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Synthesis of Innovative Materials for Food Packaging

Settore Scientifico Disciplinare CHIM06

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Abstract

Lo scopo del presente lavoro di tesi è la sintesi di materiali polimerici innovativi funzionalizzati con una porzione antiossidante legata alla catena laterale da utilizzare nella conservazione dei cibi, in particolar modo per prolungare la "shelf life" dei cibi della IV gamma cui appartengono frutta e verdura già lavata e tagliata, pronta all'uso. Generalmente questo genere di alimenti deperisce velocemente e ha una durata limitata a pochi giorni.

L'ossidazione è una delle più importanti reazioni di degradazione dei cibi e poichè ne riduce la "shelf-life", ne limita anche la conservazione con conseguente diminuzione delle vendite.

L'active packaging prolunga la "shelf-life" dei cibi e allo stesso tempo ne migliora la sicurezza e le proprietà organolettiche.

L'idea innovativa rispetto alle tecniche tradizionali è la preparazione di film bioattivi in cui la molecola antiossidante non è adsorbita sulla superficie del film con procedure di spraying, immersione o rivestimento, ma è legata covalentemente, tramite un linker, alla matrice polimerica.

In questo studio è stata evidenziata la sintesi di un PET modificato innovato che reca una catena laterale funzionalizzata con una molecola antiossidante quale il *ter*-butilidrochinone (TBHQ) o l'idrossitirosolo (Tyr-OH).

L'attività antiossidante dei monomeri di PET modificato funzionalizzati con il TBHQ e il Tyr-OH è stata testata tramite test del DPPH che ha confermato l'elevata capacità scavenger dei monomeri stessi.

Un altro obiettivo del lavoro di ricerca è stata la caratterizzazione tramite spettrometria di massa di polimeri e copolimeri funzionalizzati. Questo studio è stato svolto in collaborazione con l'Università di Akron, Ohio, USA.

Lo scopo del lavoro è stato sviluppare un protocollo di analisi di spettrometria di massa che fornisce informazioni riguardo la composizione dei poliesteri copolimeri, dei gruppi terminali, delle sequenze e della loro architettura.

Più nello specifico, l'oggetto dello studio è stato il confronto tra il copolimero lineare poli(caprolattone)-poli(etilene glicole) (PCL-PEG) e l'omopolimero poli(caprolattone).

Inoltre è stato condotto uno studio sull'energia di collisione necessaria alla frammentazione per il copolimero PCL-PEG, e gli omopolimeri PCL e PEG. Questo studio è stato condotto utilizzando spettrometri di massa MALDI Q/ToF e MALDI ToF/ToF/.

Essendo questi polimeri ampiamente usati in moltissimi campi e per differenti applicazioni, in particolare applicazioni in campo biologico, è stato condotto uno studio di degradazione in mezzo acquoso per capire come e con quale velocità questi polimeri degradano e frammentano in presenza di acqua.

Per effettuare questo studio i "frammenti" provenienti dalla degradazione sono stati separati per cromatografia liquida attraverso l'uso di uno strumento UPLC la cui uscita è accoppiata ad uno spettrometro di massa per poter facilmente individuare ed assegnare i vari frammenti.

Abstract

The aim of this PhD project is the synthesis of novel polymeric materials functionalized at the side chain with an antioxidant moiety to be used for the food active packaging, in particular to prolong the shelf life of IV range food. IV range products are washed, dried and fresh-cut vegetables and fruits and their shelf-life is limited generally at few days.

Oxidation is one of the most important degradation reactions in foodstuffs, which seriously limits their preservation reducing the shelf life of food products and consequently decreasing sales.

Active packaging prolongs the shelf life of food as well as improves its safety and organoleptic properties.

The innovative idea than traditional techniques is the preparation of a bioactive films in which the oxidant molecule is not adsorbed on the surface of the film with spray, immersion or coating procedures but it is covalently linked, via a spacer, at the polymer matrix.

In this study was underlined the synthesis of a novel modified PET bearing a side chain functionalized with an antioxidant moiety which could be *ter*-buthyl-hydroquinone (TBHQ) or hydroxytyrosol (tyr-OH).

The antioxidant activity of the modified PET monomers with TBHQ and Tyr-OH was tested by DPPH test which confirmed the high scavenger capacity of this material.

Another purpose of this research study is the characterization of functionalized homopolymers and copolymers by mass spectrometry in collaboration with the University of Akron, Ohio, USA.

The goal is to develop MS analysis protocols which provide informations about polyester copolymer composition, individual end groups, sequence and architecture. More specifically the object of this study is the comparison between the linear polycaprolactone-polyethylene glycol block copolymer (PCL-b-PEG) and the polycaprolactone (PCL) homopolymer.

Furthermore the PCL-PEG copolymer, the homo-PCL and homo-PEG collision energy study was carried out by using MALDI Q/ToF/MS and MALDI ToF/ToF/MS.

Since this kind of polymers is used in many fields and for different applications, in particular in biological application, a degradation study in aqueous moiety was carried out to understand how these polymers degrade and fragment in presence of water. To perform this study UPLC/ESI/QToF/MS was used.

PART I

Synthesis of innovative

materials for food packaging

1 Introduction

1.1 Active and Intelligent Packaging

The package as a simple instrument for the marketing of food is changing to match the needs of consumers and the food industry. New types of active and intelligent packaging have been and will be developed to meet these needs. The effectiveness of active and intelligent packaging has been demonstrated by the large number of scientific studies that confirm their application. Currently, oxygen scavenger and moisture absorbers are found on the market in increasing numbers. However, antioxidants and antimicrobial active packaging and freshness and/or ripening indicators will be increasingly important and in future demand by the food industry. The correct use of one or more types of active or intelligent packaging will increase the shelf-life and food safety.

In the recent past, food packaging was used to enable marketing of products and to provide passive protection against environmental contaminations or influences that affect the shelf life of the products. However, unlike traditional packaging, which must be totally inert, active packaging is designed to

interact with the contents and/or the surrounding environment. Active packaging systems are successfully used to increase the shelf life of processed foods and can be categorized into adsorbing and releasing systems (for example, oxygen scavengers, ethylene scavengers, liquid and moisture absorbers, flavor and odor absorbers or releasers, antimicrobials, etc.). Intelligent packaging is characterized by its ability to monitor the condition of packaged food or the environment by providing information about different factors during transportation and storage. Intelligent packaging includes time-temperature indicators, gas detectors, and freshness and/or ripening indicators. At the same time, advances in nanotechnology and the improvement of nanomaterials will enable the development of better and new active and intelligent packaging. Such packaging provides great benefits to the food industry to improve freshness, shelf-life of food, and allows monitoring to control the storage conditions from the place of production to consumption by the final consumer. (1)

The shelf life of packaged food depends on both the intrinsic nature of food and extrinsic factors. Intrinsic factors include pH, water activity (Aw), nutrient content, presence of antimicrobial compounds, redox potential, respiratory rate, and the biological structure, whereas extrinsic factors include storage temperature, relative humidity, and the surrounding gas composition. (2)

The primary purpose of food packaging is to protect the food against attack from oxygen, water vapor, ultraviolet light, and both chemical and microbiological contamination.

1.1.1 Oxygen Scavengers

Packaged foods include a certain amount of headspace gases and entrained oxygen. Permeation of oxygen into plastic containers is also of concern. Although it is desirable to keep the headspace gases to a minimum to provide reliable end closure with hermetic seals, it is also important to minimize the amount of oxygen that can react with the contents of the container. (3)

Molecular oxygen (O_2) can be reduced to a variety of intermediate species by the addition of between one and four electrons; these species are superoxide, hydroxy radical, hydrogen peroxide, and water. O_2 and water are relatively unreactive: the three intermediate species are very reactive and carbon-carbon double bonds are particularly susceptible to reaction with the intermediate species. These reactive oxygen species are free radical in nature, and the oxidative reactions in which they participate are therefore autocatalytic. Virtually any product that contains complex organic constituents will possess

such carbon-carbon double bonds or other oxygen-reactive components, and hence can undergo oxidative reactions. (4)

A high level of oxygen reduces the nutritional value of food and reduces its shelf life. The oxygen in headspace gases will react with sensitive foods. Oxygen present inside the package accelerates the deterioration of many food products (5) (meats, sausages, milk powder, or spices), degradation of vitamins, and rancidity of oils, nuts, and fatty foods, and also encourages microbial growth. Oxygen in the headspace of food packaging can be removed by vacuum sealing or by inert gas atmosphere in the packaging (N_2,CO_2), or both.

Control of the residual oxygen level in the package by use of oxygen absorbent materials limits the rate of deterioration and food spoilage. (4) (5) (6) (7) (8) (9) (10)

Oxygen scavengers reduce and actively control the residual levels of oxygen inside the package.

Oxygen scavengers have the following advantages:

- They prevent oxidation phenomena: rancidification of fats and oils and consequent emergence of off-odors and off-flavors, loss or change of colors characteristic of food, loss of oxygen-sensitive nutrients (vitamins A, C, E, unsaturated fatty acids, etc.).

- They prevent the growth of aerobic microorganisms.
- They reduce or eliminate the need for preservatives and antioxidants in food by incorporating the added value of "fresh" or "natural."
- They are an economical and efficient alternative to the use of modified atmosphere and vacuum packaging.
- They slow down metabolism of food.

The use of these systems, either alone or in combination with other traditional packaging systems, and the use of modified atmospheres can therefore extend the commercial life of a food product.

1.1.2 Ethylene Scavengers

The control of ethylene in stored environments plays a key role in prolonging the postharvest life of many types of fresh produce. (102)

Most fruits and vegetables release ethylene after they are harvested. Ethylene is a phytohormone that initiates and accelerates ripening, produces softening and degradation of chlorophylls, and inevitably leads to deterioration of fresh or minimally processed fruits and vegetables. Ethylene scavengers are useful for preserving ethylene-sensitive fruits and vegetables such as apples, kiwis, bananas, mangos, tomatoes, onions, carrots, and asparagus.

1.1.3 Liquid and Moisture Absorbers

Many food products require control of water, either in liquid or vapor. The presence of oozing liquids (water, blood, or other fluids) in meat products and fish detracts from presentation of the products. High levels of water inside packaging favor growth of microorganisms, cause the softening of dry and crunchy products, such as pasta, cookies, and biscuits, and causes caking and hardening in milk powder or freeze-dried coffee. Excessive water loss may also promote oxidation of fat. To prevent microbial growth in these nutrient-rich exudates organic acids and surfactants have been incorporated into absorbent pads. (11)

Products packaged with a high relative humidity in the headspace are susceptible to temperature fluctuations during transport or storage, which favors the formation of condensation and mist. In such foods, an antifog additive is used to reduce the interfacial tension between the condensed water and the film. This contributes to the transparency of the film and allows consumers to see packaged foods clearly, but does not affect the amount of water present inside the container. (12) (13)

The mechanism of action is based on an absorption process, which in some cases removes excess liquid water and other cases controls the relative humidity in the headspace. Highly hygroscopic and dehydrating agents are

used for these purposes. Cellulose fibers, polyacrylate salts, polypropylene glycol, carbohydrates, minerals, silica gel, montmorillonite, molecular sieves, and calcium oxide can be used.

Commercial presentations can be classified into two categories:

- Liquid absorbers (pads, sheets) generally consisting of two or more layers of micro-porous polymeric materials containing hygroscopic agents. These are used to absorb fluid oozing from cut meat and fish, to improve the appearance to the consumer, and to prevent microbial growth.
- Relative humidity regulators (sachets or tags) containing dehydrating agents. They are used to regulate humidity in a wide range of products such as cheeses, meats, nuts, and spices.

1.1.4 Flavor and Odor Absorber/Releaser

Some commercial products have been developed to eliminate, in some cases selectively, undesirable compounds or compounds that release odors.

However the removal of odors from the interior of food packages may be both beneficial and detrimental.

In Europe, article 4 (14) of Directive 1935/2004 (15) indicated: "Active materials and articles shall not bring about changes in the composition or organoleptic characteristics of food, for instance by masking the spoilage of food, which could mislead consumers." The release of odor or absorption of degradation indicators such as amines must not suggest a "better than real" quality of a product. Additionally, the regulations concerning addition of ingredients and flavors have to be maintained (Directive 89/107/EEC (109), Directive 95/2/EC (110) and amendments).

The issue of the benefits of odor/aroma removals such us activated carbon odor adsorbers is, however, significant in the realm of active packaging. Many foods such as fresh poultry and cereal products develop what are referred to as confinement odors. Very slight and generally insignificant but nevertheless detectable deterioration odors, such as sulfurous compounds from protein/amino acid breakdown or aldehyde/ketone compounds from lipid oxidation or anaerobic glycolysis, may form during product distribution. These odors are trapped within gas-barrier packaging so that, when the package is opened, they are released to be detected by consumers. These relatively harmless odors, which generally do not signal any significant spoilage, may be cause for rejection even though they dissipate into the surrounding air within seconds. One reason for odor removal from the interior

of packages would be to obviate the potentially adverse effects of these "confinement odors."

1.1.5 Antimicrobials

When inadequate processing or underprocessing occurs during manufacture, or package integrity is compromised due to a ruptured seal, puncture dents, or incomplete glass finishes, microbiological contamination due to pathogenic or spoilage bacteria may occur. (16)

Traditional methods of preserving food from the damaging effects of microbial growth include heat treatment, drying, freezing, refrigeration, irradiation, modified-atmosphere packaging, and addition of salts or antimicrobial agents. However, in Europe some of these techniques are not applicable to foodstuffs such as fresh meat, (17) and fresh fish and seafood. According to the law, (15) active substances are ingredients in the sense of the Directive 2000/13/EC. (18)

Thus, the active substances have to be allowed as additives for the respective foods.

Antimicrobial packaging include systems such as adding a sachet into the package, dispersing bioactive agents in the packaging, coating bioactive

agents on the surface of the packaging material, or utilizing antimicrobial macromolecules with film-forming properties or edible matrices. (19)

A large number of agents with antimicrobial properties (ethanol, carbon dioxide, silver ions, chlorine dioxide, antibiotics, organic acids, essential oils and spices, etc.) have been tested for the purpose of inhibiting the growth of microorganisms that can lead to deterioration of foodstuffs (bacteria can also attack the packages affecting their functions and properties). However, few such systems are commercially available. (20)

The size and capacity of the sachet containing the ethanol-releasing agent will depend on the weight of food, water activity, type of food, and shelf-life required. When food is packaged with an ethanol-releasing agent, the moisture is absorbed and ethanol is released to the headspace of the package. (2)

In commercial preparations, a sachet containing ethanol on a base of finely divided silica with an adequate degree of humidity is placed on the inside of the package.

Antimicrobial packaging can play an important role in reducing the risk of contamination of food by pathogens, (21) as well as in extending the shelf life of foods, but should never replace the use of quality raw materials and the proper use of good manufacturing practices. (22) On the other hand, some

foods can have their shelf-life extended with incorporated volatile antimicrobial compounds in the headspace of the package.

1.1.6 Antioxidants

Oxidation of food can be avoided by use of oxygen scavengers and antioxidant agents in the packaging. Such packaging is intended to prevent or slow down the oxidation reactions that affect the quality of food (23) (24). However, radicals, mainly oxo, hydroxyl, and superoxide, are originated from oxygen and they are the main initiators of oxidation. Thus, oxidation can be avoided by eliminating radicals as soon as they are formed. Some natural compounds act this way and react very efficiently with the radicals by trapping them, thus avoiding further oxidation. In such a case, neither high-barrier nor vacuum packaging materials would be required to avoid oxidation, but only the presence of a radical scavenger to protect the food against the oxidation process. (25)

In turn, the materials containing radical scavengers do not need to be protected or activated before using.

The additives that are gaining increased attention are natural antioxidants such as vitamin E and natural extracts rich in phenolic compounds and/or terpenes

(barley, rosemary, clove, oregano, cinnamon, ginger, etc.). (26) (23) (24) (25) (27) (28)

Spices contain large amounts of phenolic compounds such as flavonoids and phenolic acids, which exhibit antimicrobial and antioxidant properties. (29) (30)

Cellulose films have been incorporated with cysteine and sulpfite and used to cover apples slices. Brighter apples and less browning was obtained with these cellulosic films. (31)

The antioxidant content decreases during storage due to diffusion of the antioxidant through the film and its subsequent evaporation at the surface. This decrease in the concentration of the antioxidant can be prevented by adding an extra layer of film that has a low permeability to antioxidant (32) (33) or through the use of cyclodextrins. (34) Antioxidants can be used for oil, nuts, butter, fresh meat, meat derivatives, bakery products, fruits and vegetables, among others.

Traditional antioxidants applied to delay lipid oxidation by reacting with intermediate products of lipid oxidation, i.e., by interrupting free radical chain reactions, have been incorporated into package materials for many years.

A research showed that by coating the interior surface of a polyethylene film package with t-butylhydroquinone (TBHQ) the shelf life of contained deep-

fried noodles is extended. (35) It was also demonstrated that when butylated hydroxy toluene (BHT) was incorporated into high density polyethylene film, the shelf life of a contained oat cereal was increased. This research asserted that the applications of the antioxidants into the plastic film of the package were more effective than adding the same antioxidant directly into the contained food products. (36) Oxidation of fats is one of the most important mechanisms leading to food spoilage, second only to growth of microorganisms. The oxidation of lipids in food leads to a reduction in shelf-life due to changes in taste and/or odor, deterioration of the texture and functionality of muscle foods, and a reduction in nutritional quality. (23)

The antioxidant could be incorporated into two-ply or three-ply structures. An example would be two layers of PVdC sandwiching the antioxidant. Also cited as antioxidants were butylated hydroxyl anisole (BHA), BHT, and dihydroguaretic acid, among others, with the preference being for n-propyl gallate.

The antioxidants were incorporated on the interior surface of the film from a liquid solution in which the solvent was propylene glycol, glycerol, or edible oil. The antioxidant in the solvent was trapped between the two film layers.

1.2 Intelligent Packaging

The headspace of food packages undergoes changes in their composition over time. Devices capable for identifying, quantifying, and/or reporting changes in the atmosphere within the package, the temperatures during transfer and storage, and the microbiological quality of food provide valuable information both to the final consumer and producer and/or marketer about the effectiveness of the conservation strategies used in the marketing chain. For packaging intended to be used in the food industry, some very strict requirements must be fulfilled: the indicators should be easily activated and exhibit a change (or show an indication) that is easily measurable and irreversible, time- and temperature-dependent changes must be reproducible and ideally matched or readily correlated with the food quality, and also provide information regarding the status of the package. (37)

1.2.1 Time-Temperature Indicators

The best-before date printed on food packaging is only an indicative value and does not take into account possible fluctuations in temperature that food may suffer during storage. The best-before date must therefore be within the shelf-life of the food to ensure that food is safe to consume.

Time-Temperature Indicators fall into two types: visual indicators or radio frequency identification (RFID) tags.

The basic idea underlying visual indicators is that the quality of food deteriorates more rapidly at higher temperatures because chemical reactions, biochemical reactions, and microbial growth are speeded up. The indicators change color in response to cumulative exposure to temperature. The main mechanisms of action include enzymatic reactions, polymerization, or chemical diffusion. These products are used to monitor exposure to unsuitable temperatures during transport and storage and are an indication of quality for the producer because they ensure that the product reaches the consumer in optimal conditions.

It is important that the indication is irreversible.

1.2.2 Seal and Leak Indicators

The gas composition in the package headspace often changes as a result of the activity of the food product, leaks, nature of the package, or environmental conditions. O_2 and CO_2 can be used to monitor food quality, as seal indicators (leaks), or to verify the effectiveness of an oxygen absorber. Most O_2 or CO_2 indicators change color as a result of chemical or enzymatic reactions. A color

change indicates when the oxygen concentration exceeds the limit established in a sealed food package. (38)

A major problem with such indicators is that they require storage under anaerobic conditions, since they quickly deteriorate in air, ceasing to work in a few hours as the strong reducing agent is used up in a direct, or indirect, reaction with oxygen. (39)

A new approach is the use of intelligent ink/indicator that changes its color in the presence of oxygen. This ink must be activated with UV light and is stored in aerobic conditions. (40)

1.2.3 Freshness and/or Ripening Indicators

Freshness and/or ripening indicators provide an indication of the deterioration or loss of freshness of packaged goods. They are described as indicating different mechanisms of volatile metabolites, such as diacetyl, amines, carbon dioxide, (41) ammonia and hydrogen sulfide, produced during the aging of foods. (42)

Changes in the concentration of hydrogen sulfide or organic acids such as n-butyrate, L-lactic, D-lactate, and acetic acid during storage are offered as

viable indicators of the formation of metabolites in meat products, fruits, and vegetables (43). Indicators based on color changes due to changes in pH are of great potential use as indicators of microbial metabolites and ripeness. (41) (44) Products formed during microbial growth (carbon dioxide and hydrogen sulfide) and biogenic amines are of great potential use in indicating the freshness of meat and fish. (42) (45)

Biogenic amines (putrescine, cadaverine, histamine, and others) are formed by degradation of protein-containing food to amino acids and by enzymatic decarboxylation of the latter. Thus, biogenic amines are an indicator of food deterioration and only an indirect indicator of food freshness in meat and fish.

2 Results and discussion

The introduction of functional groups on PET monomers is a novel and sample method to obtain polymeric materials with antioxidant activity.

The creation of a polymeric film with scavenger activity is interesting for the conservation of IV group food.

IV range products are washed, dried and fresh-cut vegetables and fruits. They are “ready to use foods” and then they don’t require any action before consumption.

IV range products shelf-life is limited generally at few days because of some stability factors combination.

Preliminary operations IV range food undergoes produce mechanical and physiological damages that induce chemical and enzymatic reactions and consequent the manifestation of undesidered events, such as enzymatic browning, loss of tissues consistence, oxidation processes.

The employment of suitable plastic films for conservation of IV range food can remarkably increase its shelf-life.

Oxidation is one of the most important degradation reactions in foodstuffs, which seriously limits their preservation reducing the shelf life of food products and consequently decreasing sales.

Active packaging prolongs the shelf life of food as well as to improve its safety and organoleptic properties.

Food packaging has evolved from simply a container to hold food to something that can play an active role in food quality.

The innovative idea than traditional techniques in the synthesis of the antioxidant materials is the preparation of a bioactive films in which the oxidant molecule is not adsorbed on the surface of the film with spray, immersion or coating procedures but it is covalently linked, via a spacer, at the polymer matrix.

As shown in the figure 1 the main chain is basically the polyethylene terephthalate, the linker (L) is an alkylic chain and the antioxidant group (A) can be TBHQ or hydroxytirosol.

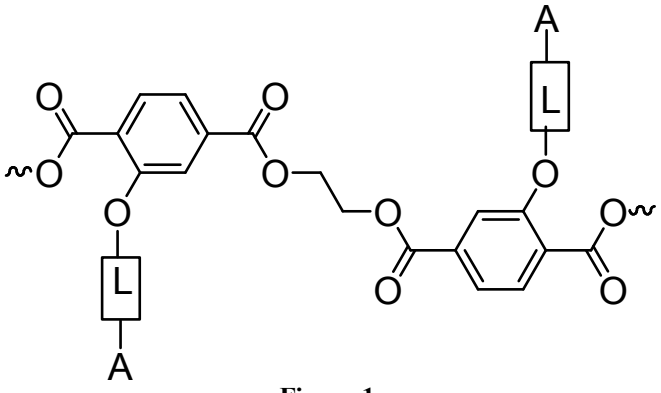
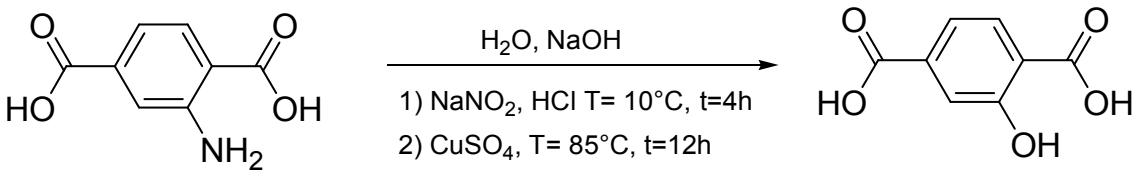


Figure 1

The synthesis of 2-hydroxy-terephtalic acid was realized starting from the 2-amino-terephtalic acid which is commercially available (Scheme 1.1; the preparation is reported in the experimental section).

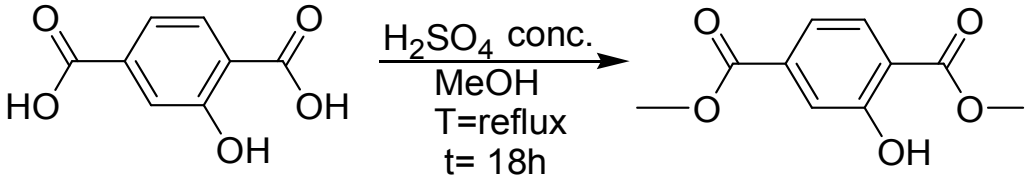


1

Scheme 1.1

The product **1** was obtained with high yield (85%) and it didn't require purifications.

The synthesis of 2-hydroxyterephthalic acid was followed by the protection of the carboxylic function of the molecule.



2

Scheme 1.2

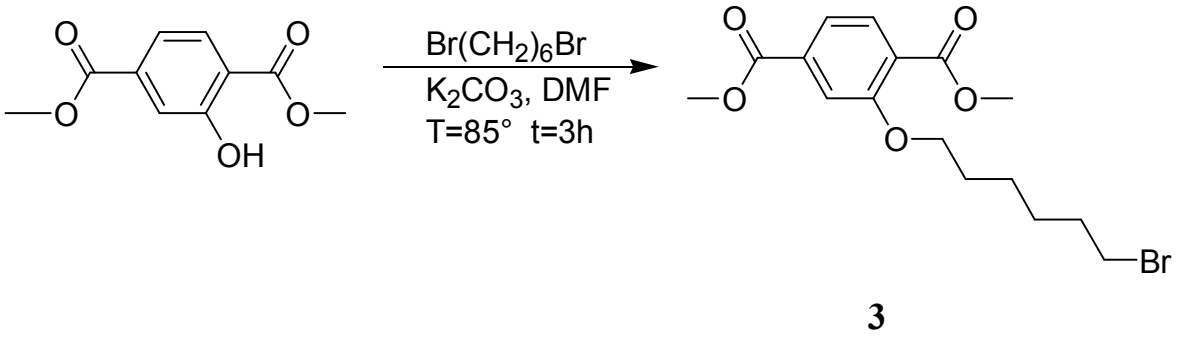
The scheme 1.2 shows the synthetic procedure for the preparation of the 2-

hydroxyterephthalic acid methyl ester **2**. The experiment was carried in MeOH in presence of H_2SO_4 conc. and under reflux conditions. The preparation of product **2** is better described in the experimental section.

This product was characterized by GC-MS and IR.

There are two different synthetic strategies for the linking of the alkyl linker.

In the first one the alkyl spacer was linked at the hydroxyl group of 2-hydroxyterephthalic acid methyl ester according to the scheme 1.3.



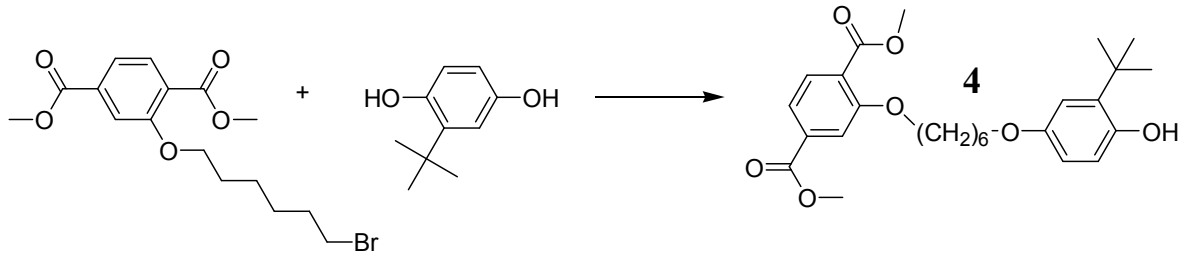
3

Scheme 1.3

The reaction mixture was monitored, and after 3h GLC and TLC analyses revealed the formation of the desired product, which was purified by chromatographic column, separated and characterized by IR, NMR and GC-MS.

The linking of 2-hydroxytherephthalic acid methyl ester with the spacer was followed by the coupling with the antioxidant moiety. In the first synthetic plan the antioxidant molecule was the *ter*-butylhydroquinone (TBHQ).

The synthetic procedure is showed in scheme 1.4. Details of the preparation are reported in the experimental section.

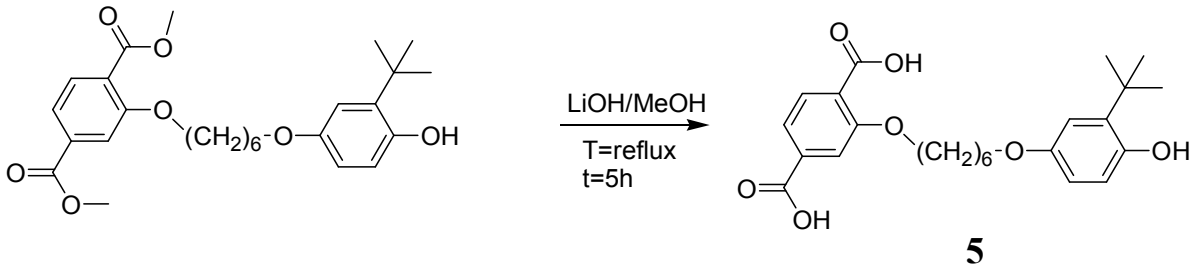


Scheme 1.4

The demethylation of the carboxylic functions of carboxylic functions was carried in MeOH by adding LiOH in a ratio of 18:1 for each carboxyl group.

The synthetic procedure is showed in the scheme 1.5 and all details about the preparation are explained in the experimental section.

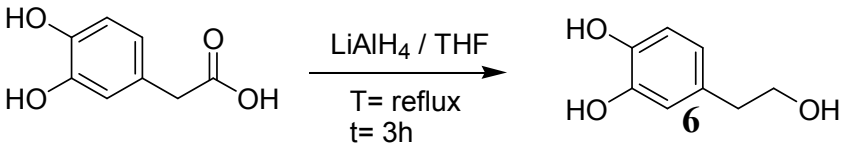
The final product (**5**) didn't need purifications and it was completely characterized by NMR and IR. This product is one of the antioxidant monomers which was later polymerized.



Scheme 1.5

In the second synthetic plan is the antioxidant molecule to be alkylated with the linker before the coupling with the 2-hydroxyterephtalic acid methyl ester.

As shown in the scheme 1.6 the hydroxytyrosol was synthesized from the (3,4-dihydroxyphenil)acetic acid by reducing with $LiAl_4$.

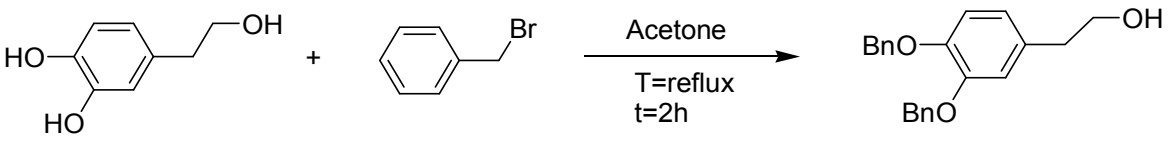


Scheme 1.6

The mixture was purified by a flash chromatography giving the final product **6** with a high yield. The synthetic procedure is better explained in the experimental section.

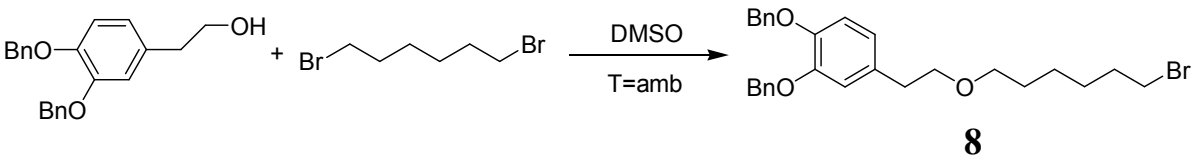
Before the linking with the alkyl chain the oxydril groups of hydroxytyrosol were protected with benzyl groups (Scheme 1.7) in order to have only one reactive function in the coupling reaction and the product **7** was obtained.

Subsequently it was alkylated by the reaction with dibromohexane (Scheme 1.8).



7

Scheme 1.7

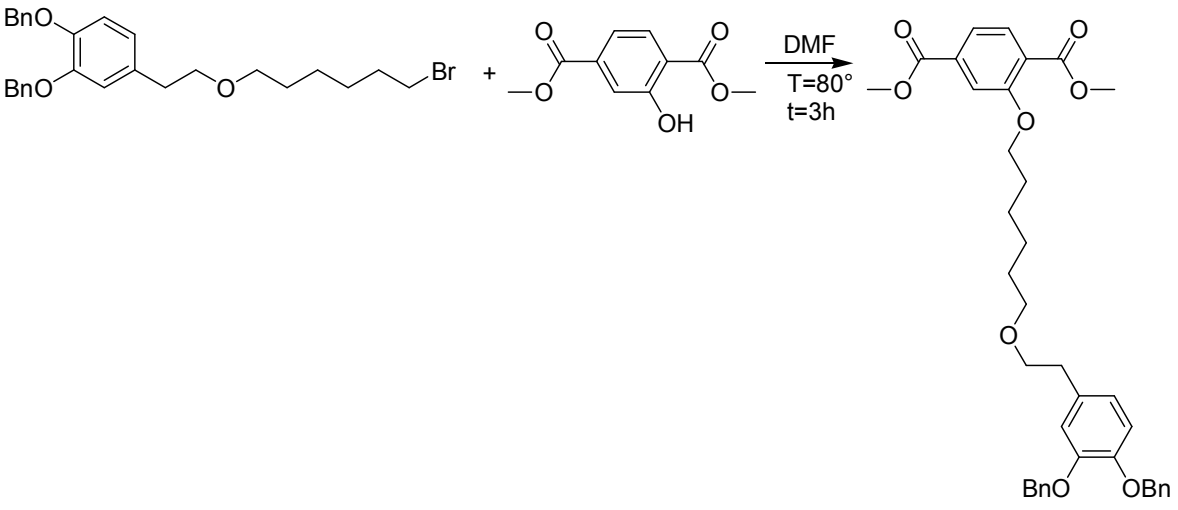


8

Scheme 1.8

The product of the reaction 1,2-Bis-benzyloxy-4-[2-(6-bromo-hexyloxy)-ethyl]-benzene (**8**) was fully characterized by GC-MS and NMR.

The next step in the synthesis of the monomer with Hydroxytyrosol (**9**) as antioxidant moiety was the coupling with the antioxidant molecule (Scheme 1.9).

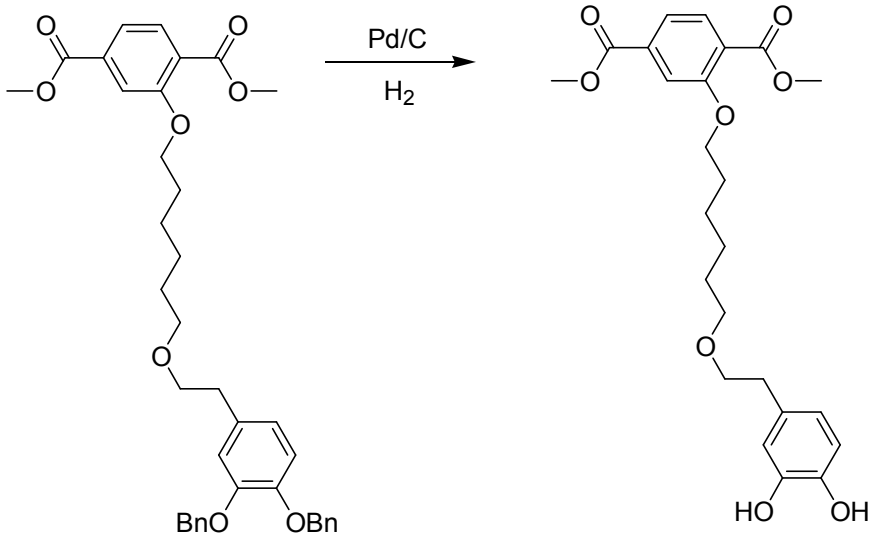


9

Scheme 1.9

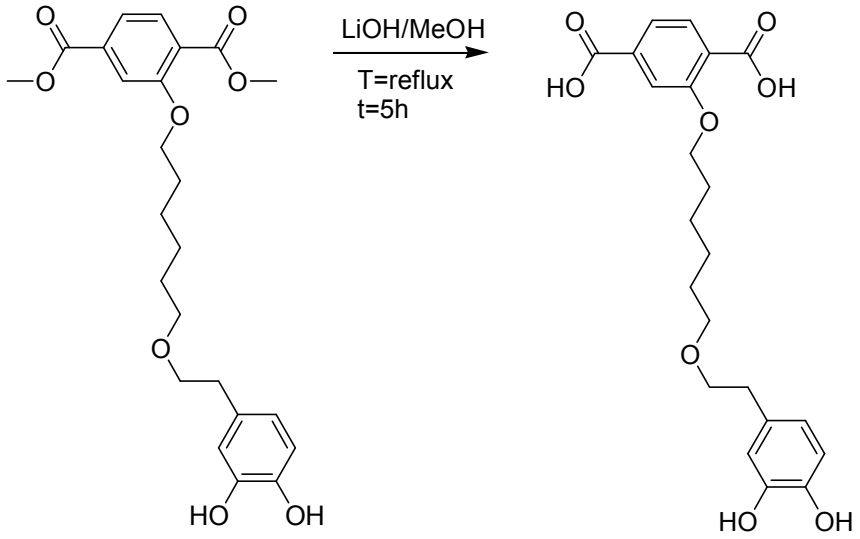
Benzyl groups on Hydroxytyrosl were removed by catalytic hydrogenation with Pd/C (Scheme 1.10) to obtain product **10** which was subsequently

deprotected on the carboxylic groups (Scheme 1.11) to obtain the final product (**11**), a monomer with antioxidant activity which was later polymerized.



10

Scheme 1.10

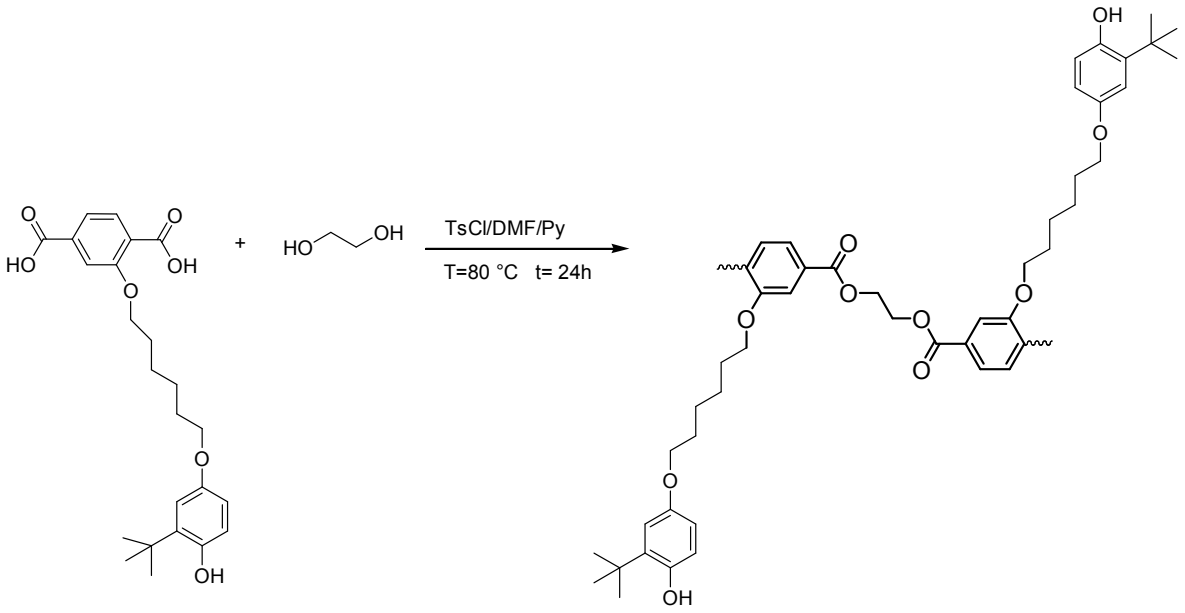


11

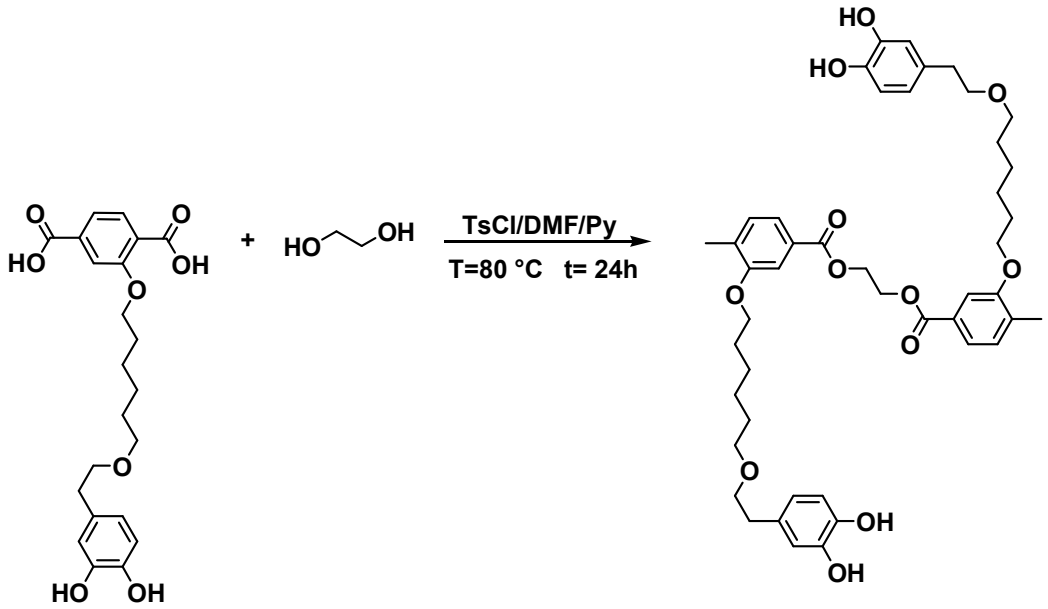
Scheme 1.11

The polymerization of the monomers having as antioxidant moiety TBHQ or Tyr-OH with Ethylene Glycole was carried out in solution using TsCl/DMF/Py as condensing agent. (Scheme 1.12 and scheme 1.13)

The synthetic procedure is better explained in the experimental part.



Scheme 1.12



Scheme 1.13

2.1 Antioxidant activity of the synthesized monomers

The in vitro antioxidant activity of the synthesized monomers was determined by evaluating their free radical scavenging activity with respect to DPPH at four different concentrations according to a known protocol (46). Thus, 2 MeOH solutions containing respectively known amounts of **10** and **11** and DPPH, were prepared, and the colorimetric decrease in absorbance of DPPH was measured at 517 nm after a period of 30 min since the preparation.

The absorbance of each sample was measured against blank at 517 nm. Experiments were carried out in triplicate, and TBHQ and Hydroxytyrosl weres used as the positive reference antioxidants. The percent of scavenging activity on DPPH was calculated according to the formula:

$$\text{DPPH scavenging activity (\%)} = 100\ (\ A_{blank} - A_{sample}) / A_{blank}$$

Good antioxidant properties were recorded using the DPPH test.

The method based on the disappearance of colored synthetic radicals such as DPPH was chosen because of its simplicity and versatility.

It measures the ability of antioxidant compounds for trapping free radicals by donating hydrogen atoms or electrons, producing in consequence the bleaching of the colored radical solution of DPPH.

The antiradical capacity of the modified PET monomer, determined in the DPPH assay, indicate a good ability to scavenge the radical in the methanolic medium. The results obtained, given as dose–response histograms, are shown in Figures 1 and 2. The tert-butylhydroquinone (TBHQ) and Tyr-OH radical scavenging activities are also shown for comparison.

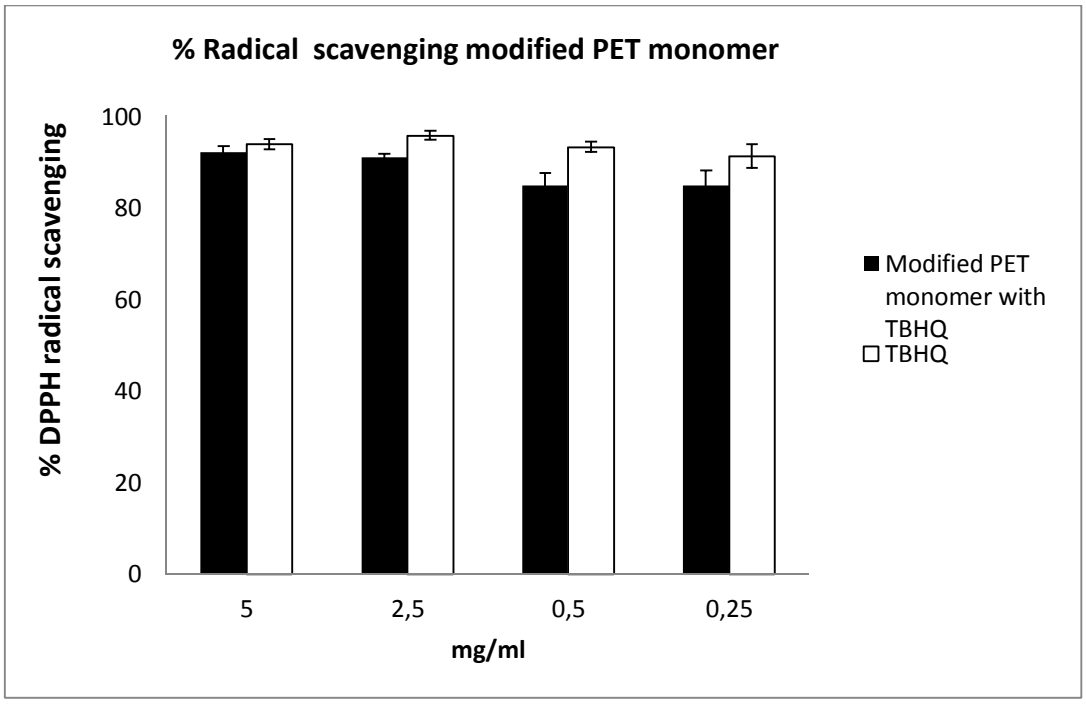


Figure 2

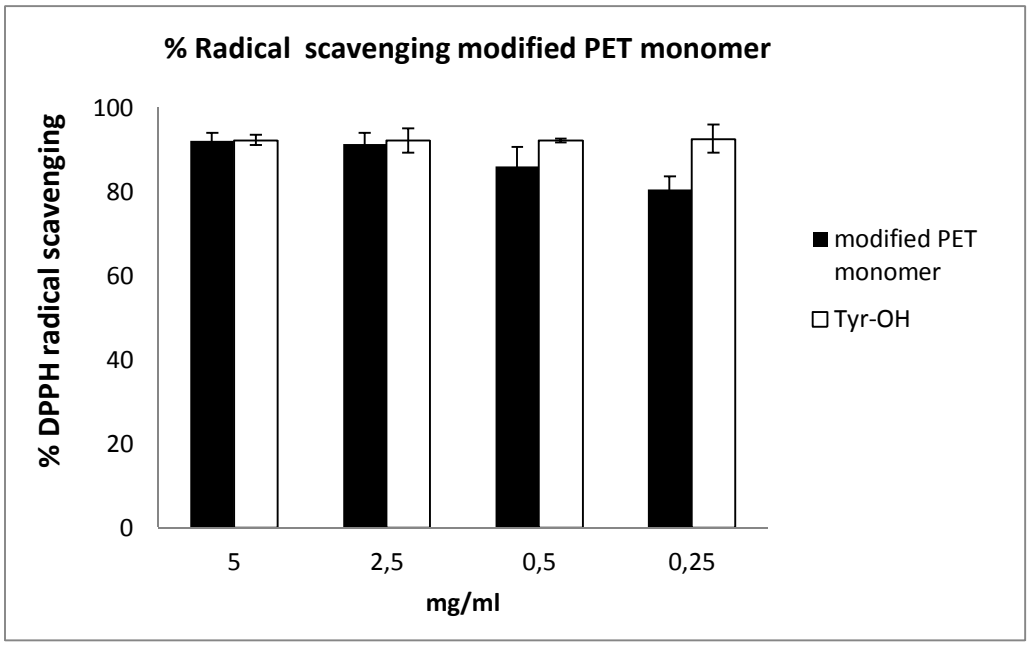


Figure 3

As can be observed from Figures 2 and 3, in all cases the values of the radical scavenging activity of the monomers **10** and **11** increase with concentration. More specifically, the values of radical scavenging activity of **10** varied from 92.2% for the concentration of 5 mg/ml to 84.9% for the concentration of 0.25 mg/ml.

The synthetic antioxidant TBHQ was used as a positive control and displayed radical scavenging activities from 93.9 % to 91.3%.

The values of radical scavenging activity of modified PET monomer **11** varied from 92.1% for the concentration of 5 mg/ml to 80.6% for the concentration of 0.25 mg/ml.

Tyr-OH was used as a positive control and displayed radical scavenging activities from 92.2 % to 91.7%.

Interesting evidence of this study is that both the monomers have a high radical scavenging activity and the two modified PET monomers have comparable antioxidant activity.

3 Experimental section

3.1 General Experimental Methods.

^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C in $CDCl_3$ solutions at 300 or 500 MHz and 75 or 125 MHz, respectively, with Me_4Si as internal standard. Chemical shifts (δ) and coupling constants (*J*) are given in ppm and in Hz, respectively. IR spectra were taken with an FT-IR spectrometer. Mass spectra were obtained using a GC-MS apparatus at 70 eV ionization voltage or a mass spectrometer equipped with a turbo ion spray ionization source in the positive mode [ion spray voltage (IS) 4500 V; curtain gas 10 psi; temperature 25 °C; ion source gas (1) 20 psi; declustering and focusing potentials 50 and 400 V, respectively]. Microanalyses were carried out at our analytical laboratory. All reactions were analyzed by TLC on silica gel 60 F_{254} and by GLC using a gas chromatograph and capillary columns with polymethylsilicone + 5% phenylsilicone as the stationary phase or using a gas chromatograph and a capillary columns with diethyl tertbutylsilyl-β-cyclodextrine as the stationary phase. Column chromatography was performed

on silica gel 60 (70-230 mesh). Evaporation refers to the removal of solvent under reduced pressure.

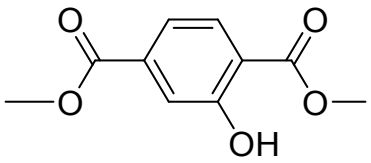
3.2 Synthesis of 2-hydroxyterephthalic acid

2-hydroxyterephthalic acid was synthesized from 2-aminoterephthalic acid. (47)

4.0 g of 2-aminoterephthalic acid were suspended in 60 ml of water. Then, 4.0 g of a 50 wt.-% NaOH solution in water were added. When the solution became clear, it was cooled down to 10 °C and 2.3 g of $NaNO_2$ dissolved in 3.3 ml of water were added. The solution was then diluted with 32 ml of water and 24 ml of 18.6 % HCl were added subsequently. The resulting vanilla coloured suspension was stirred at a temperature of 10 to 15 °C for further 4h. Thereafter, a small amount of $CuSO_4$ (covering the tip of a spatula) was added and the mixture was heated slowly to 85 °C for 12 h (or until gas evolution stops). The yellow tainted solid was filtrated off, washed two times with 10 ml of water and dried under vacuum at 100 °C for 12 h to give 2-hydroxyterephthalic acid **1** in 85% yield (3.4 g).

3.3 Synthesis of dimethyl-2-hydroxy-terephtalic acid dimethyl ester

The carboxylic functions of 2-hydorxyterephthalic acid were protected as methyl esters (48). The reaction was carried dissolving product **1** (2.42 g, 13.3 mmol) in 250 ml of MeOH. Conc. H_2SO_4 (5 ml) was added and the solution was refluxed overnight (~ 18 h). Upon cooling the reaction to room temperature, the MeOH was removed under vacuum. The remaining solution was neutralized with saturated $NaHCO_3$ (aq) solution and the product was extracted with CH_2Cl_2 (3×50 ml). The organic layer was isolated, washed with brine (4×15 ml), and the CH_2Cl_2 was removed under vacuum to obtain a white solid **2**. Yield: 2.65 g (95%).

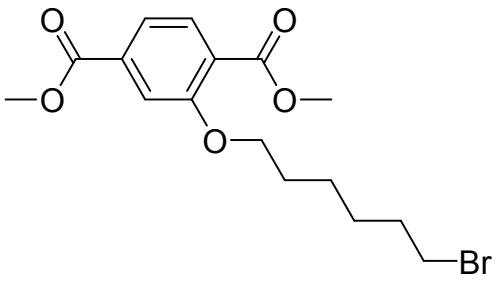


2-hydroxyterephthalic acid dimethyl ester

$C_{10}C_{10}O_5$ (210.18). IR (KBr): ν = 3438 (s, b), 3221 (w), 3014 (w), 2959 (w), 1720 (s), 1676 (s), 1622 (m), 1578 (w), 1503 (w), 1434 (m), 1384 (w), 1320 (m), 1273 (w), 1209 (s), 1106 (m), 985 (w), 959 (w), 908 (w), 887 (w), 823 (w), 795 (m), 754 (s), 692 (w), 569 (w), 441 (w).

3.4 Procedure for the alkylation of 2-hydroxy-terephthalic acid dimethyl ester

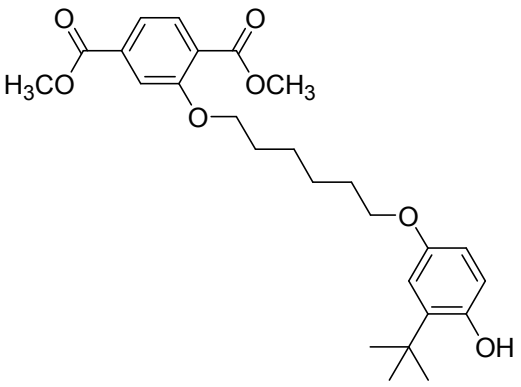
The product **2** (1 g, 4.8 mmol) was dissolved in dry DMF [0.2] mM. 1,6-dibromexane (880 µl, 5.7 mmol) and K_2CO_3 (1.32 g, 9.6 mmol) were added and the mixture was stirred at 80 °C for 3 h. After cooling to room temperature, the K_2CO_3 was filtered off and the solvent removed under vacuum to reveal slightly yellow oil which was purified by a chromatographic separation using a 9:1 hexane/ethyl acetate mixture as eluent to give the product **3** with a yield of 40%.



$C_{16}H_{21}BrO_5$ (373.24). IR (KBr) ν= 3437 (w), 3003 (w), 2948 (s), 2859 (m), 1727 (s), 1610 (m), 1574 (m), 1498 (m), 1435 (m), 1389 (m), 1293 (s), 1231 (s), 1190 (w), 1114 (m), 1083 (m), 1008 (w), 965 (w), 878 (w), 818 (w), 796 (w), 756 (m), 645 (w), 561 (w) cm^{-1}. GC-MS (EI, 70 eV): *m/z* = 373 [M^+] (3), 372 (3), 343 (5), 341 (6), 223 (8), 211 (9) 210 (50), 178 (100), 147 (3), 119 (17), 83 (8), 55 (12).

3.5 Coupling between 2-(6-bromo-hexyloxy)-terephthalic acid dimethyl ester and the antioxidant molecule *ter*-butylhydroquinone.

Product **3** (430 mg, 1.15 mmol) was dissolved in anhydrous DMF [0.2] mM. *ter*-buthylhydroquinone (TBHQ) (0.192 mg, 1.15 mmol) and K_2CO_3 (0.318 g, 2.3 mmol) were added and the mixture was stirred overnight at 80 °C. After cooling to room temperature, the K_2CO_3 was filtered off and the solvent removed under vacuum to reveal slightly brown oil which was purified by a chromatographic separation using a 9:1 hexane/ethyl acetate mixture as eluent to give the product 2-[6-(3-*tert*-butyl-4-hydroxy-phenoxy)-hexyloxy]-terephthalic acid dimethyl ester **4**.



[6-(3-tert-Butyl-4-hydroxy-phenoxy)-hexyloxy]-terephthalic acid dimethyl ester.

$C_{26}H_{34}O_7$ (458.54). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.79 (d, J = 8.4,1 H, H-6 on terephthalic ring), 7.63-7.61 (m, 2 H, H-3+H-5 on terephthalic ring), 6.85 (d, J =

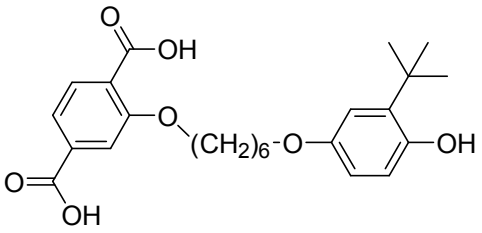
1.9, 1 H, H-2 on tyrosol ring), 6.62-6.55 (m, 2 H, H-5+H-6 on tyrosol ring), 4.89 (s, 1 H, O*H*), 4.10 (t, *J* = 6.3, 2 H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 3.94-3.88 (m, 8 H, $2CO_2CH_3$ + $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 1.90-1.77 (m, 4 H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 1.56-1.54 (m, 4 H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 1.39 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.4, 158.3, 153.0, 148.3, 137.6, 134.3, 131.3, 121.1, 116.8, 114.8, 114.0, 111.4, 69.0, 68.5, 52.4, 52.2, 29.6, 29.5, 29.0, 25.8, 25.6.

IR (KBr) ν= 3490 (s), 2951 (s), 2914 (w), 2870 (w), 1734 (s), 1713 (s), 1608 (w), 1574 (m), 1510 (m), 1443 (w), 1417 (m), 1388 (w), 1299 (s), 1228 (s), 1199 (s), 1123 (m), 1081 (s), 1046 (w), 1025 (s), 985 (w), 965 (w), 929 (w), 902 (w), 875 (w), 812 (m), 784 (m), 753 (s), 730 (w), 695 (w), 615 (w), 522 (w), 506 (w). GC-MS (EI, 70 eV): *m/z* = 458 [M^+] (90), 426 (29), 371 (8), 261 (17), 211 (27), 179 (40), 166 (88), 151 (100), 123 (23), 83 (21), 55 (44).

3.6 Removal of methyl esters from 2-[6-(3-*tert*-Butyl-4-hydroxy-phenoxy)-hexyloxy]-terephthalic acid dimethyl ester

Product **7** (0.110 g, 0.24 mmol) was suspended in MeOH [0.2] M. LiOH (0.206 g, 8.64 mmol) was added and the resulting mixture was allowed to reflux for 5 h. After removal of the solvent under vacuum, H_2O (10 ml) and

Et_2O (10 ml) were added and the organic layer was separated. The aqueous layer was acidified to pH 2 with 1 N aq HCl and then extracted with Et_2O (3 × 20 ml); then the combined organic layers were dried (Na_2SO_4). After filtration, the solvent was removed by rotary evaporation to give 2-[6-(3-*tert*-Butyl-4-hydroxy-phenoxy)-hexyloxy]-terephthalic acid (**5**) (49) (50)



2-[6-(3-tert-Butyl-4-hydroxy-phenoxy)-hexyloxy]-terephthalic acid.

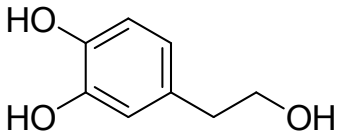
$C_{24}H_{30}O_7$ (430.49). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.85 (s, br, 1 H, OH), 7.69-7.57 (m, 3 H, on terephthalic ring), 6.67-6.59 (m, 3 H, on tyrosol ring), 4.10-4.01 (m, 2 H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 3.90-3.83 (m, 2 H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 1.80-1.67 (m, 4 H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 1.58-1.40 (m, 4 H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 1.32 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.6, 156.9, 151.2, 149.5, 136.2, 134.4, 130.1, 120.9, 116.3, 113.8, 113.5, 111.3, 68.4, 67.7, 29.2, 28.7, 28.4, 25.2, 25.1.

IR (KBr) ν= 3433 (s, b), 2174 (w), 1645 (s), 1499 (w), 1402 (m), 1384 (m), 1295 (w), 1240 (w), 1121 (w), 1087 (w), 788 (w), 756 (w), 622 (w), 566 (w).

3.7 Synthesis of Hydroxytyrosol

The hydroxytyrosol was synthesized from 3,4-dihydroxyphenilacetic acid by reducing with $LiAlH_4$.

(3,4-Dihydroxyphenyl)acetic acid (1,5 g, 0.9 mmol) was dissolved in dry THF (50 ml)and $LiAlH_4$ (1g, 26 mmol) was slowly added. After the addition was complete, the suspension was heated under reflux for 3 h then the mixture was cooled down to 0 °C in an ice bath and excess of $LiAlH_4$ was destroyed by the careful addition of H_2O. HCl 10% was added until pH 2 and then the mixture was extracted with ethyl acetate (3 x 30 ml). The combined organic extracts were dried and concentrated in vacuo. Careful flash chromatography (Chloroform/Methanol) gave the hydroxityrosol (**6**) as yellow oil. Yield 83%

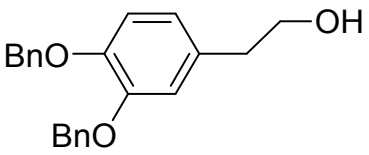


Hydroxytyrosol

$C_8H_{10}O_3$ (154.16). IR (KBr) ν= 3434 (s, br), 2077 (w), 1634 (s), 1529 (m), 1443 (w), 1384 (m), 1287 (m), 1261 (m), 1119 (w), 1044 (w).

3.8 Synthesis of 2-(3,4-bis-benzyloxy-phenyl)-ethanol

To a solution of pure hydroxytyrosol (0.8 g – 5.2 mmol) in anhydrous acetone (25 ml), benzyl bromide (1.4 ml – 11.8 mmol) and potassium carbonate (2.9 g – 20.8 mmol) were added and the resulting mixture heated to reflux for 24 h. The obtained suspension was filtered and concentrated to yield a crude residue, which was further purified by column chromatography, using a 9:1 mixture of hexane/ethyl acetate as eluent. The desired product 2-(3,4-Bis-benzyloxy-phenyl)-ethanol (**7)** was obtained as a white solid (60%). (51)

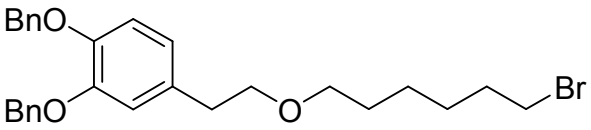


2-(3,4-bis-benzyloxy-phenyl)-ethanol

$C_{22}H_{22}O_3$ (334.41). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45-7.29 (m, 10 H, phenyl rings), 6.88 (d, J = 8.1,1 H, H-5 on hydroxytyrosol ring), 6.81 (d, J = 2.0, 1 H, H-2 on hydroxytyrosol ring), 6.72 (dd, J = 8.1, 2.0, 1 H, H-6 on hydroxytyrosol ring), 5.15 (s, 2 H, CH_2C$_6$H$_5$), 5.13 (s, 2 H, CH_2C$_6$H$_5$), 3.75 (t, J = 6.5, 2 H, CH_2OH), 2.73 (t, J = 6.5, 2 H, CH_2CH$_2$OH), 1.46 (s, 1 H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 149.1, 147.9, 137.5, 137.4, 132.0, 128.5, 127.8, 127.4, 127.3, 122.0, 116.4, 115.7, 71.6, 71.5, 63.6, 38.7. GC-MS (EI, 70 eV): m/z = 334 [M^+] (1), 243 (5), 211 (1), 181 (7), 92 (8), 91 (100), 89 (1), 77 (1), 65 (6), 44 (2).

3.9 Alkylation of 2-(3,4-bis(benzyloxy)phenyl)ethanol

For the alkylation a mixture of 2-(3,4-bis(benzyloxy)phenyl)ethanol **5** (334 mg, 1 mmol), KOH (335 mg) and dibromohexane (3 mmol) in anhydrous methyl sulfoxide (12 ml) was stirred at room temperature until completion of reaction (TLC). 3M HCl (25 ml) was added and the mixture extracted with $CHCl_3$. The organic phase was washed with 2% $NaHSO_3$ (25 ml) and water (25 ml), dried over Na_2SO_4, filtered and evaporated. The desired product **6** was purified by flash column chromatography over silica gel with hexane/ethyl acetate as eluents.



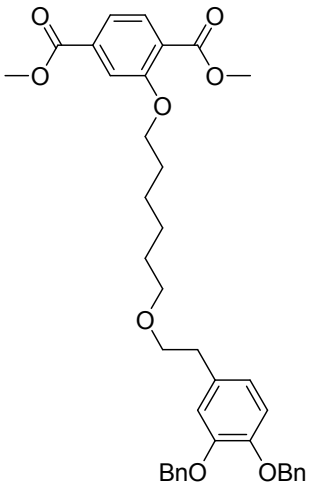
$C_{28}H_{33}BrO_3$ (497.46). GC-MS (EI, 70 eV): *m/z* = 498 [M^+] (5), 405 (2), 316 (1), 281 (1), 225 (3), 181 (6), 135 (1), 91 (100), 83 (2), 65 (4), 55 (3).

IR (KBr): ν = 3031 (w), 2924 (s), 2858 (s), 1724 (w), 1589 (w), 1513 (s), 1454 (w), 1428 (m), 1380 (m), 1262 (s), 1138 (m), 1112 (m), 1025 (m), 852 (w), 804 (w), 735 (m), 696 (m), 644 (w), 562 (w).

3.10 Coupling between 2 and the antioxidant molecule Tyr-OH

Product **2** (115 mg, 0.54 mmol) was dissolved in dry DMF [0.2] mM under nitrogen.

Product **6** (320 mg, 0.64 mmol) and K_2CO_3 (146 mg, 106 mmol) were added and the mixture was stirred overnight at 80 °C. After cooling to room temperature, the K_2CO_3 was filtered off and the solvent removed under vacuum to reveal product **8** as slightly yellow oil.

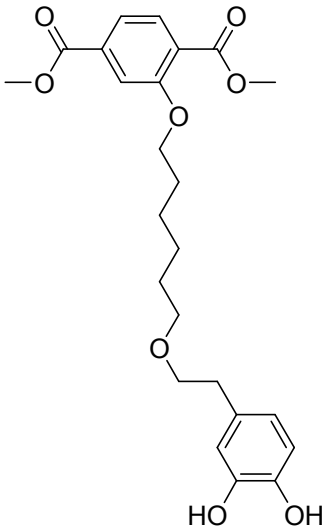


2-{6-[2-(3,4-Bis-benzyloxy-phenyl)-ethoxy]-hexyloxy}-terephthalic acid dimethyl ester

$C_{38}H_{42}O_8$ (629.29). IR (KBr): ν = 2952 (m), 2924 (m), 2855 (w), 1733 (s), 1707 (s), 1615 (w), 1573 (w), 1496 (w), 1438 (s), 1423 (m), 1293 (s), 1238 (s), 1123 (m), 1090 (w), 968 (w), 820 (w), 751 (s), 695 (w).

3.11 General procedure for cleavage of Bn protective groups

Palladium over charcoal (Pd-C) was added to a solution of the product **8** (445 mg- 0.7 mmol) in THF/MeOH (7ml + 7ml) and the mixture was hydrogenated at 1 atm with magnetic stirring. After 24 h at room temperature the catalyst was filtered off and solvent was evaporated in vacuum, yielding the desired compound **9** that was purified by column chromatography.



2-{6-[2-(3,4-Dihydroxy-phenyl)-ethoxy]-hexyloxy}-terephthalic acid dimethyl ester

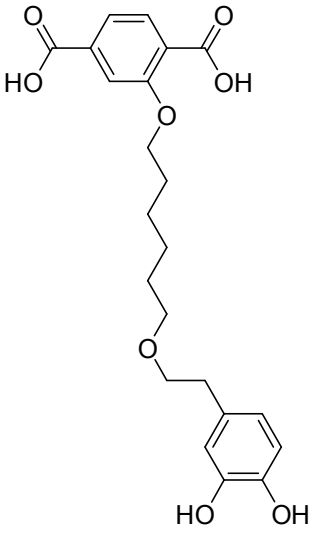
$C_{24}H_{30}O_8$ (446.49). NMR (300 MHz, $CDCl_3$): δ = 7.79 (d, J = 8.4,1 H, H-6 on terephthalic ring), 7.62-7.60 (m, 2H, H-3+H-5 on terephthalic ring), 6.78-6.62 (m, 2 H, H-2+H-5 on hydroxytyrosol ring), 6.61 (dd, J = 8.1, 1.8, 1 H, H-6 on hydroxytyrosol ring), 6.22 (s, br, 2 H, O*H*), 4.04 (t, J = 6.5, 2 H,

OC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCH$_2$CH$_2$), 3.94 (s, 3 H, CO$_2$C*H*$_3$), 3.90 (s, 3 H, CO$_2$C*H*$_3$), 3.75 (t, *J* = 6.5, 2 H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OC*H*$_2$CH$_2$), 3.60 (t, *J* = 6.8, 2 H, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$OCH$_2$CH$_2$), 3.44 (t, *J* = 6.5, 2 H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCH$_2$C*H*$_2$), 1.81-1.76 (m, 2 H, CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$O), 1.61-1.56 (m, 2 H, CH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$O), 1.38-1.26 (m, 4 H, CH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$O); ^{13}C NMR (75 MHz, CDCl$_3$): δ = 166.6, 138.4, 143.8, 142.3, 134.3, 131.3, 121.0, 116.0, 115.1, 114.0, 71.8, 70.8, 52.6, 52.3, 35.7, 29.7, 29.4, 29.0, 25.8, 25.6.

IR (KBr) ν= 3422 (m, b), 2940 (m), 2859 (m), 2368 (w), 1718 (s), 1616 (w), 1570 (w), 1519 (w), 1437 (m), 1391 (w), 1299 (m), 1263 (w), 1227 (w), 1191 (w), 1119 (m), 1084 (m), 792 (w), 751 (s).

3.12 Removal of methyl esters from the modified PET monomer with Tyr-OH

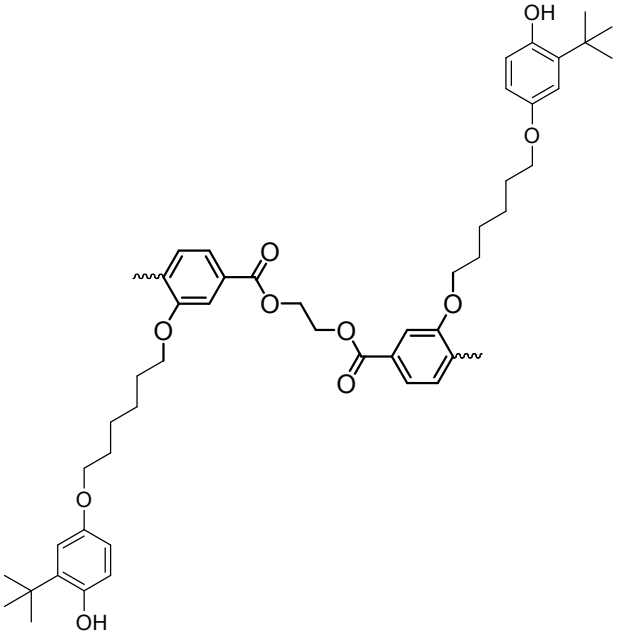
The removal of methyl esters from product **9** was carried out in the same conditions of the removal of methyl esters from product **7** (49) (50). The products **10** was completely characterized by NMR and IR.



$C_{22}H_{26}O_8$ (418.44). IR (KBr) ν= 3416 (w, b), 3069 (w), 2936 (m), 2859 (m), 2654 (w),2583 (w), 1698 (s), 1611 (w), 1570 (w), 1503 (w), 1442 (w), 1309 (s), 1242 (s), 1089 (m), 1023 (w), 930 (w), 874 (w), 756 (s).

3.13 Polymerization of the monomers

A mixture of TsCl (1.2 mmol) and DMF (0.3 mmol) in pyridine [0.6] M, prepared and aged at room temperature for 30 min, was reacted with a pyridine [0.2] M solution of each monomer (0.15 mmol) at room temperature for 10 min and then at 80 °C for 10 min. A pyridine [0.2] M solution of Ethylene Glycole (0.15 mmol) was added to the resulting solution and the mixture was heated at 80 °C for 24h. The polymers **11** and **12** were isolated by diluting the reaction mixture with pyridine and pouring into MeOH. NMR spectra were recorded in $CF_3COOH/CDCl_3$ (1/3, v/v). (52)



IR (KBr) ν= 3432 (s, b), 3135 (w), 2059 (w), 2946 8w), 2869 (w), 1729 (s), 1636 (m), 1493 (m), 1294 (w), 1227 (s), 1171 (s), 1119 (s), 1023 (m), 1012 (m), 818 (w) 680 (m), 578 (m).

3.14 Antioxidant activity of the synthesized monomers

The in vitro antioxidant activity of the synthesized modified PET monomer was determined by evaluating their free radical scavenging activity with respect to DPPH at four different concentrations according to a known protocol (46). Thus, 2 MeOH solutions containing respectively known amounts of **10** and **11** and DPPH, were prepared, and the colorimetric decrease in absorbance of DPPH was measured at 517 nm after a period of 20 min since the preparation.

More specifically, a standard methanolic solutions of **10** (5 mg in 1 ml of MeOH) and DPPH (3.9 mg in 10 ml of MeOH, corresponding to 1 mM) were prepared. The standard methanolic solution of **10** was subsequently diluted to give solutions with concentration respectively of 2.5 mg/ml, 0.5 mg/ml and 0.25 mg/ml. Same thing for sample **11**.

100 μl of the DPPH solution and 100 μl of modified PET monomer solutions were added for each concentration to 2.8 ml of MeOH until a final total volume of 3 ml.

The mixtures were shaken vigorously and allowed to stand at room temperature for 30min.

The absorbance of each sample was then measured against blank (the DPPH solution obtained by diluting 100 μL of the DPPH standard solution with 2.9

ml of MeOH to give a final volume of 3ml) at 517 nm. Experiments were carried out in triplicate, and TBHQ was used as the positive reference antioxidant. The percent of scavenging activity on DPPH wascalculated according to the formula:

$$\text{DPPH scavenging activity (\%)} = 100 \left(A_{blank} - A_{sample} \right) / A_{blank}$$

4 Conclusions

In conclusion we have reported a new method for the synthesis of novel materials with antioxidant activity to be used for active packaging of IV range food and other easily oxidizable foods.

This new material is a modified PET carrying on its side chain an antioxidant moiety which could be TBQ or Tyr-OH.

PART II

Characterization of poly(ε-caprolactone)-b-poly(ethylene glycol) copolymers by mass spectrometry techniques

1 Introduction

1.1 Mass Spectrometry and Polymers

Biodegradable polymers have found increased interest in biomolecular and industrial fields. They break down into simpler components owing to the presence of cleavable bonds within their backbone.

Polyesters are widely used in industry because of their different applications. They are employed in the production of plastics, composite materials, coatings and fibers; because of their biodegradable properties, recently they have also been used for biomedical applications. Their synthesis is straightforward; they are synthesized by condensation reactions between diols and dicarboxylic acids via step growth polymerization. The common acids and diols involved in the generation of this class of materials are ethylene glycol, neopentyl glycol and trimetylol propane for the diols, and phtalic acid isomers, adipic acid, sebacic acid and lactic acid for the dicarboxylic acids. Diverse architectures and end groups arise from these polymerization reactions. (53)

Mass spectrometry can helps to detect oligomers resulting from different architectures and end groups by single-stage mass spectrometry experiments

(MS). Structural composition or oligomeric sequence can then be derived by performing tandem mass spectrometry (MS/MS) experiments.

Single-stage mass spectrometry, also referred to as one-dimensional MS, provides the mass-to-charge ratio (*m/z*) of each polymer component, from which the corresponding mass can be obtained depending on the size of the polymer and the mass accuracy of the instrumentation used. For new or known polymers prepared via established synthetic procedures, the mass information is often sufficient to derive the elemental composition of the product's constituents and predict their structure. If new synthetic concepts are evaluated or the origin of the sample is unknown, mass data alone may not permit unequivocal compositional or structural assignments. In such cases, two-dimensional or tandem mass spectrometry (MS/MS or MS^2) offers a means to collect additional analytical information, so that the problem can be solved. In MS^2, ions of the same *m/z* ratio corresponding to a specific *n*-mer are first isolated and then energetically activated in order to undergo structurally diagnostic fragmentations. Detection and interpretation of the resulting fragment ions allows one to reconstruct the primary structure (connectivity) of the selected *n*-mer. The successive isolation and fragmentation events can take place either in physically separated regions of the mass spectrometer (MS^2 in space) or in the same location at different times

(MS^2 in time), depending on the type of mass spectrometer available. MS^2 in space is performed with beam instruments, while the ions travel from the source to the detector; inversely, MS^2 in time is performed in ion traps (ITs), while the ions are stored in the trapping region. The MS^2 in-space experiment requires the coupling of at least two mass analyzers having a collision cell or different excitation section in between.

The first analyzer is set to transmit only ions of a specific *m/z* ratio. After exiting this analyzer, the selected ions (precursor or parent ions) enter the excitation section, where their internal energy is perturbed by collisions with an inert gas, or other reactive species (for example, ions of opposite charge), by collisions with a surface, or by photons. This process increases the ions' internal energy, so that they undergo unimolecular fragmentations. Consecutively, the newly formed fragments and any residual precursor ions travel to the second mass analyzer, which deconvolutes and transmits them to the detector according to their *m/z* ratio. Instruments performing MS^2 in space may contain quadrupole (Q), time-of-flight (ToF), or IT mass analyzers. An advantage of the trapping instruments, compared to the beam instruments, is that the isolation/fragmentation processes can be iterated on the fragment ions in triple-stage MS experiments (MS/MS/MS or MS^3). Theoretically, *n* stages

(MS^n) can be carried out in the same trapping analyzer, provided a sufficient number of ions remain trapped to yield measurable signals.

Determination of molecular structure by mass spectrometry is analogous to solving a puzzle. A molecule or a mixture of molecules is ionized with sufficient internal energy to break it into several pieces which are then individually and reassembled according to valence rules to determine the structure of the original ion. Thus fragmentation of molecular ions is central to the characterization by tandem mass spectrometry. (54)

Coupling MS to a separation technique such as size exclusion chromatography, gas chromatography, capillary electrophoresis, liquid chromatography, or ion mobility spectrometry provides separation of the compounds before their MS analysis, leading to higher sensitivity and selectivity. Liquid chromatography (LC) is the most applicable technique for separation of nonvolatile mixtures into their components, which are eluted one after another based on their physicochemical and chemical interactions with the mobile phase and the stationary phase. Although LC has high separation power, it is not good at identifying compounds.

On the other hand, MS is suitable for molecular identification, but it is not efficient for the separation of a complex mixture into its components.

Therefore, interfacing both methods creates a very powerful analytical tool with higher sensitivity and specificity than either of the individual methods.

Nowadays, the most two frequently employed MS ionization methods are matrix assisted laser desorption ionization (MALDI) and electrospray ionization (ESI). Since in ESI the analyte flows continuously to the mass spectrometer in the form of liquid solution, this permits coupling with LC.

LC-ESI/MS has become a widely used technique in many types of laboratories for the separation and identification of complex polymeric samples and mixtures.

Reverse phase chromatography is employed to separate the oligomers by adsorption/desorption equilibria. Hydrophobic chains were retained inside the column for longer times than hydrophilic ones which were swept by the mobile phase more easily and thus eluted earlier.

1.2 Dissociation of ions by collisional activation

With the advent of collision-induced dissociation (CID) and tandem mass spectrometry it became important to study collision energy related effects. For a molecule to fragment within the time frame of the experiment, it needs more internal energy than the thermochemical minimum (the activation energy), to

drive the reaction sufficiently fast. This excess energy has been called the "kinetic shift", (55) and this is known to increase with precursor ion mass. For this reason internal energy needs to be increased significantly to fragment large molecules, and this has also been called the DoF effect. When the common CID technique is used, the energy imparted into the molecule can be increased, up to a limit, by increasing the collision energy and/or by increasing the number of collisions.

The degree of fragmentation depends on the activation parameters (energy and entropy), on the molecular size, and on the degree of excitation. In order to study the influence of molecular size on the fragmentation, the activation parameters should be constant; and the degree of fragmentation should be studied as a function of the degree of excitation (which, in turn, depends on the collision energy).

Collisionally activated dissociation (CAD), also known as collision-induced dissociation (CID), is the most common fragmentation method in use nowadays. Activation of the mass-selected precursor ions is effected by accelerating them, so that they undergo energizing collisions with gaseous targets, commonly argon or helium atoms. During the collision, a fraction of the kinetic (translational) energy of the precursor ion is converted into internal energy, which is redistributed rapidly among the rotational–vibrational

degrees of freedom of the ion before fragmentation occurs (ergodic process). As a consequence, the weakest bonds in the precursor ion have a higher predisposition for cleavage. Also, unimolecular rearrangement mechanisms are triggered, because they tend to produce stable fragment ions and, hence, have favorable energy requirements.

The survival yield (SY) is a convenient quantitative measure to describe the efficiency of fragmentation. (56)

The SY is defined according to eq 1:

$$SY = I_M / I_M + \sum I_F \qquad (1)$$

where I_M is the intensity of the ionized molecule (parent ion signal) and $\sum I_F$ is the sum of all fragment ions intensity.

Using SY analysis, the total ion current can be quantified as the sum of surviving precursor ions and newly formed product ions can be related to bond types and relative bond strengths.

The precursor ion mass represents the size of the molecule and is linearly correlated with the number of degrees of freedom. (57) (58)

1.3 Poly(ε-caprolactone) (PCL) and poly(ε-caprolactone)-poly(ethylene glycol) block copolymer (PCL-b-PEG)

In the group of biodegradable (synthetic) polymers, polyesters play the most important part. (59) Many are already available on the market and their degradation has been studied extensively, especially in soil or compost (60). Until a few years ago, these polyesters were of aliphatic nature only. Well known are the bacterial polyesters [e.g., PHB and BIOPOL IOPOL] and synthetic materials [e.g., PCL, polylactic acid (PLA) and BIONOLLE IONOLLE (co-polymer from starch, cellulose, lactic acid and succinic acid)] (61). In the 1990s, a new, somewhat more empirical approach was made to improve the use and the processing properties of aliphatic polymers while maintaining their biodegradability. This led to combinations of biodegradable aliphatic polymers with aromatic polyesters, with the resulting polymers having better properties in both regards.

Since this group of polyesters has biodegradable properties, it has been investigated closely in recent years, with degradation in soil and compost being the main feature of interest. This is mostly due to the market situation for these materials, since they have major applications in packaging and agriculture.

Poly(ε-caprolactone) (PCL) is a biodegradable, hydrophobic and semi-crystalline polyester with a relatively low melting point (60 °C) but a glass transition temperature (Tg) around -60 °C. The crystallinity in the polyester accounts for this property balance. It is made by metal catalyzed ring opening polymerization of epsilon caprolactone. PCL degrades with a slow rate. PCL is widely used as a PVC solid plasticizer or for polyurethane applications, as polyols. But, it finds also some applications based on its biodegradable character in domains such as biomedicine (e.g. drugs controlled release) and environment (e.g. soft compostable packaging).

Errore. Il segnalibro non è definito. Modifications on poly(ε-caprolactone) by adding poly(ethylene glycol) (PEG) increase the degradation rate and gives at the copolymer a better biodegradation.

This kind of polyester-polyether block polymer has a superior amphiphilic property as compared with the parent PCL homopolymer.

The object of this study was the MS characterization of the linear poly(caprolactone)-poly(ethylene glycol) block copolymer (PCL-b-PEG) and the comparison between this and the polycaprolactone (PCL) homopolymer.

Furthermore the PCL-PEG copolymer and the PCL and PEG homopolymers collision energy study was carried out.

The PCL-PEG and PCL degradation in an aqueous moiety was also investigated.

2 Results and discussion

The development of soft ionization techniques such as matrix assisted laser/ionization (MALDI) and electrospray ionization (ESI) has enabled the mass spectrometry characterization of much type of synthetic polymers. These soft ionization methods minimize the fragmentation of large molecules, thereby permitting the observation of intact synthetic polymers. The time of flight (TOF) mass analyzer, which is usually coupled to MALDI, has allowed the detection of the mass to charge ratios (m/z) of large molecules, because its unlimited mass range.

The sample preparation for the MALDI analysis requires a dilute sample solution to be mixed with a more concentrated matrix solution. The matrix is a small organic molecule with absorbance capacity at the laser's wavelength. The large molar excess of matrix not only disperses the sample molecules but also prevents cluster formation and protects the sample molecules from photo-induced decomposition when the laser beam hits the solid solution of the sample in the matrix. A few microliters of the resulting mixture solution of sample and matrix solutions is deposited on a sample plate and allowed to dry so that the matrix crystals encapsulate the analyte molecules. Absorption of

laser energy leads to evaporation and ionization of the matrix creating a gas-phase plume, in which sample molecules are also trapped, thus reaching the gas phase. It is believed that the sample molecules are ionized by gas phase proton transfer reactions with matrix ions. In the case of samples that do not protonate easily, a salt solution is added to the mixture of matrix and sample to promote ionization by metal ion adduction, for example, of Na^+, Li^+ or Ag^+ ions. The nature of the salt is strictly dependent on the particular functional groups present on the molecules under investigation.

2.1 Data Analysis.

Data analysis involves rationalization of the spectra obtained from the various experiments conducted. Several pieces of data can be obtained by evaluating the mass spectra; what monomer or monomers were used, the initiating and terminating groups, existence of un-reacted products, side reactions, etc. When MS/MS is applied, definitive structure information is obtained for the selected oligomer based on the fragmentation pieces observed.

Whenever possible the all ^{12}C peak is selected for MS/MS analysis. Selection of this peak allows the utilization of monoisotopic masses which in turn

provides the most accurate information about the exact molecular weight and composition of the observed species. The difference in parts per million (ppm) between theoretical weight of an assumed structure and the observed weight provides a measure for the confidence that the observed peak corresponds to the structure assumed. Whenever possible, this information is corroborated with information provided with the sample which allows for a high degree of confidence in the interpretation. By calculating the nominal mass difference between two adjacent oligomers, one can determine the repeat mass of the polymer and thus determine the type of polymer or dominant component in a copolymer.

If the all ^{12}C isotopic peak is resolved the following logic is applied to determine the end groups. First, we take the observed mass of the all ^{12}C isotope and subtract the monoisotopic mass of the ionizing cation which gives the mass of the oligomer. Next, we divide that value by the monoisotopic mass of the previously determined monomer repeat unit (see above), to obtain a value representative of the number of potential repeat units and the size of the ends groups. Next, we subtract the whole number, which is less than or equal to the number of monomer repeat units (n), leaving a fraction which in then multiplied by the mass of the monoisotopic repeat unit. This value is the lowest value possible for the mass of the combined ends

groups (plus any in-chain substituents). If this value is not large enough for the expected initiating and terminating groups, we can take monomer units away from the whole number we subtracted previously until a mass value is reached that is representative of the initiating and terminating groups.

For example:

observed *m/z* = 1365.905 Da of a PCL standard $[C_5H_{11}O_2\text{-}(C_6H_{10}O_2)_n\text{-}H]^{Li+}$,

n = ?

1365.905	(Observed all ^{12}C, Li peak)
- 7.01600	(Theoretical monoisotopic value of Li)
1358.889	(Product mass of the all ^{12}C isotope of the oligomer after the removal of the Na cation)
÷ 114.0681	(Calculated monoisotopic value for $C_6H_{10}O_2$)
11.9130	
- 11	(n ≤ 11 for this oligomer)
0.9130	
x 114.06805	
104.1404	(Nominal mass of the combined end groups $C_5H_{11}O_2$ and H)

Nominal mass of $C_5H_{11}O_2$ + H = 104.15 Da

Therefore, we have $[C_5H_{11}O_2\text{-}(C_6H_{10}O_2)_{11}\text{-}H]^{Li+}$

2.2 Characterization of Poly(ε-caprolactone)-b-poly(ethylene glycol) Copolymers by Mass Spectrometry Techniques

My research period abroad utilized MALDI-TOF mass spectrometry to characterize functionalized homopolymers and copolymers. For homopolymers the mass of the monomer unit and combined end groups can usually be determinate from the *m/z* ratios observed in the mass spectrum of the polymer. The characterization of copolymers presents additional challenges, arising from complexities in their structure, composition and molecular weight distribution. Single-stage mass spectrometry (MS) does not provide enough information to determine all structure features of particular types of copolymers. In this study, the characterization of synthetic polyester homopolymers and polyester copolymers was investigated by combined MS and tandem mass spectrometry (MS/MS) techniques.

The goal is to develop MS analysis protocols which provide informations about polyester copolymer composition, individual end groups, sequence and architecture. More specifically the object of this study was the MS characterization of the linear poly(caprolactone)-poly(ethylene glycol) block copolymer (PCL-b-PEG) and the comparison between this and the polycaprolactone (PCL) homopolymer.

Furthermore the PCL-PEG copolymer and the PCL homopolymer collision energy study was carried out.

The PCL-PEG and PCL degradation in an aqueous moiety was also investigated. Since this kind of polymers is used in many fields and for different applications, in particular in biological application, this study was carried out to understand how these polymers degrade and fragment in presence of water.

The MALDI mass spectrum of the PCL-PEG block copolymer shows different polymer distributions, each one having a difference in mass between two consecutive oligomers of either 114 Da or 44 Da, which are the masses of one PCL monomer unit (calculated mass 114.06805 Da) and one PEG monomer unit (calculated mass 44.0262 Da), respectively (figure 1). In order to determine the exact compositions of the n-mers that reconstitute the various polymer distributions, MS/MS experiments have been carried out. MALDI MS/MS spectra show that the main fragments arise from 1,5-H rearrangement along the polyester chain leading to losses of PCL oligomers with the structure $HO[CH_2CH_2CH_2CH_2CH_2COO]_xH$. The resulting fragments carry always the PEG block and its original end group, $-OCH_3$, plus the end resulting from the rearrangement.

The 1,5-H rearrangement is observed at all ester groups of the PCL block; therefore, counting the fragments produced via this mechanism also unveils the length of PCL block. The smallest fragment ion produced by the 1,5-H

rearrangement contains no PCL unit and, thus, identifies the PEG block length (figure 2).

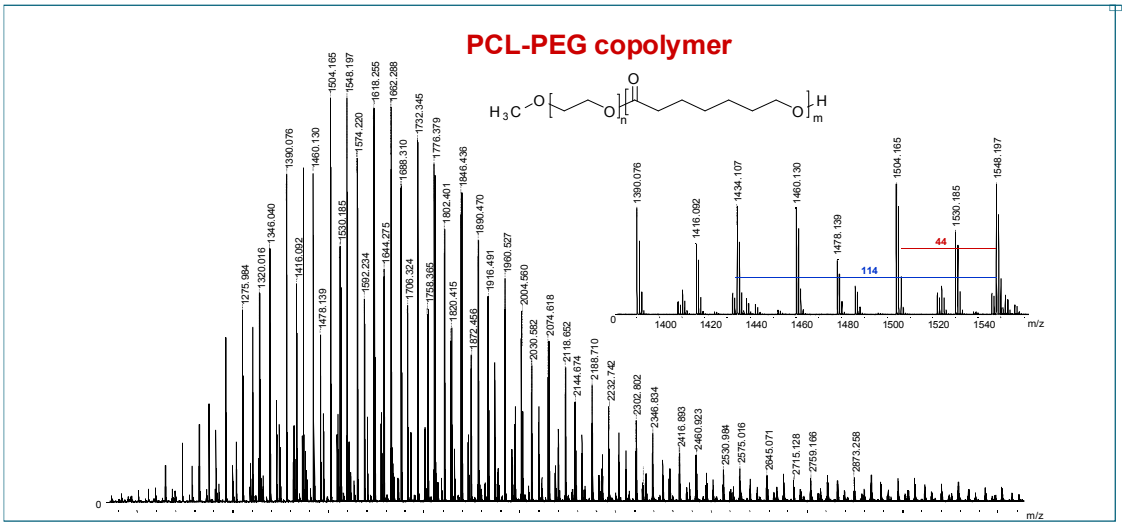


Figure 1. MALDI spectrum of PCL-PEG

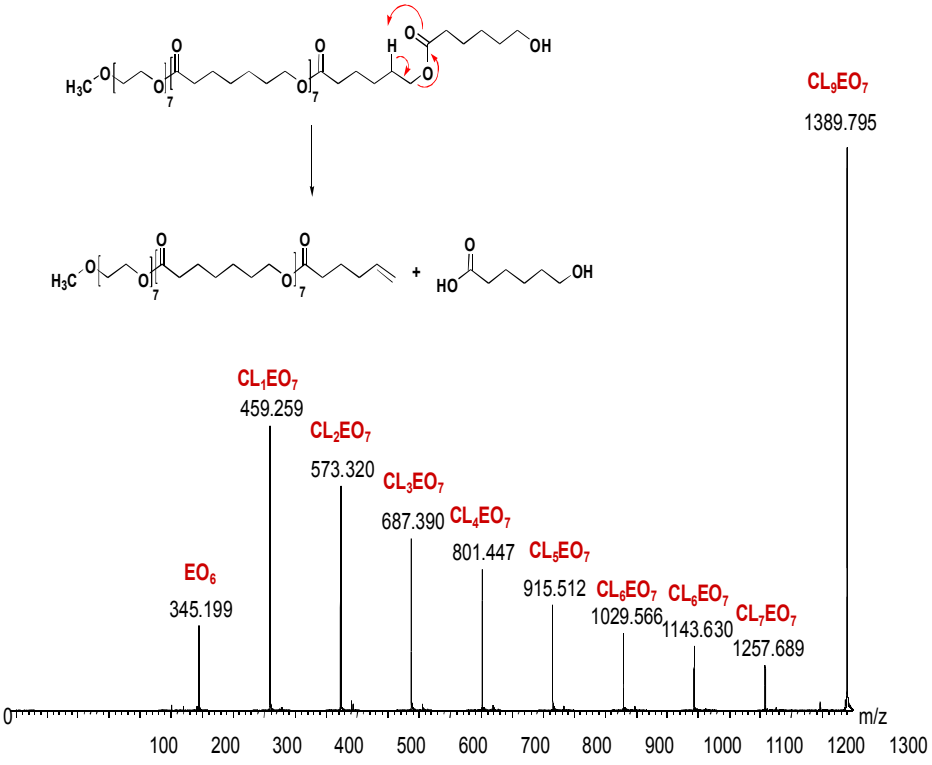


Figure 2. MS/MS spectrum of PCL-PEG copolymer 1389.795 parent

The PCL MALDI spectrum in figure 3 shows the monomers repetition. The MS/MS of the 11-mer, shown in figure 4, explains better how the polymer fragment by 1,5 H rearrangement. Then, as was expected, the 1,5-H rearrangement dominates also with the PCL homopolymer. With either sample, consecutive fragmentations via the same mechanism lead to the same fragments as the one step processes.

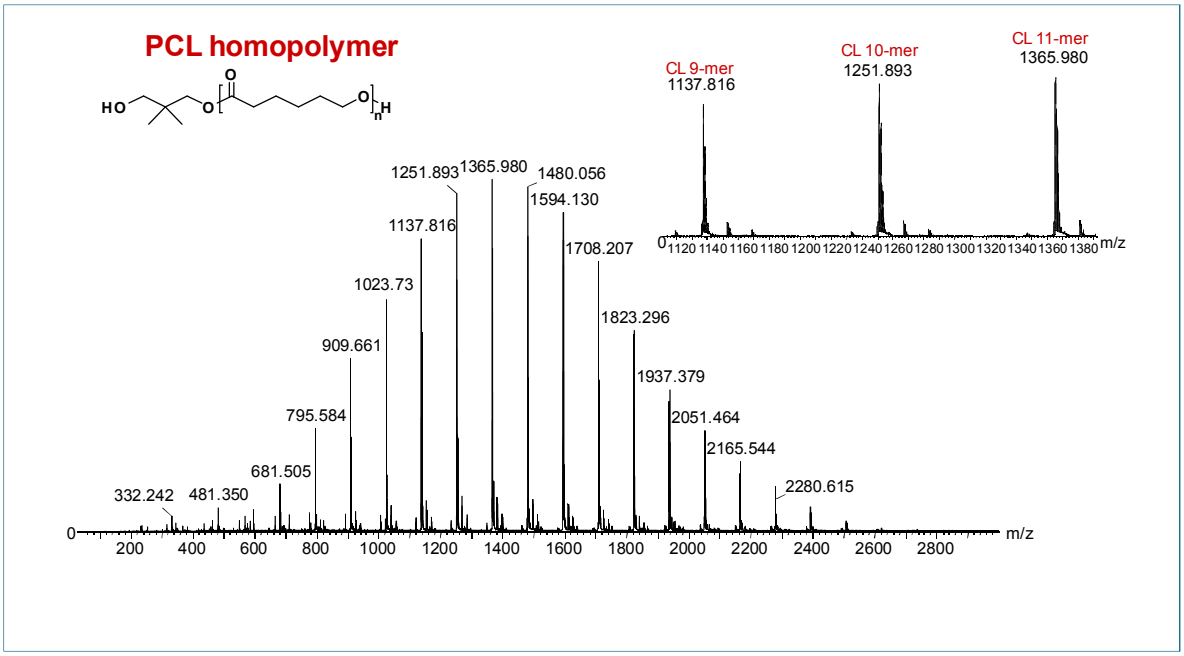


Figure 3. MALDI spectrum of PCL homopolymer

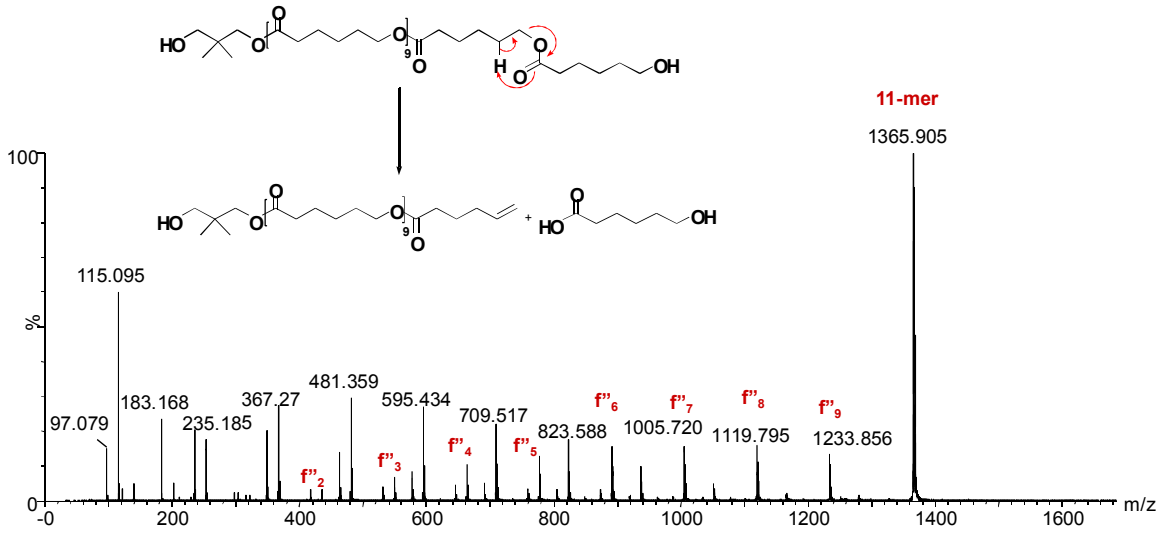


Figure 4. MS/MS spectrum of PCL homopolymer 1365.905 parent

A detailed nomenclature scheme which describes the fragmentation of polyesters was previously described by Wesdemiotis et al. (62)

To better understand the polyester-polyether fragmentation pathway the PCL-PEG copolymer, PCL homopolymer and PEG homopolymer were fragmented under different and increasing collision energies in order to probe the effects of variable collision energies and to study the relative abundances of the MH^+ precursor ion and its fragment ions.

Figures 5, 6, and 7 evidence that the survival yield (SY) curves are shifted to higher excitation energy when the number of monomeric units is increased.

The results indicate that PEG requires really high energy to fragment comparing with PCL homopolymer and PCL-PEG copolymer.

Using SY analysis, the total ion current can be quantified as the sum of surviving precursor ions and newly formed product ions can be related to bond types and relative bond strengths. The precursor ion mass represents the size of the molecule and it is linearly correlated with the number of degrees of freedom. (57) (58)

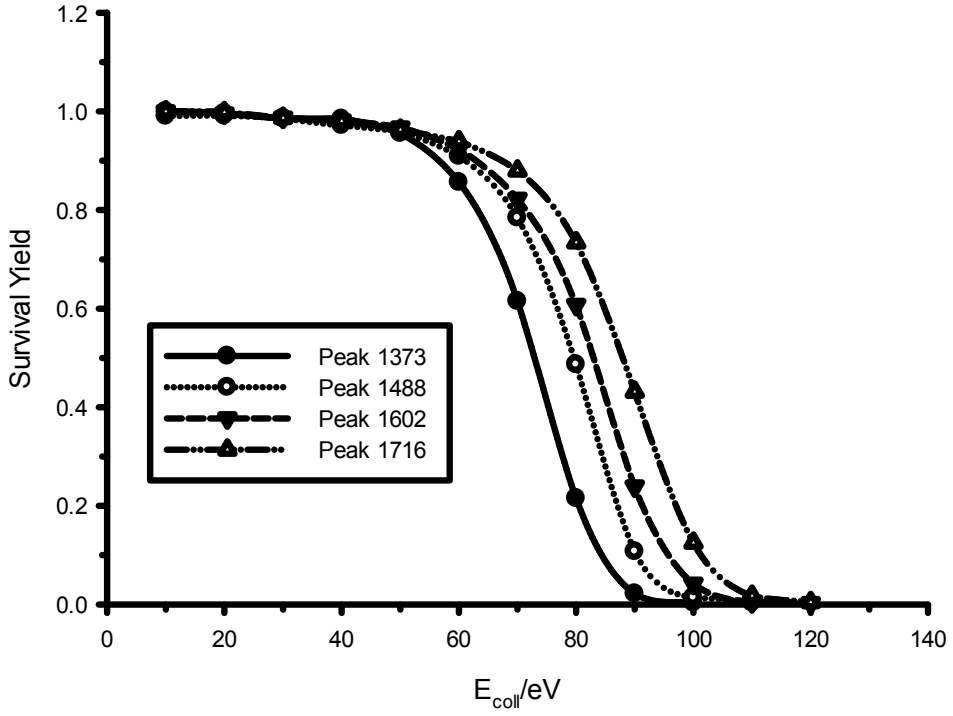


Figure 5. PCL-PEG E_{coll}/SY

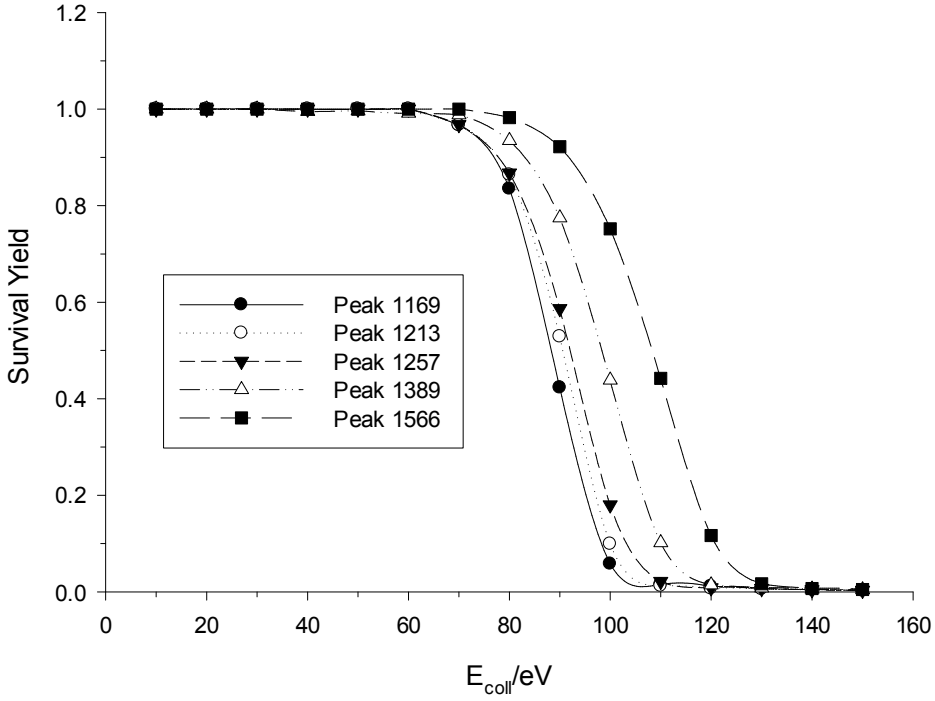


Figure 6. PEG E_{coll}/SY

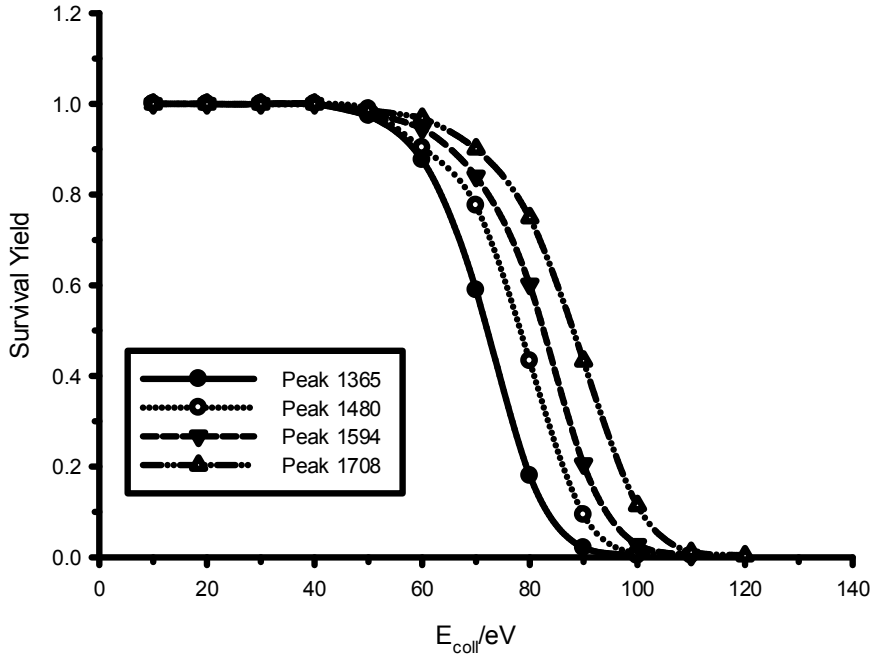


Figure 7. PCL E_{coll}/SY

As the molecules get larger, there are an increased number of vibrational modes where energy can be stored that do not lead to decomposition. Hence, the energy transferred during one collision decreases with the ion size.

In mass spectrometric experiments, the relevant energetic quantity to consider is the center-of-mass collision energy (E_{com}) which is the the maximum energy transferred during a single collision

$$E_{com} = E_{lab} * M_g / (M_i + M_g)$$

where E_{lab} is the laboratory frame collision energy, M_g is the molecular weight of the collision gas and M_i is the precursor ion mass.

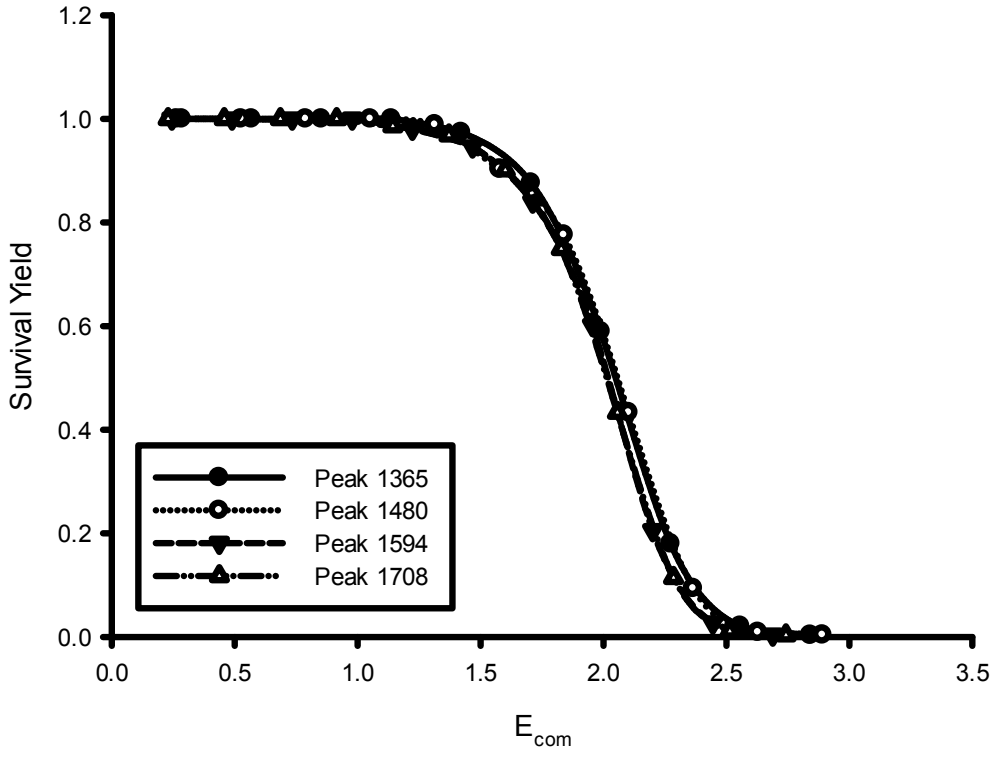


Figure 8. PCL homopolymer E_{com}/SY

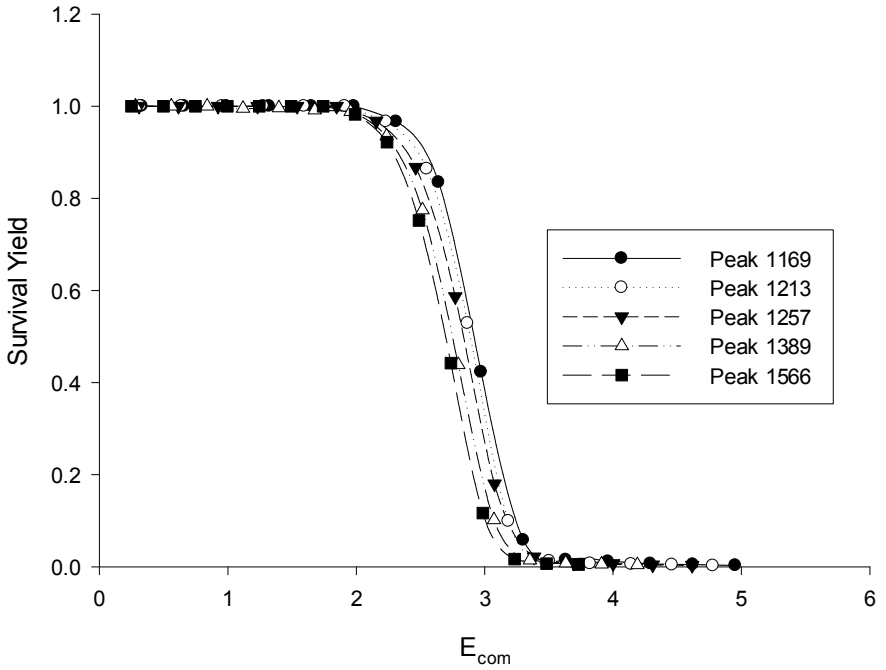


Figure 9. PEG homopolymer E_{com}/SY

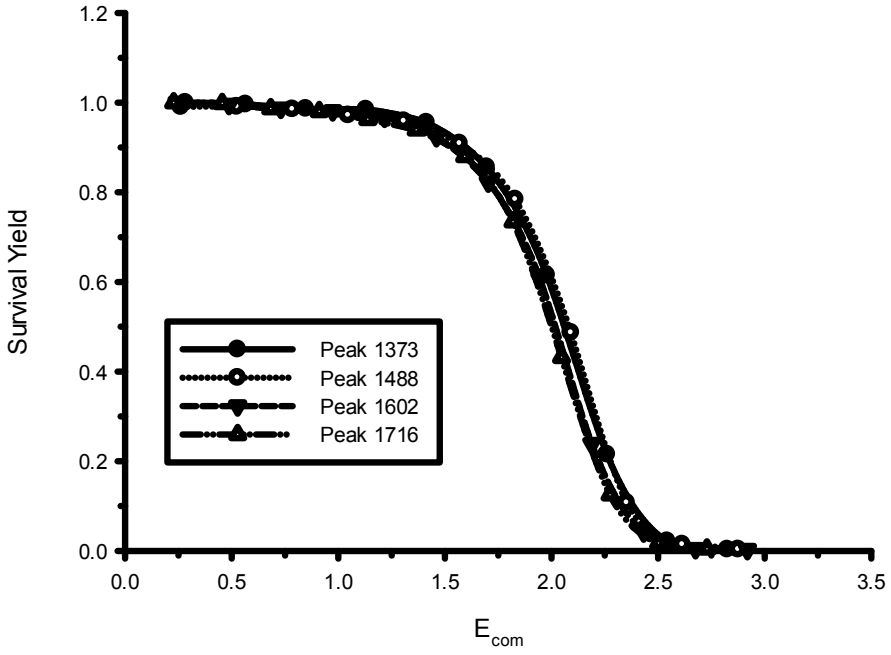
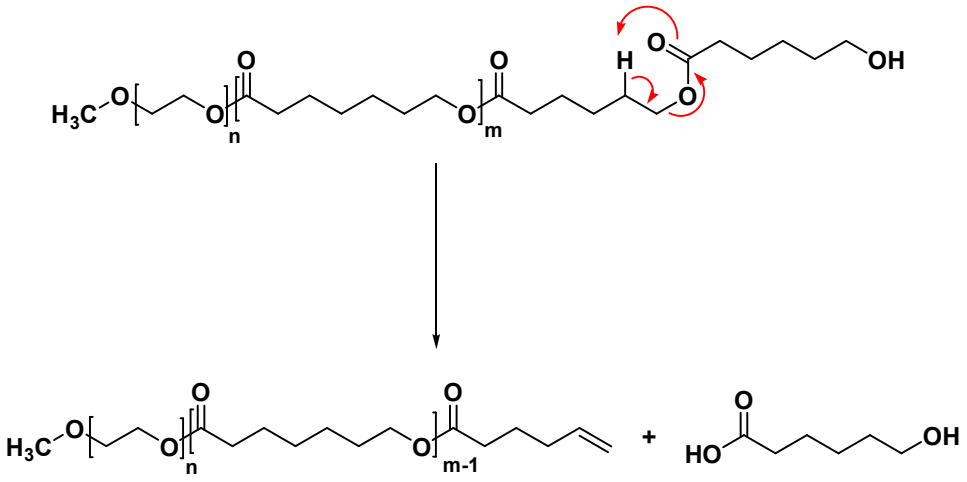


Figure 10. PCL-PEG copolymer E_{com}/SY

We can see from the curves that the homopolymer PCL need lower collision energy to fragment comparing with PEG homopolymer. This could be explained by the nature of the polymer in which the presence of the ester moiety allow the molecule to fragment easily by 1,5-H re-arrangements (1,5-rH). In this kind of polymers is preponderant the charge remote cleavage. In PEG, on the other hand, is preponderant the charge induced cleavage even though it isn't the only one. (62)

For the copolymer PCL-PEG the E_{com} is lower than PEG homopolymer and higher then PCL homopolymer. From the fragmentation study on the copolymer it's clear that PCL-PEG fragments mainly by 1,5 H rearrangement along the polyester chain (scheme 1) but the presence of PEG increase E_{com} because of its tendency to fragment both by charge induced and charge remote cleavage.



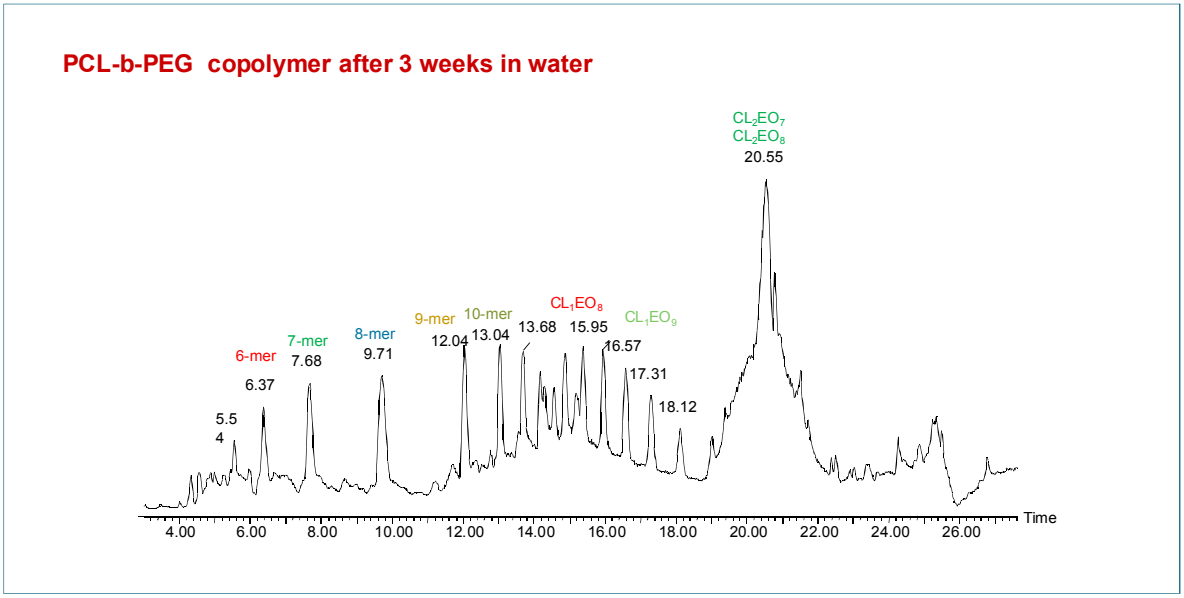
Scheme 1. 1,5-H rearrangement in PCL-PEG

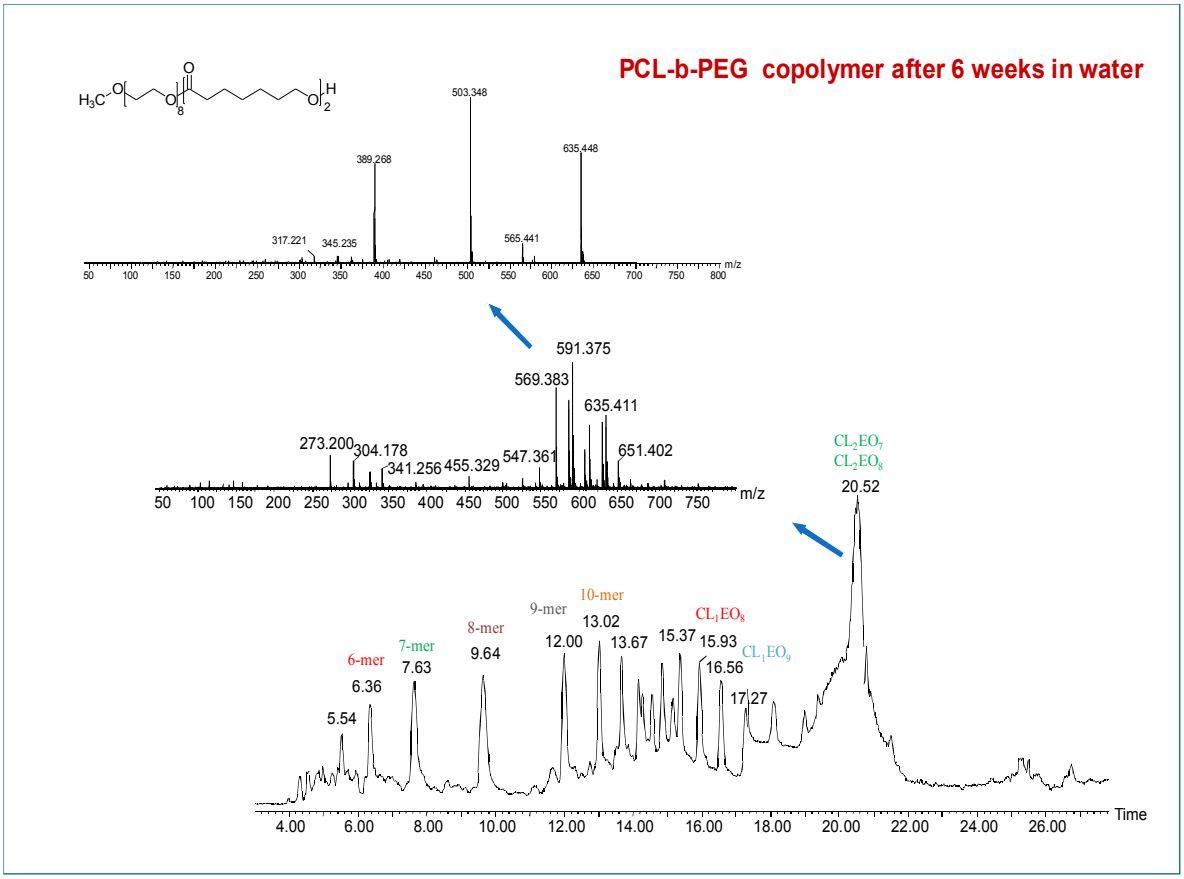
2.3 Degradation study

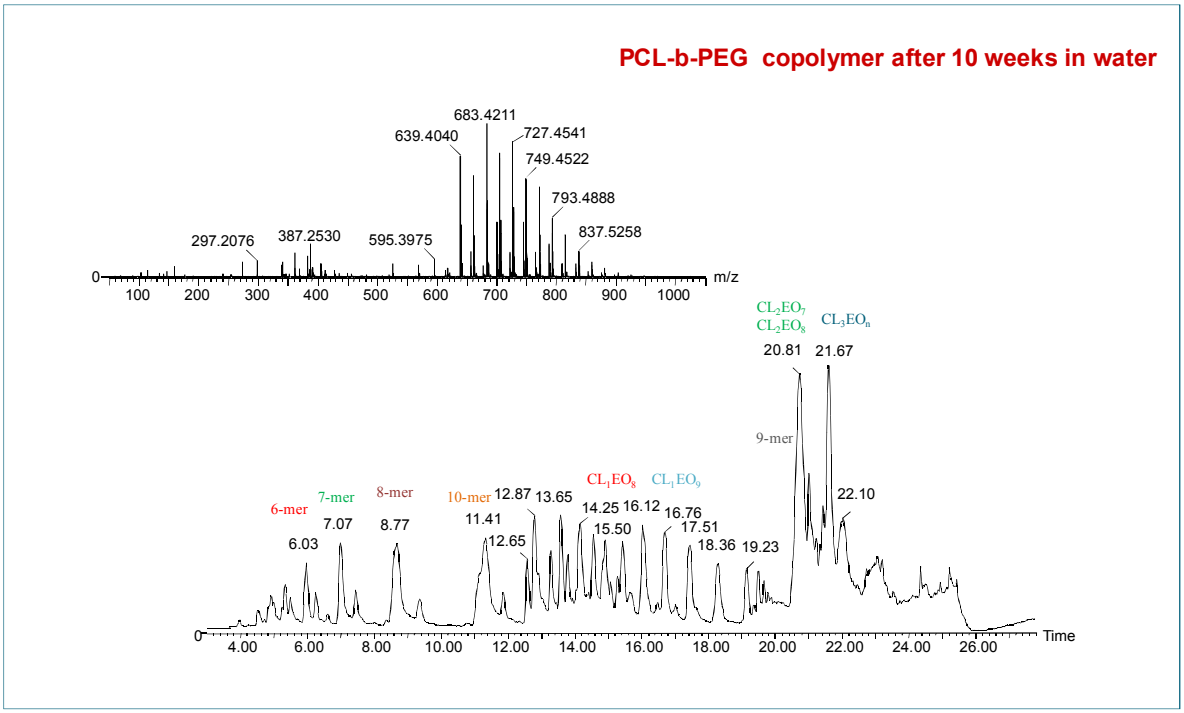
The degradation study of PCL-PEG copolymer and PCL homopolymer was carried out storing the samples in pure water for an established time. This study confirms that the form of degraded material depends on the chain composition and on the PEG/PCL ratio. A high number of PEG sequences considerably increases the hydrophilicity of the copolymer as compared with PCL homopolymer.

All PEG/PCL samples were from a commercial PEG(350)-PCL(1200) copolymer and with a PEG/PCL ratio of 0,75.

UPLC/ESI/qToF analyzer was used to determine the release of oligomers from the polymer in the water medium.




PCL-b-PEG copolymer after 6 weeks in water
503.348
389.268
635.448
317.221
345.235
565.441
m/z
591.375
569.383
635.411
273.200
304.178
341.256
455.329
547.361
651.402
m/z
CL2EO7
CL2EO8
20.52
10-mer
9-mer
13.02
8-mer
12.00
13.67
15.37
15.93
CL1EO8
CL1EO9
16.56
17.27
6-mer
7-mer
7.63
9.64
6.36
5.54
Time


PCL-b-PEG copolymer after 10 weeks in water
683.4211
639.4040
727.4541
749.4522
793.4888
837.5258
297.2076
387.2530
595.3975
m/z
CL2EO7
CL2EO8
CL3EO8
20.81
21.67
9-mer
22.10
CL1EO8
CL1EO9
10-mer
12.87
13.65
14.25
16.12
16.76
17.51
18.36
19.23
11.41
12.65
15.50
6-mer
7-mer
8-mer
7.07
8.77
6.03
Time

Possible reason for degradation is the chemical hydrolysis of the ester groups of PCL due to the aqueous solution. Furthermore the presence in the copolymer of hydrophilic PEG segments is responsible to attract hydroxyl groups from the environment causing hydrolysis. (63)

Due to the high hydrophilicity of PEG, water fraction should be rich in PEG oligomers.

The water fraction is supposed containing the PEG portion of the copolymer that is the hydrophilic moiety. The degradation study of the copolymer in water shows that the presence of PEG in the copolymer increases the rate of degradation. UPLC analysis demonstrates that week by week the copolymer degrades by releasing at first PEG monomers into the aqueous medium and then by losing slowly CL monomers.

The study of the aqueous medium shows that until 6 weeks the degradation of the copolymer involves mainly the loose of PEG monomers and it releases only few monomers of PCL. Only after 12 weeks the sample starts to release more monomers of caprolactone and this confirms that is the presence of PEG that heads the degradation.

3 Experimental section

3.1 Mass Spectrometry Sample Preparation

PEG-PCL, PCL and PEG samples were commercially available.

PCL-PEG copolymer was from a commercial PEG(350)-PCL(1200) copolymer with a PEG/PCL ratio of 0,75.

Trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]-malononitrile (DCTB) was used as matrix for the preparation of MALDI samples.

Two cationizing salts were utilized; sodium trifluoroacetate (NaTFA) and lithium trifluoroacetate (LiTFA). Selection of salt was based on the experiments were to run. Sometimes spectra were acquired using both NaTFA and LiTFA in order to compare them. LiTFA was utilized for ToF/ToF due to its higher binding affinity versus NaTFA and the ability to perform both MS and MS/MS experiments on the Ultraflex without the need for further sample preparation. All materials were dissolved in tetrahydrofuran (THF).

Materials were used as purchased with no modifications. For the matrix, 20 mg/mL solutions were prepared, and for the salt and the samples, 10 mg/mL solutions were prepared. These solutions were mixed in a 10:2:1 ratio

(matrix:sample:salt). For the Q-ToF and Ultraflex ToF/ToF experiments ~ 0.5μL of solution was deposited per target spot. A Bruker Ultraflex III ToF/ToF and a Waters Ultima Q/ToF mass spectrometer were used to analyze homo-PCL, homo-PEG as well as PCL-PEG block copolymer.

To analyze the water fractions of PCL-PEG degradation in water an Acquity UPLC system manufactured by Waters, equipped with a binary pump, auto sampler, and degasser was used. The Acquity UPLC system was coupled with the Synapt HDMS Q/ToF mass spectrometer.

The Waters Acquity UPLC BEH C-18 1.7 μm 1.0x150 mm column with pore size of 130 Å was used in this study. The flow rate was 0.25 ml/min with a linear gradient of water w/ 0.1 % of formic acid and acetonitrile mixture running from 0% to 100% acetonitrile in 30 min.

3.2 Hydrolysis

The different networks were exposed to hydrolytic degradation in pure water at 37 °C. Approximately 20 mg of polymer sample and 10 mL deionized water was added to 20-mL glass vials which were subsequently sealed with septa. The sample vials were placed in a thermostatically controlled incubator with temperature set to 37 °C. Samples were withdrawn from the test medium after

1 day, 7, 21, 49, 70 days of hydrolysis, and were centrifuged in order to separate the insoluble solid fraction from the aqueous surnatant. The different networks were analyzed after each degradation time. (64) (65)

4 Conclusions

Tandem mass spectrometry (MS/MS) investigation was carried on biodegradable amphiphilic copolyesters, containing poly(ethylene oxide) and poly(carpolactone) blocks. The results provided strong evidence that MS/MS can reveal the identity of the comonomers and copolymer block lengths.

In tandem mass spectrometry, structural information is obtained by inducing ionized polymers to undergo unimolecular fragmentation in the gas phase.

The MALDI MS and MALDI MS/MS show that PCL homopolymer and PCL-PEG copolymer fragment mainly by 1,5-H rearrangement along the polyester chain. Furthermore the degradation study of the same samples shows that the presence of the amphiphilic PEG in the copolymer considerably increases the degradation when it is in an aqueous environment.

Appendix

1. List of publications:

   - Synthesis and antioxidant activity of modified PET monomers (in preparation)
   - Characterization of poly(ε-caprolactone)-b-poly(ethylene glycol) copolymers by mass spectrometry techniques (in preparation)
   - Degradation study of poly(ε-caprolactone)-b-poly(ethylene glycol) copolymers in water by UPLC/ESI/MS (in preparation)
   - *In vitro* antioxidant activity of extracts of Sybaris liquorice roots from Southern Italy – Gabriele B., Fazio A., Carchedi M., Plastina P., *Natural Product Research*, **2012**, 1-6, iFirst

2. Participation in PhD Schools and Conferences

   - American Society Mass Spectrometry International Conference, Minneapolis, MN- USA, 9-13 giugno 2013
   - 16° Corso di Spettrometria di Massa, Certosa di Pontignano, Siena, 19-23 marzo 2012
   - International Congress Alimed 2011, Palermo, 22-27 maggio 2011

3. Scientific contribution in Conferences

- Carchedi M., Wesdemiotis C., Gabriele B., Fazio A., Sindona G., "Characterization of poly(ε-caprolactone)-b-poly(ethylene glycol) copolymers by mass spectrometry techniques". ASMS Conference, Minneapolis, MN – USA, 9-13 giugno 2013
- Carchedi M., Fazio A., Gabriele B., Plastina P., "Antioxidant activity of extracts from Sybaris licorice roots". International congress Alimed 2011, 22-27 maggio 2011.

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