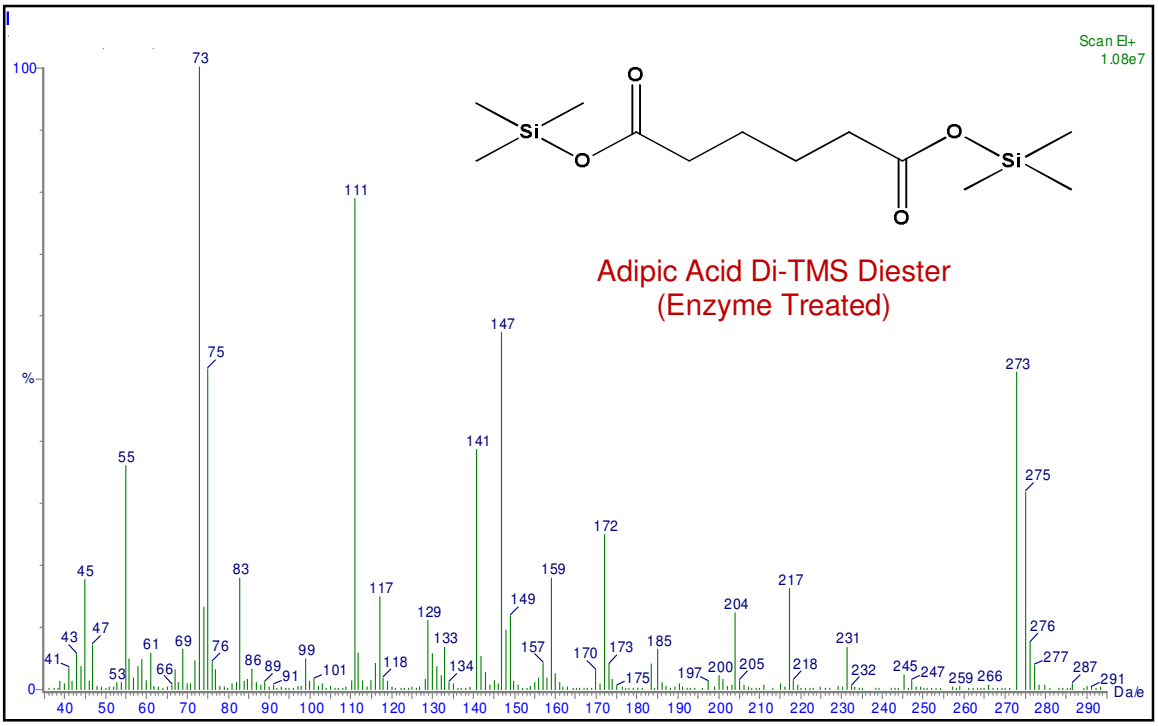


Figure 83: Mass Spectrum (EI) of Adipic Acid-Di-TMS Diester After the Enzyme Treatment

VI.C.3. 1,6-Hexanediol Adipate Cyclic Diester

The in vitro metabolic fate of 1,6-Hexanediol Adipate Cyclic Diester followed the same path as that of Diethylene Glycol Adipate and 1,4-Butanediol Adipate. The porcine esterase enzyme treatment at 37 ℃ for 1 h led to complete metabolism of 1,6-Hexanediol Adipate Cyclic Diester. The enzyme treatment caused breakdown of ester linkages leading to the formation of the starting monomers 1,6-Hexanediol and Adipic Acid in their derivatized forms. As discussed previously, it was confirmed by comparing the mass spectrum of the enzyme treated extract with individual reference mass spectrum of derivatized Adipic Acid and 1,6-Hexanediol (**Fig. 85** was compared to **Fig. 84** and **Fig. 87** was compared to **Fig. 86**).

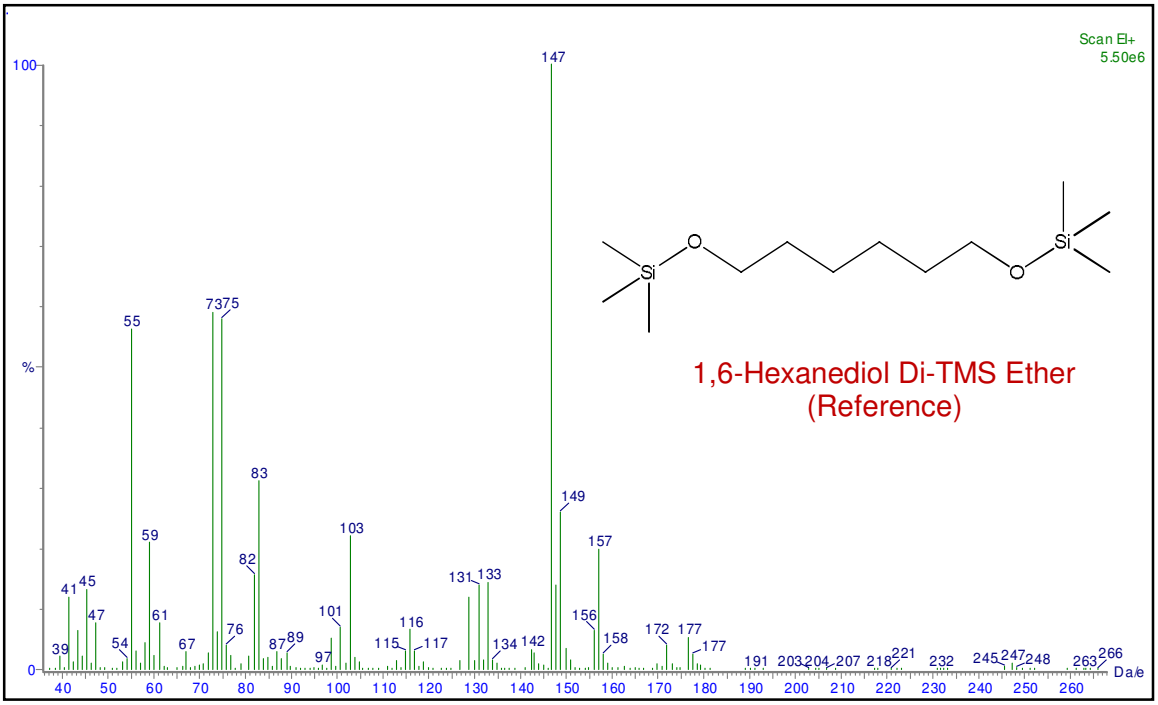


Figure 84: Reference Mass Spectrum (EI) of 1,6-Hexanediol- Di-TMS Ether

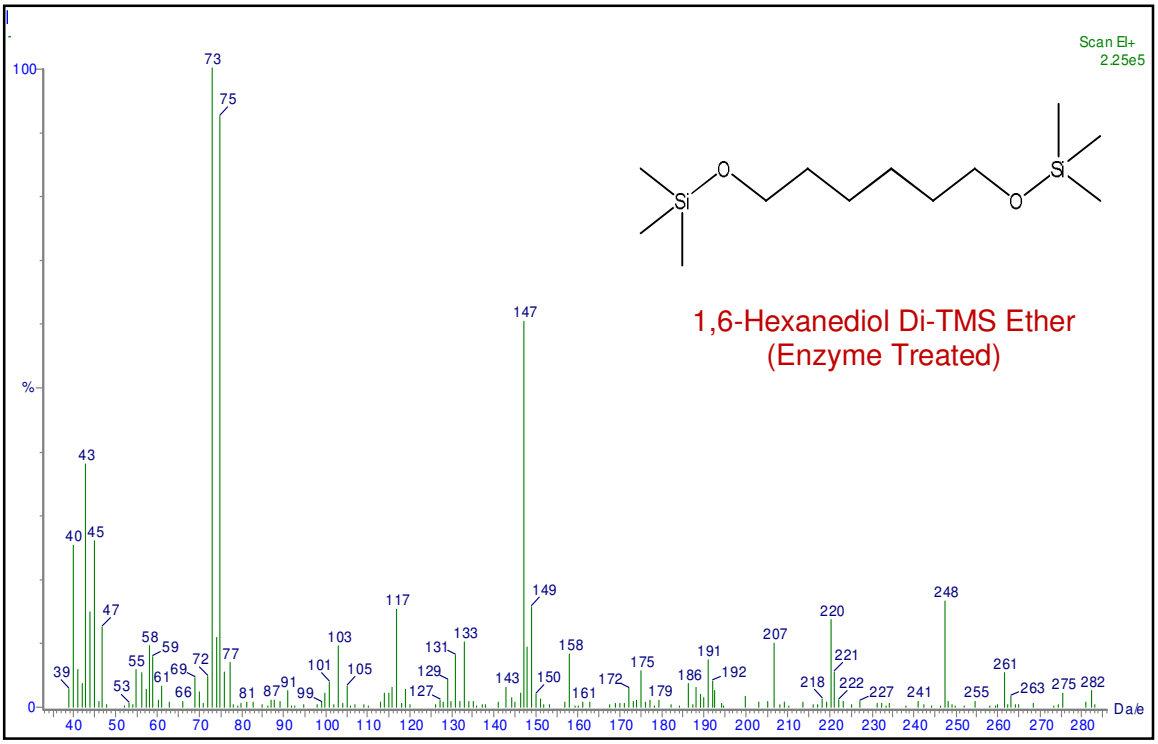


Figure 85: Mass Spectrum (EI) of 1,6-Hexanediol-Di-TMS Ether After the Enzyme Treatment

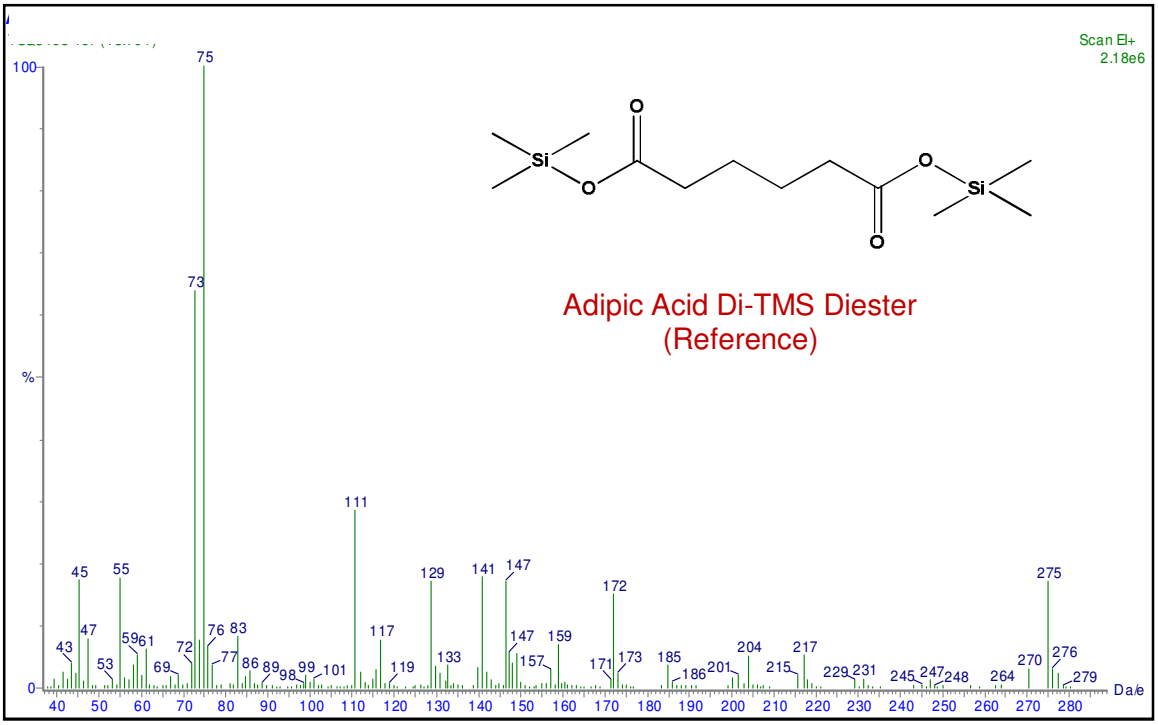


Figure 86: Reference Mass Spectrum (EI) of Adipic Acid-Di-TMS Diester

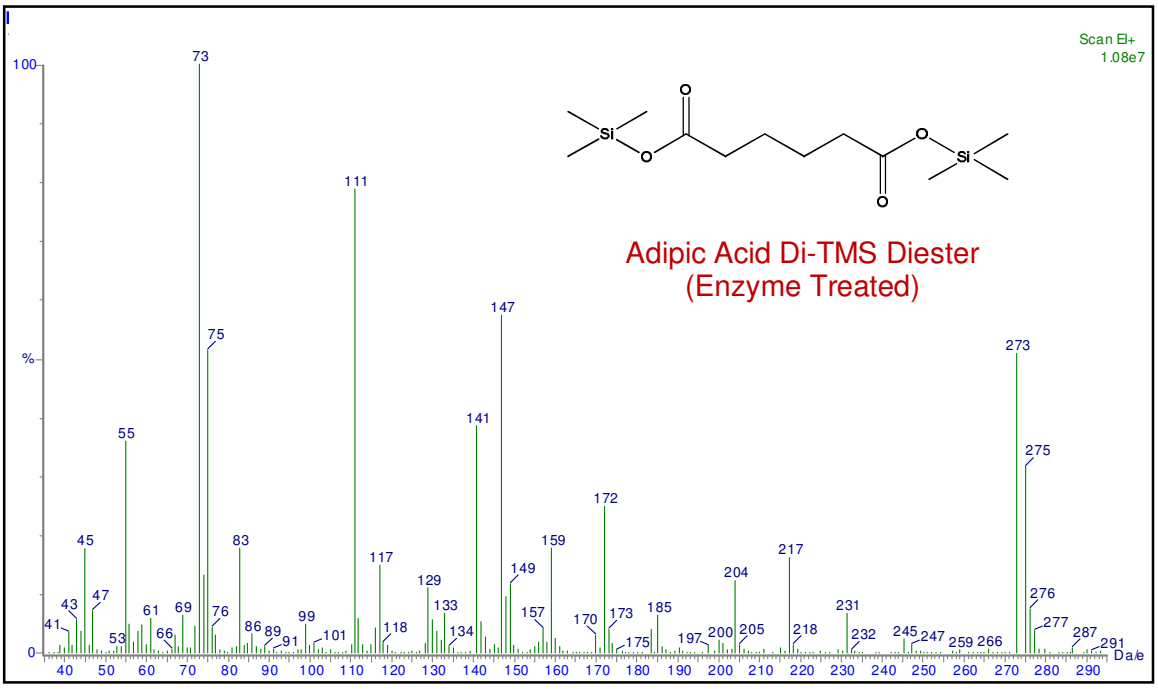


Figure 87: Mass Spectrum (EI) of Adipic Acid- Di-TMS Diester After the Enzyme Treatment

VI.C.4. Diethylene Glycol Phthalate Cyclic Diester

It was observed that Diethylene Glycol Phthalate Cyclic Diester followed the same in vitro metabolic fate as the previously discussed cyclic polyester oligomers. Exposure to porcine esterase enzyme at 37 ℃ for 1 h led to complete hydrolysis of Diethylene Glycol Phthalate Cyclic Diester, forming its constitutional monomers, Diethylene Glycol and Isophthalic acid in their derivatized form. The cleavage of ester bonds to form glycol and dicarboxylic acid was confirmed by comparing the mass spectrum of enzyme treated derivatized Diethylene Glycol and enzyme treated derivatized Isophthalic acid to the mass spectrum of their respective reference standards **(Fig. 89** was compared to **Fig. 88** and **Fig. 91** was compared to **Fig. 90**).

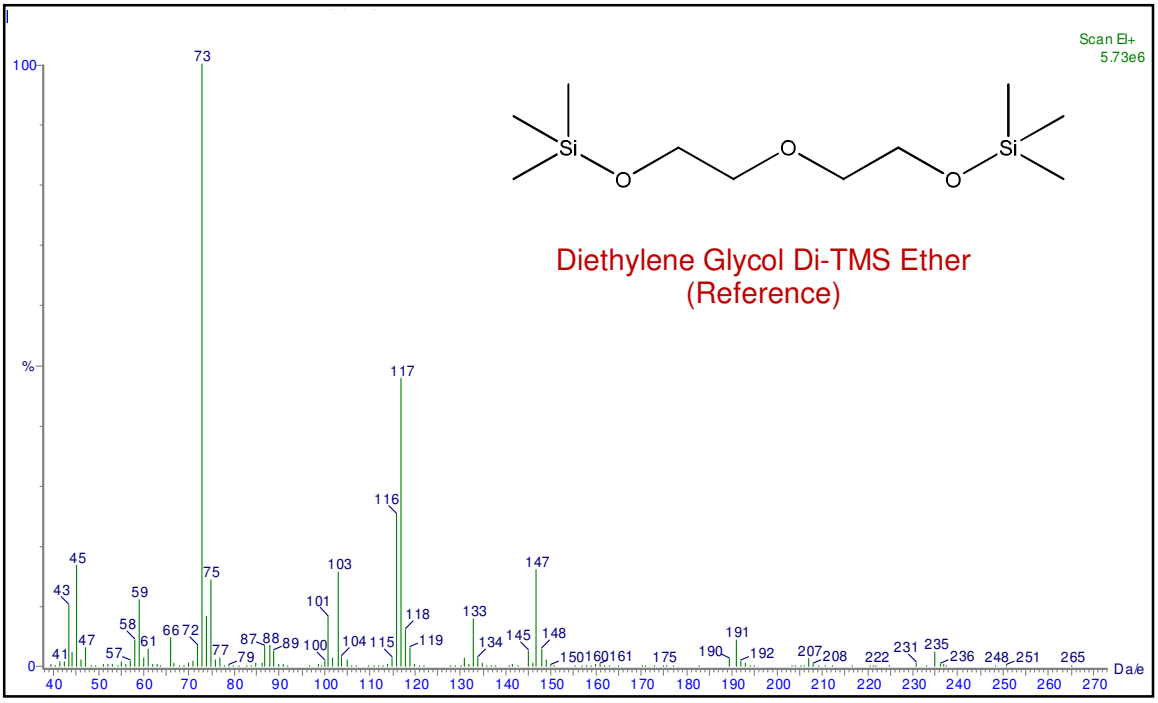


Figure 88: Reference Mass Spectrum (EI) of Diethylene Glycol- Di-TMS Ether

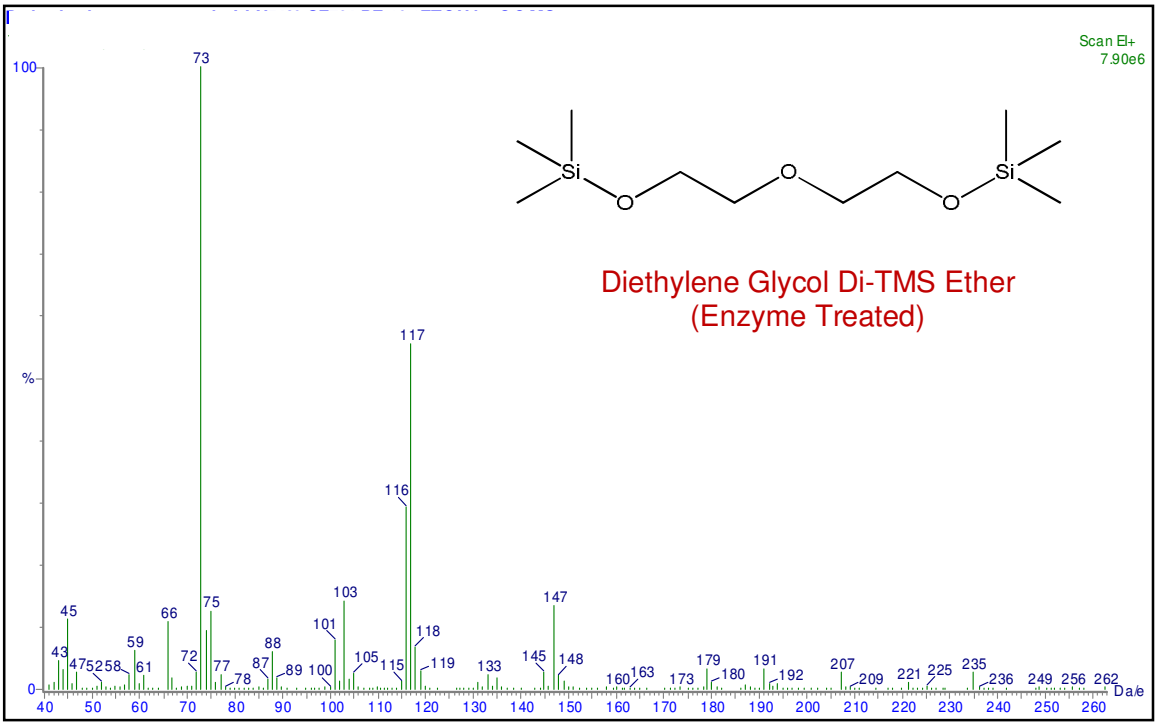


Figure 89: Mass Spectrum (EI) of Diethylene Glycol-Di-TMS Ether After the Enzyme Treatment

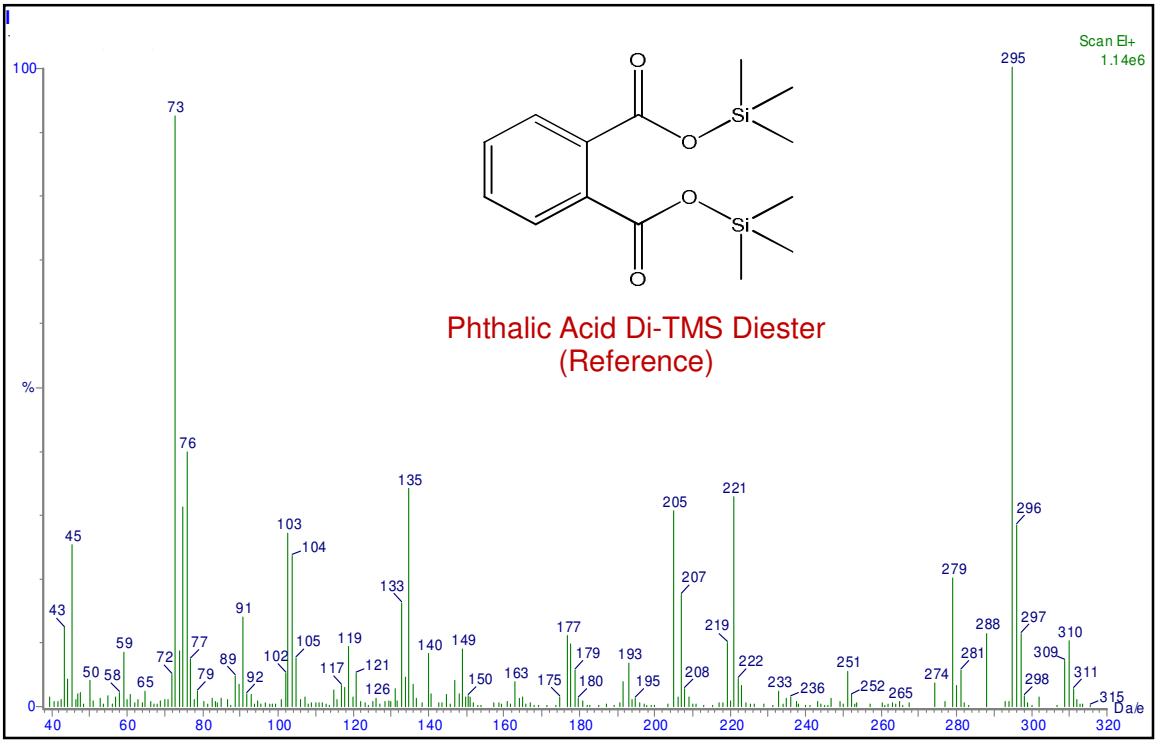


Figure 90: Reference Mass Spectrum (EI) of Phthalic Acid-Di-TMS Diester

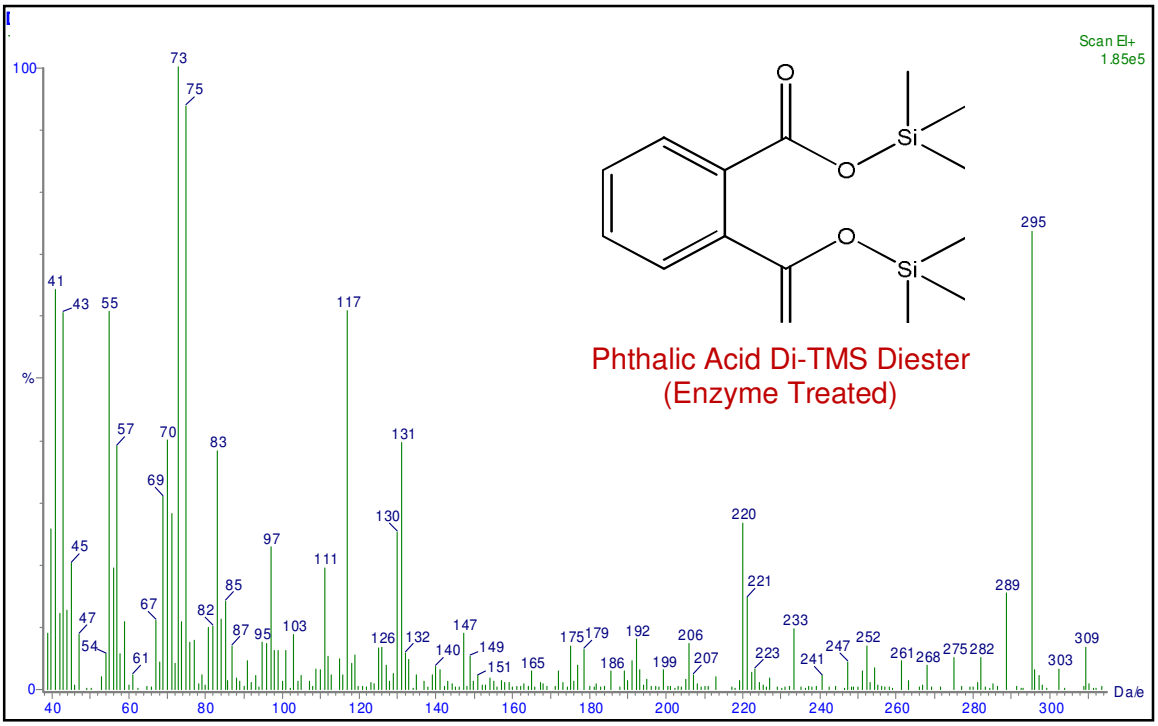


Figure 91: Mass Spectrum (EI) of Phthalic Acid-Di-TMS Diester After the Enzyme Treatment

VI.C.5. Neopentyl Glycol Adipate Cyclic Diester

When Neopentyl Glycol Adipate was treated with porcine esterase enzyme at 37 ℃ for 1 h, there was an indication that it broke down yielding its constitutional monomers – Neopentyl Glycol and Adipic Acid, in their derivatized forms. As discussed in the previous examples, this was verified by comparing the mass spectrum of the enzyme treated sample with the derivatized individual mass spectrum of Neopentyl Glycol and Adipic Acid (**Fig. 93** was compared to **Fig 92** and **Fig. 95** was compared to **Fig. 94**). It was noted that mass spectrum of Neopentyl Glycol Di-TMS Ether after the enzyme treatment did not match very closely to its reference spectrum and hence this assignment is tentative (**Fig. 93 and Fig. 92**).

Since, the pure reference standards of Neopentyl Glycol Adipate Cyclic Diester were not available; the enzyme studies were performed on trace amounts of Neopentyl Glycol Cyclic Diester in sealant-side laminate extracts, which reduced the sensitivity of the analysis. This partially explains the imperfect spectral match of Neopentyl Glycol Di-TMS Ether with its reference compound.

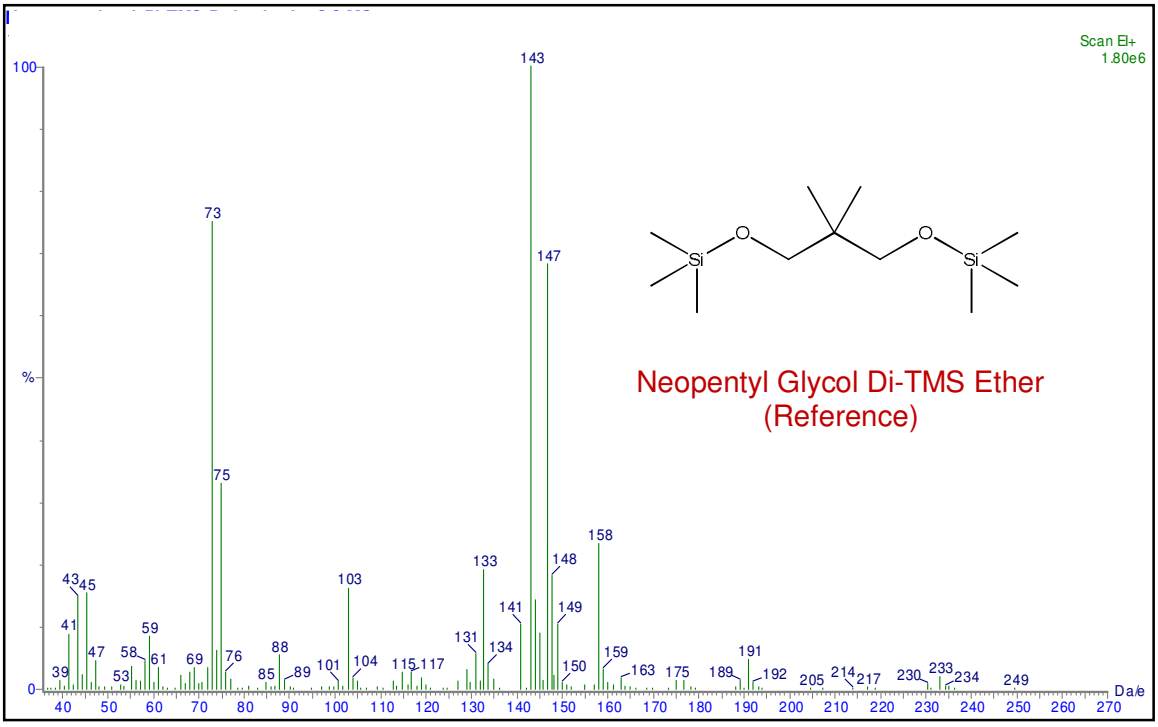


Figure 92: Reference Mass Spectrum (EI) of Neopentyl Glycol-Di-TMS Ether

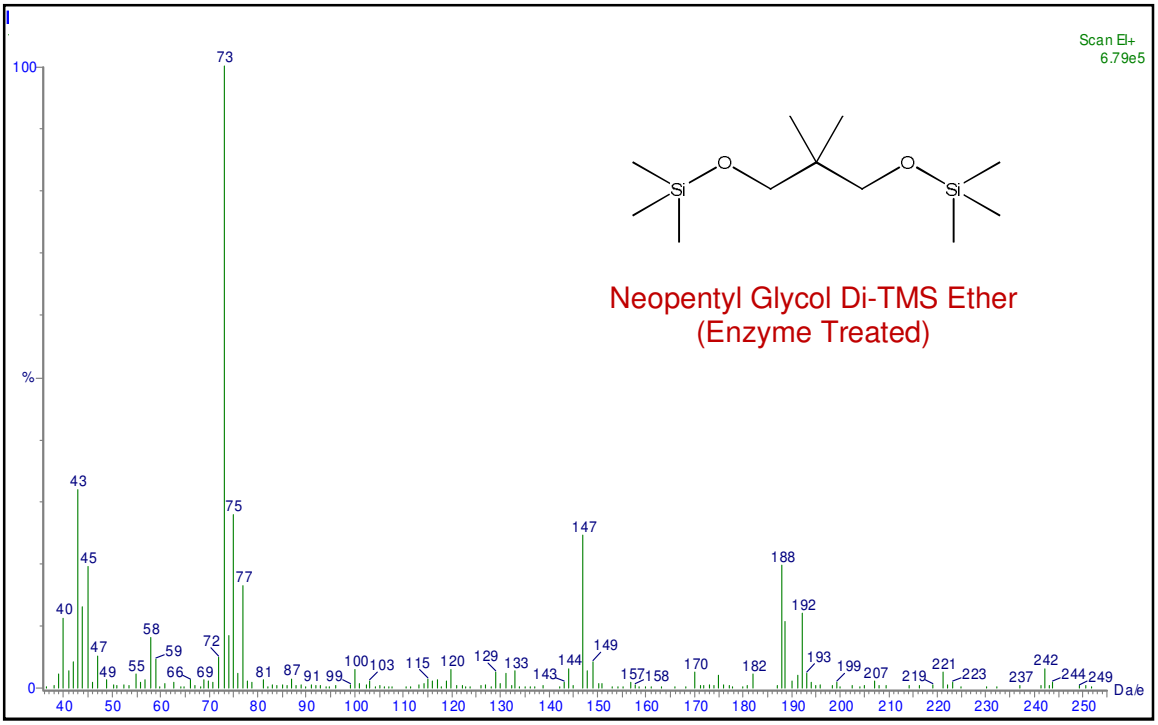


Figure 93: Mass Spectrum (EI) of Neopentyl Glycol-Di-TMS Ether After the Enzyme Treatment

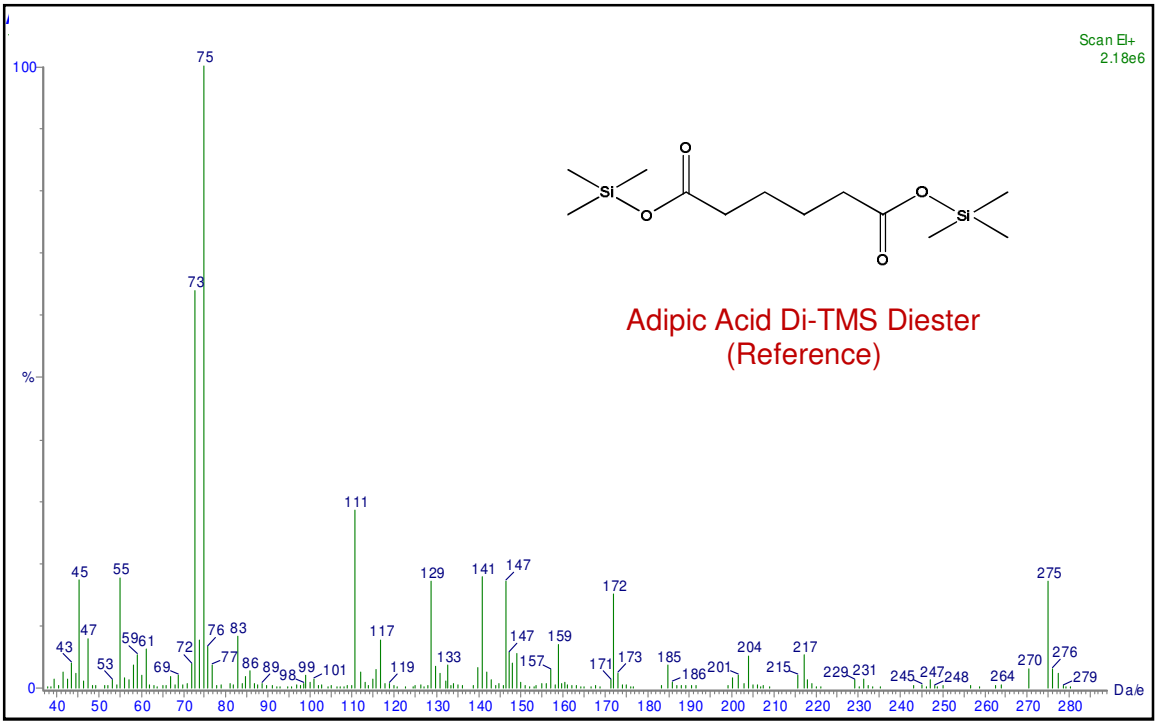


Figure 94: Reference Mass Spectrum (EI) of Adipic Acid-Di-TMS Diester

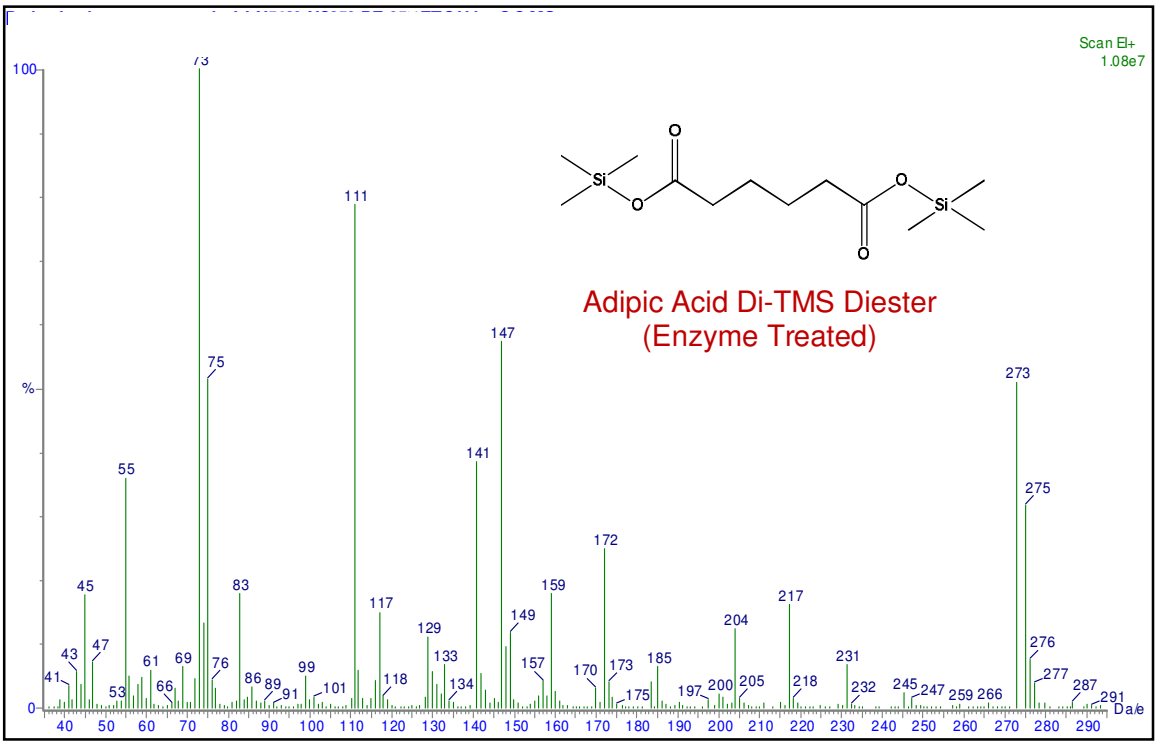
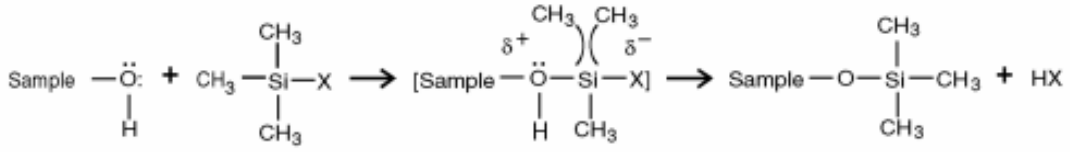


Figure 95: Mass Spectrum (EI) of Adipic Acid-Di-TMS Diester After the Enzyme Treatment

Derivatization using silylation reagents modified the chemical structures of dicarboxylic acids and diols by replacing active Hydrogen with trimethylsilyl group. The reaction occurred as a nucleophilic attack resulting in the bimolecular transition state, shown in the mechanism below (Knapp, 1979).



When trimethylchlorosilane (TMCS) was used for silylation, HCl was generated as a byproduct of the reaction. The acid thus formed has the potential to attack the esters. However, since pyridine is a base, it acts as an acid scavenger and prevents the acid from attacking the esters.

Although, the research has shown that the short chain cyclic polyester oligomers yielded their constitutional monomers – dicarboxylic acid and diols, on exposure to the esterase enzyme, the efficiency of conversion of cyclic oligomers into their monomers was not known. Even though, no intact cyclic oligomers were found to be present in the mass spectrum of enzyme treated sealant side laminate extracts, it could not be concluded that the enzyme treatment converted all the cyclic oligomers completely into their constitutional monomers. This was because the presence of monoesters could not be verified without having their reference standards for the comparison. Hence, this is still a preliminary work and needs further research.

All the 10% and 95% EtOH sealant-side laminate extracts were analyzed prior to the esterase enzyme treatment as control samples. However, it was noted that no presence of monomers (free glycols and free carboxylic acids) was detected until after the enzyme treatment.

VII. Related Findings

In our research we identified different short chain cyclic polyester oligomers migrated from adhesives used in the construction of laminates. When similar migration testing was conducted on dual chamber pouches, the presence of some short chain cyclic polyester oligomers was detected in the food simulant, which belong to the same class as the compounds detected, identified and analyzed in this research. The purpose of including the mass spectra of these compounds was to bring this class of compounds together, in the hope that the chemistry and migratory properties would help the regulatory scientists to assess their toxicology potential.

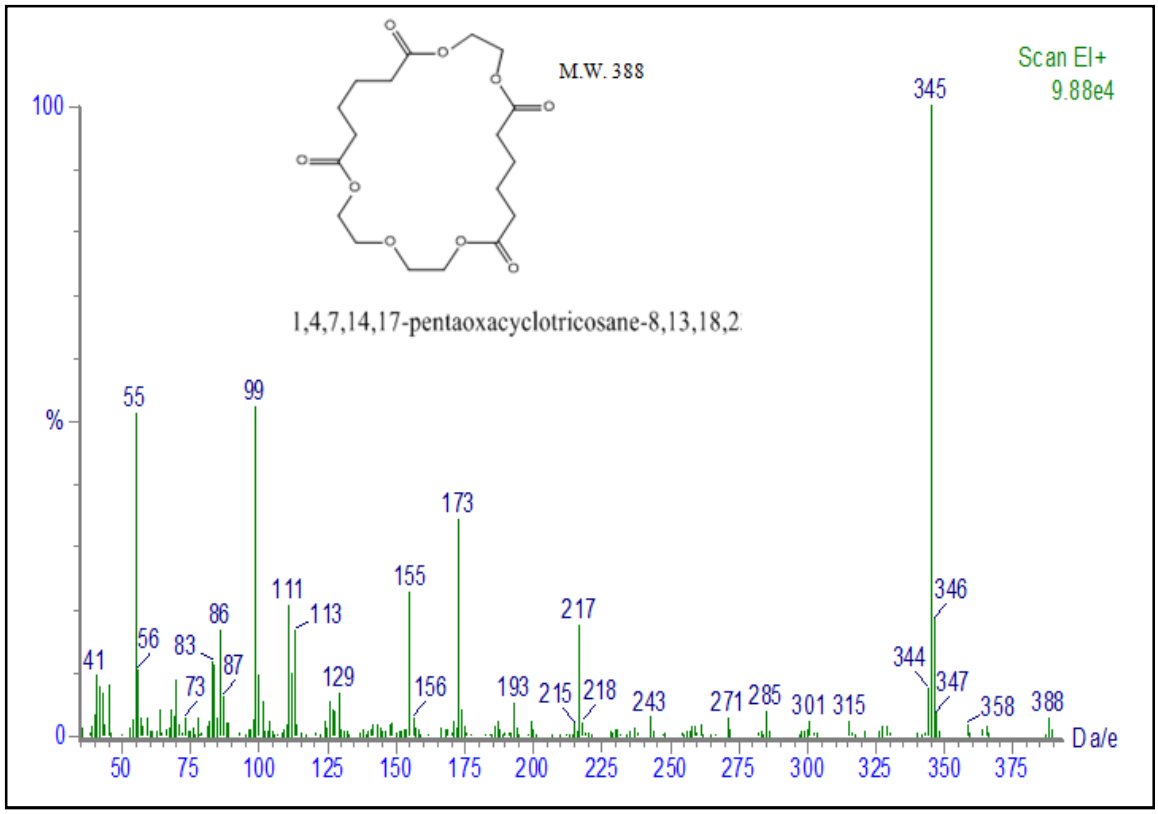


Figure 96: Mass Spectrum (EI) of Ethylene Glycol-Adipic-Diethylene Glycol- Adipic Acid Cyclic Ester (M.W. 388)

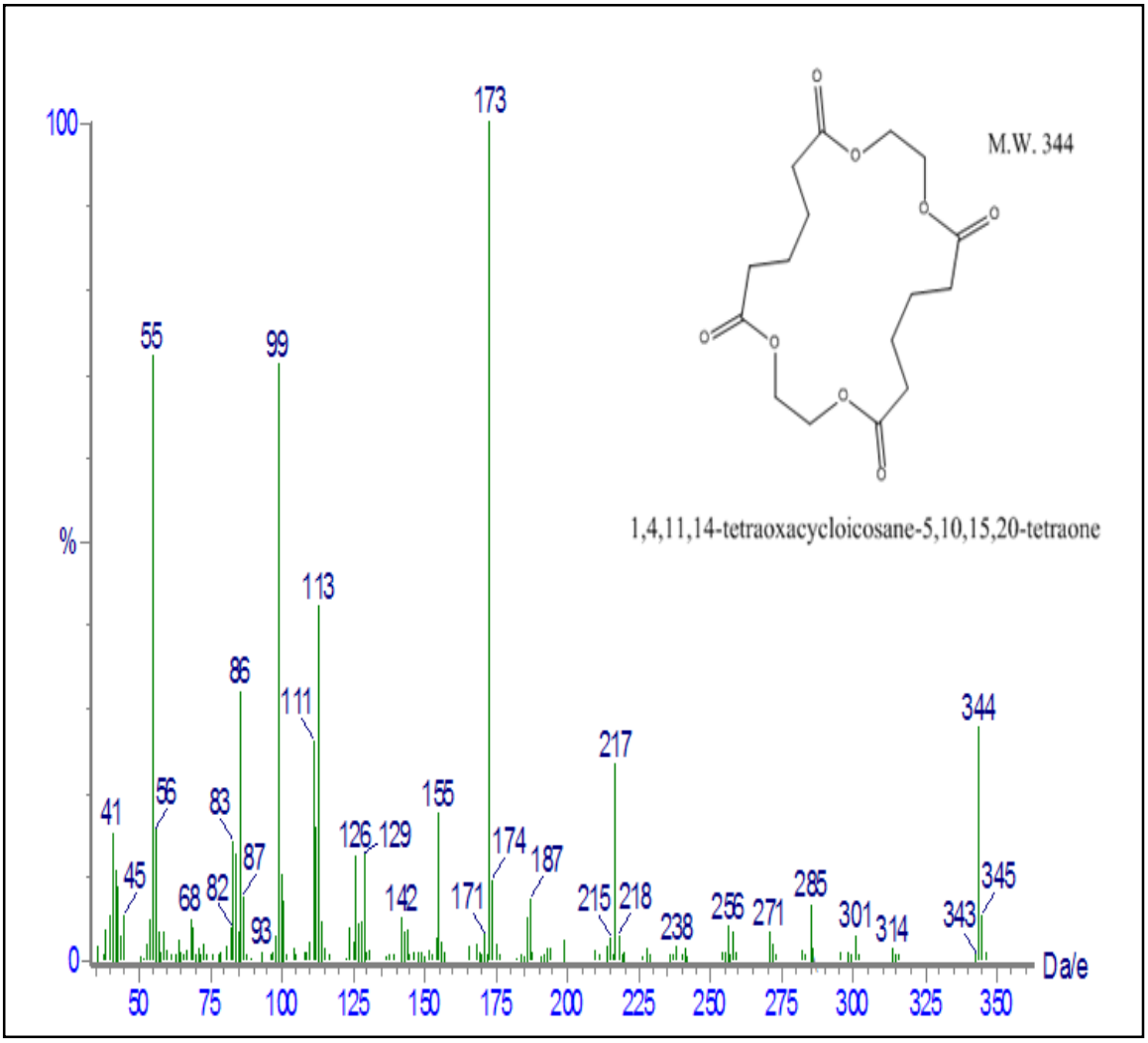


Figure 97: Mass Spectrum (EI) of Di-(Ethylene Glycol, Adipic Acid, Cyclic Diester) (M.W. 344)

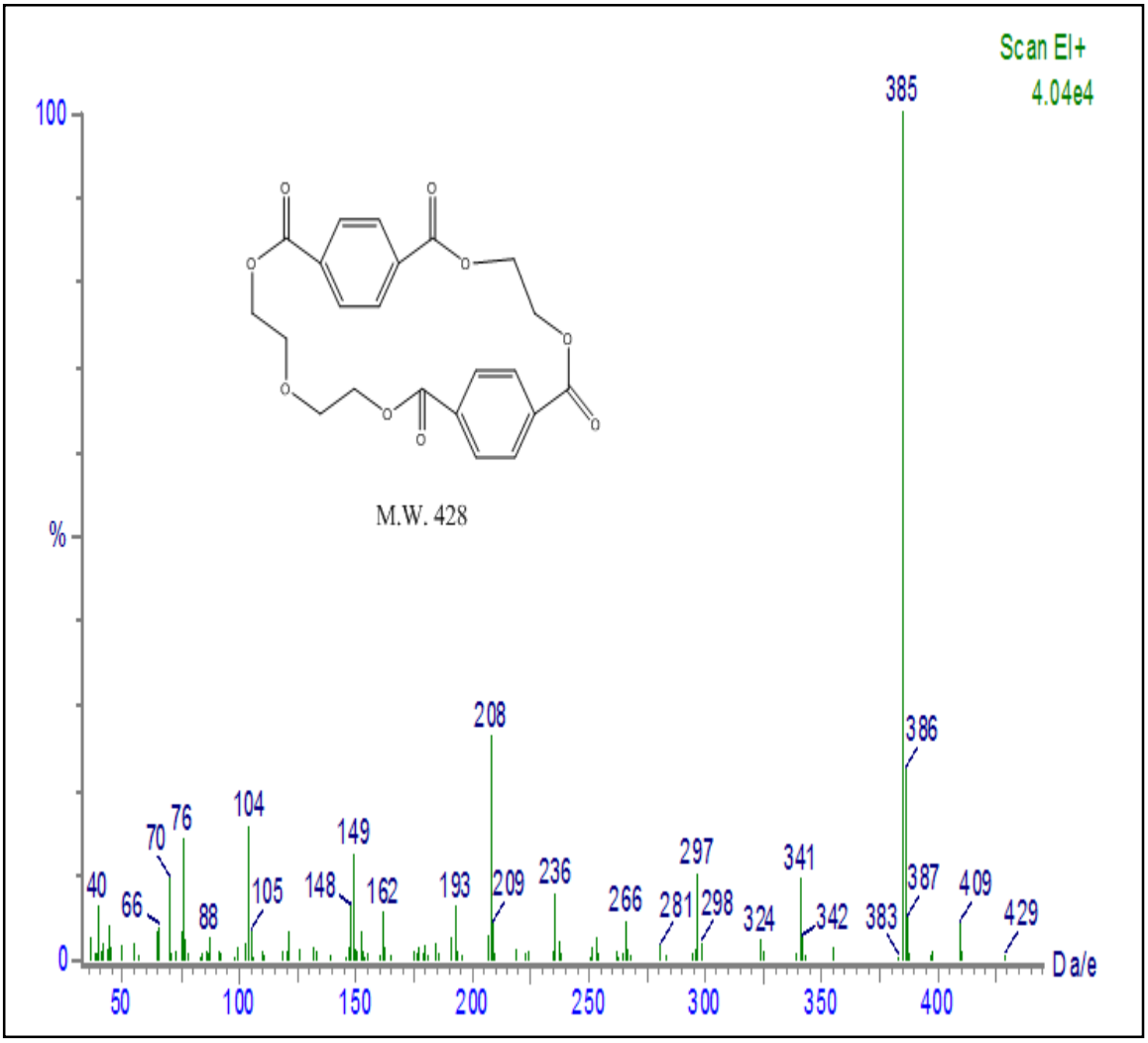


Figure 98: Mass Spectrum (EI) of Polyethylene Glycol Terephthalate (M.W. 428)

VIII. Conclusions

Migration behavior of short chain cyclic polyester oligomers from the sealant-side of the laminate packaging into food simulants was studied in this research. Migration testing was conducted on commercially manufactured laminates bonded together by polyurethane adhesive. The food simulants used for the migration testing experiments were 10% ethanol and 95% ethanol for the aqueous foods and the fatty foods respectively, as recommended by the USFDA. Single side extraction cell was used for the purpose of extraction of migrants from the sealant side.

Short chain cyclic polyester oligomers migrated in the food simulants in the mass range of 170 g/mol – 480 g/mol were identified and characterized using GC-MS analysis. Chemistry, migratory parameters, electron ionization (EI) and chemical ionization (CI) mass spectra were determined. Chemical ionization mass spectra confirmed the molecular weights of the oligomers. Further the characteristic fragmentation patterns from the EI mass spectra assisted in elucidating the chemical structures of these cyclic polyester oligomers. Possible mechanisms associated with the fragmentation of individual compounds were also proposed. GC retention time indices were calculated by normalizing their retention times to the mixture of closely eluting n-paraffin alkanes. The migratory concentrations of the migrants were calculated by integrating the peaks in the mass spectrum and from the known amount of internal standard spiked in the experiments.

It was also reported in the literature that the compounds with molecular weight below 1000 Da were important from a toxicological view as they could be potentially absorbed in the gastrointestinal tract (Commission Regulation (EU) N. 10/2011, 2011). We predicted that, if these compounds were absorbed in the blood stream via dietary intake,

they would be metabolized by the non-specific esterase enzyme present ubiquitously in blood and tissues. In our research, migrated short chain cyclic oligomers were subjected to porcine esterase enzyme treatment for 1 h at 37 ℃, in order to investigate their in vitro metabolic fate under the influence of non-specific esterase enzyme. Samples were then derivatized using silylation reagent Sylon BFT and analyzed by GC-MS.

Our research has shown that porcine esterase enzyme treatment at 37 ℃ for 1 h completely metabolized the short chain cyclic diesters and oligoesters back into their original corresponding constitutional monomers – dicarboxlic acids and diols precursors, suggesting the occurrence of complete hydrolysis. Since, the degree of conversion of cyclic oligomers into their constitutional monomers was not known and possibility of the existence of monoesters could not be verified, this area needs further research.

We are hopeful that the chemical structure deduced in this research will aid chemists to investigate the structural properties of this class of compounds, thus providing a good understanding about their potential interactions in the food systems. Retention time indices obtained from the GC-MS analysis in combination with the fragmentation patterns and the mechanisms would be a useful tool to characterize the short chain cyclic polyester oligomers. The electron ionization mass spectra of the compounds could be used as a reference for the identification of cyclic polyester oligomers in future. Also, the in vitro metabolic fate data would assist the regulatory scientists and toxicologists to assess the safety of these packaging borne migrants.

IX. References

Adhesion and Cohesion. http://www.adhesives.org/TrainingEducation/StudentResources/AdhesionandCohesion.aspx.

Ahjopalo, L., Osterholm, H., Jarrvinen, H. and Pietila L.O. "Cyclic oligomers in saturated polyesters." *Polymer* , 2000: 8283-8290.

Applications: Mitsui Chemicals America, Inc. http://www.mitsuichemicals.com/adm_app1.htm.

Bisswanger, H. *Practical Enzymology.* 2004.

Bradley, E.L., Driffield, M., Guthrie, J., Harmer, N. and Castle, L. "Analytical approaches to identify potential migrants in polyester-polyurethane can coatings." *Food Additives and Contaminants* , 2009: 1602-1610.

Brugel, E.G. USA Patent 6,646134 B2. 2003.

Bushendorf, P. USA Patent US 7,754,334 B2. 2008.

"Chemical Migration from Food Packaging." In *Food Chemical Safety: Contaminants*, by Castle. Woodhead Publishing Limited, 2005.

Chapman, J. R. *Practical Organic Mass Spectrometry.* Wiley, 1993.

"Commission Regulation (EU) N. 10/2011." *Official Journal of the European Union*, 2011: 15.

Comission Regulation(EC) No 1565/2000 "Flavooring Group Evaluation 1: Branched chain aliphatic saturated aldehydes, carboxylic acids and reated esters of primary alcohols and branched-chain carboxylic acids."

Cottrel, J. and Greathead, R.J. "Extending the Mass Range of a Sector Mass Spectrometer." In *Mass Spectrometry Reviews*, 215-247. 1986.

Dordick, J.S. "Designing Enzymes for Use in Organic Solvents." *American Chemical Society and American Institute of Chemical Engneers*, 1992.

"FDA, Preparation of premarket submissions for food contact substances: chemistry recommendations." 2007.

Hamdani, M., Thil, L., Gans, G. and Feigenbaum, A.E. "Safety Assessment of Polymeric Additives for Food Packaging: Hydrolysis of Polymeri Plasticizers by Didestive Fluids." *Journal of Applied Polymer Science* , 2002 : 956-966.

Harrison, A.G. "Analysis of cyclic oligomers of poly(ethylene terephthalate) by liquid chromatography ." *Polymer Communications*, 1997.

Hartman, T.G. and Pace, G. "Migration studies of 3-chloro-1,2-propanediol (3-MCPD) in polyethylene extrusion-coated paperboard food packaging." *Food Additives and Contaminants*, 2010: 884-891.

Holland, B.J. and Hay, J.N. "Analysis of comonomer content and cyclic oligomers of poly(ethylene terephthalate)." *Polymer*, 2002: 1797-1804.

Ikada, Y. and Tsuji, H. "Biodegradable polyesters for medical and ecological applications ." *Macromolecular Rapid Communications*, 2000: 117-132.

"Polyester Polyols for Elastic Polyurethanes." In *Chemistry and Technology of Polyols for Polyurethanes*, by M. Ionescu, 276. Rapra Technology, 2005.

Kim, D.J. and Lee, K.T. "Determination of monomers and oligomers in polyethylene terephthalate trays and bottles for food use by using high performance liquid chromatography-electrospray ionization-mass spectrometry." *Polymer Testing*, 2012: 490-499.

Knapp, D. R. *Handbook of Analytical Derivatization Reactions.* John Wiley & Sons, Inc., 1979.

Kováts, E. "Gas-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone." *Helvetica Chimica Acta*, 1958.

Laine, O., Osterholm, H., Jarvinen, H., Wickstrom, K. and Vainiotalo, P. "Qualitative and quantitative end-group analysis of a small molecular weight polyester by matrix-assisted laser desorption/ionization time of flight mass spectrometry." *Rapid Communications in Mass Spectrometry*, 2000: 482-495.

Laine, O., Osterholm, H., Selantaus, M., Jarvinen, H. and Vainiotalo, P. "Determination of cyclic polyester oligomers by gel permeation chromatography and matrix-assisted laser desorption/ionization time of flight mass spectrometry." *Rapid Communications in Mass Spectrometry*, 2001: 1931-1935.

Lattimer, R.P., Polce, M.J. and Wesdemiotis, C. "MALDI-MS analysis of pyrolysis products from segmented polyurethane." *Journal of Analytical and Applied Pyrolysis*, 1998: 1-15.

Lawson, G., Barkboy, C.T. and Lawson, C. *Fresenius journal of analytical chemistry*, 1996: 354.

Lawson, G., Bartram, S., Fitchner, S. and Woodland, E.D. "MALDI-MS and colorimetric analysis of diisocyanate and polyol migrants from model polyurethane adhesives used in food packaging." *The Royal Society of Chemistry*, 2000: 115-118.

Lim, B.H., Kwon, S.H., Kang, E., Park, H., Lee, H.W. and Lim, W. "Isolation and Identification of Cyclic Oligomers of th Poly(ethylene terephthalate)-Poly(ethylene isophthalate) Copolymer." *Journal of Polymer Science*, 2002.

Longlang, R.C., Shilling, W.H. and Gangolli, S.D. "The Hydrolysis of Flavoring Esters by Artificial Gastrointestinal Juices and Rat Tissue Preparations." *Toxicology* 8 (1977): 197-204.

Mattson, H. and Volpenhein, R.A. "Relative rates of hydrolysis by rat pancreatic lipase of esters of C12 - C18 fatty acids with C1-C18 primary n-polyols." *Journal of Lipid Research* 10 (1969): 271-276.

Montaudo, G. "Mass Spectral Determination of Cyclic Oligomer Distributions in Polymerization and degradable Reactions ." *American Chemical Society*, 1991: 5829-5833.

Nasser, Lopes, Eberlin M.N., Monteiro M. "Identification of oligomers in polethyleneterephthalate bottles for mineral water and fruit juice." *Journal of Chromatography A.*, 2005: 130-137.

Oertel, G. *POlyurethane Handbook.* Hanser Gardner Publications, 1985.

Packaging Solutions: Glenory Inc. http://www.glenroy.com/packaging/.

Petrie, E.M. "An Introduction to Adhesives and Sealants." In *Handbook of Adhesives and Sealants*. McGraw Hill, 2000.

Piringer, Anita Gruner and Otto. "Component Migration From Adhesives Used In Paper And Paperboard Packaging For Foodstuffs." *Packging Technology and Science*, 1999: 19-28.

Sasano, S., Igarashi, S. USA Patent US 6,908,981 B2. 2005.

Schaefer, A., Ohm, V.A., Simat, T.J. "Migration from can coatings: Part 2. Identifcation and quantification of migrating cyclic oligomers below 1000 Da." *Food Additives and Contaminants* , 2004: 377-389.

Schafer, A., Simat, T.J. and Steinhart, H. "Migration from can coatings: Part 1. A size-exclusion chromatographic method for the simultaneous determination of overall migration and migrating substances below 1000 Da." *Food Additives and Contaminants*, 2004: 287-301.

Seong Jae Yoo, Gregory V. Pace, Bin K. Khoo, Joseph Lech and Thomas G. Hartman. "Mass Spectrometry and Gas Chromatographic Retention Indices of Selected UV/EB-Curable Monoers and Photo intiators Commonly Used in Food Packaging Print and Coating Formulations." 2004.

Smith, J. S., Hui, Y.H. *Food Processing: Principles and Application.* Blackwell , 2004.

Szycher, M. *Szycher's Handbook of Polyurethanes.* CRC Press, 1999.

Thiebaut, B., Lattuati-Derieux, A., Hocevar, M. and Vilmont, L. "Application of headspace SPME-GC-MS in characterization of odorous volatile organic compounds emitted from magnetic tape coating based on poly(urethane-ester) after natural and artificial ageing." *Polymer Testing*, 2007: 243-256.

"USA, Code of Federal Regulation; Title 21, Parts 170.39."

"USA, Code of Federal Regulation; Title 21, Parts 174.5."

"USA, Code of Federal Regulation; Title 21, Parts 175.105."

"USA, Code of Federal Regulation; Title 21, Parts 176.170.(c)."

Van Den Dool, H. and Kratz, P. "A generization of the retention index system including linear temeratured programmed gas liquid partition chromatography." *Journal of Chromatography*, 1963: 463-471.

"Additives to design and improve the performance of multilayer flexible packaing." In *Multilayer Flexible Packaging*, by J.R. Wagner. Elsevier Inc. , 2010.