Synthesis, Characterization and Biodegradation of Poly(α-Methyl β-Propiolactone)

Poli(α-Metil β-Propiolakton)'un Sentezi, Karakterizasyonu ve Biyobozunurluğu

Research Article

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ABSTRACT

Poly(α-methyl β-propiolactone) (PmPL) was synthesized through anionic ring-opening polymerization of the corresponding lactone. Structural characterization of PmPL was performed by using FTIR, ^{1}H-NMR and MALDI-MS spectrometry. Thermal behavior of the polymer was investigated by using DSC and TGA. Glass transition, melting and thermal degradation temperatures of PmPL were determined as -36.7, 95.5 and 242.4 °C, respectively. Biodegradation studies were conducted at 37 °C in three different media, that is, pH 7.4 phosphate buffered saline (PBS), PBS containing pancreatic trypsin and PBS containing pancreatic lipase. The biodegradation kinetics and mechanism of the polymer were followed by gravimetric measurements and LC-MS analysis. Up to 65% mass loss in 5-week period was recorded for the buffer media in the presence of lipase and trypsin. LC-MS analysis of the residues in the media revealed that biodegradation undergoes mainly through ester bond hydrolysis.

Key Words
polyhydroxyalkanoates, ring-opening polymerization, biodegradation, aliphatic polyesters.

ÖZ

Poli(α-metil β-propiyolakton) (PmPL) karşılık gelen laktonun anyonik halka açılması polimerizasyonu ile sentezlendi. PmPL'un yapısal karakterizasyonu FTIR, ^{1}H-NMR ve MALDI-MS spektrometri ile gerçekleştirildi. Polimerin termal davranışı DSC ve TGA ile incelendi. Camsı geçiş, erime ve termal bozunma sıcaklıkları sırasıyla -36.7, 95.5 ve 242.4 °C olarak belirlendi. Biyobozunma çalışmaları 37 °C de pH 7.4 fosfat tamponu (PBS), pancreatic tripsin içeren PBS ve pankreatik lipaz içeren PBS gibi üç farklı ortamda yürütüldü. Polimerin bozunma kinetiği ve mekanizması gravimetrik ölçümler ve LC-MS analizi ile takip edildi. Lipaz ve tripsin içeren tampon ortamlarında, beş haftalık peryotta % 65'e varan kütle kayıpları kaydedildi. Ortamlardan alınan kalıntıların LC-MS analizi ile biyobozunmanın büyük oranda ester bağının hidrolizi üzerinden yürüdüğü belirlendi.

Anahtar Kelimeler
polihidroksialkanoatlar, halka açılması polimerizasyonu, biyobozunma, alifatik poliesterler.

Article History: Received: Feb 28, 2018; Revised: May 28, 2018; Accepted: Jul 6, 2018; Available Online: Oct 4, 2018.

DOI: 10.15671/HJBC.2018.251

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INTRODUCTION

Many polymers have been used as disposable products, assistant materials in surgery and temporary or permanent prostheses in medical applications. However, polymers used in direct contact with living cells of human body are called polymeric biomaterials. An important class in polymeric biomaterials is biodegradable polymers that have been widely used as absorbable sutures, bone fixation aids, carriers in gene therapy, scaffolds in tissue engineering and matrices for controlled drug delivery. Biodegradable synthetic polymers have attracted attention for various medical applications for several decades [1-3], because physical and chemical properties of final product can be improved by changing the parameters like polymerization condition, type(s) and ratio of monomer(s). Novel biodegradable polymers are still needed for especially the applications as tissue engineering and gene therapy.

Polyhydroxyalkanoates (PHAs) are aliphatic biopolyesters, synthesized and stored within cells as energy storage materials by many microorganisms [4,5]. Polyhydroxyalkanoats (PHAs) are most widely known biodegradable [6,7], biocompatible [8,9] and bioresorbable [10] polymers used as biodegradable biomaterials. The biodegradation of PHAs takes place either under anaerobic or aerobic conditions [11,12] Rate and extent of their biodegradation depend upon many factors such as molecular weight, crystallinity, chemical composition, environmental conditions and activity of degrading agent [13].

As shown in Figure 1, poly(α-methyl β-propiolactone) (PmPL) is structurally similar to both poly(hydroxybutyrate) (PHB) and poly(lactic acid) (PLA) considering that being an aliphatic poly(beta-ester) as in PHB and having a methyl group at alpha position as in PLA. PmPL draw attention as a new potential biomaterial due to the structural similarity. Although the similarity has been mentioned in the literature [14], there has been no study on its application as biomaterials. This study was performed to reveal its biodegradability. Although hydrolytically degradable polymers are generally preferred as implant materials and matrices in drug delivery systems, enzymatic degradation is generally required for scaffolds in tissue engineering to replace extracellular matrix. That's why the *in vitro* degradation study of PmPL was carried out in buffer medium with (or without) enzyme.

MATERIALS and METHODS

Materials

Methacrylic acid (Sigma 99%), HBr in acetic acid (Sigma-Aldrich 33 % w/w), anhydrous sodium carbonate (Sigma-Aldrich 99.9), chloroform (Sigma-Aldrich 99.5%), $(C_4H_9)_4NBr$ (Sigma-Aldrich 98%), dibenzo-18-crown-6-eter (Aldrich 98%), potassium acetate (Sigma 99%), THF (Sigma-Aldrich 99.9%), PBS tablets (Sigma), sodium azide (Sigma-Aldrich 99.5%), Trypsin from porcine pancreas (Sigma, 100-200 BAEE units/mg solid) and Lipase from Porcine Pancreas (Sigma type II, 100-500 units/mg protein) were obtained commercially. THF was dried on sodium and distilled before use.

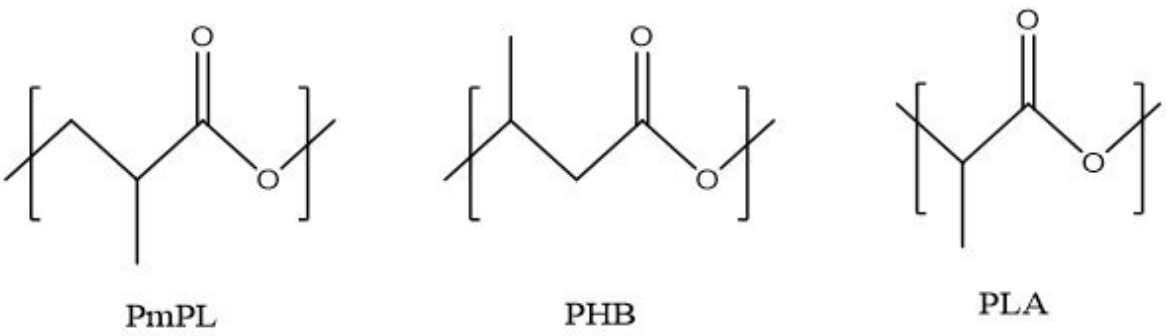


Figure 1. Chemical structure of poly(α-methyl β-propiolactone) (PmPL), poly(hydroxybutyrate) (PHB) and poly(lactic acid) (PLA), respectively.

Synthesis and Measurement

Synthesis of PmPL was carried out as in the literature [14,15]. Chemical structure, molar mass distribution and thermal properties of PmPL were determined using FTIR, ^{1}H-NMR, MALDI-MS, DSC and TGA techniques. FTIR spectra were recorded with Shimadzu IRaffinity 1 spectrometer in the range of 750-4000 cm^{-1}. ^{1}H-NMR spectra of mPL and PmPL in $CDCl_3$ were obtained using by Bruker AVANCE III 400 MHz NMR Spectrometer. Thermal analysis was performed using TA Instruments Q2000 and Q600 at a heating rate of 10◦ C/min in the nitrogen atmosphere. MALDI mass spectra were acquired on a Voyager-DE™ PRO MALDI-TOF mass spectrometer (Applied Biosystems, USA) equipped with a nitrogen UV-Laser operating at 337 nm. Spectra were recorded in positive ion and linear mode with average of 500 shots. MALDI matrix, 2,5-dihydroxybenzoic acid (DHB) was prepared in THF:ACN mixture (1:1, v/v) at a concentration of 20 mg/mL. The solution of cationization reagent, LiTFA (10mg/mL in THF), was added to the matrix solution (1.0 % (v/v) of the total solution). All samples were dissolved in THF and initially spotted (1.0 μL) on MALDI target. After air-drying, the matrix solution (1.0 μL) was deposited on each sample spot. Sample spots were finally allowed to air-dry prior to the MALDI-MS analyses.

Biodegradation

Solution-cast films of PmPL were prepared using 20% (w/v) polymer/chloroform solutions. Films were dried at room temperature under 0.1 mmHg vacuum for 16h. For hydrolytic degradation study, a set of polymer films was immersed in phosphate buffer saline (PBS) solutions (pH=7.4) containing NaN_3 as antimicrobial agent. Two sets of films were immersed into the previously defined solutions containing pancreatic trypsin or pancreatic lipase for investigation of enzymatic biodegradation. Biodegradation study was performed by a shaker operating with 90 rpm frequency at 37 °C for 5 weeks. Samples were removed from the degradation media at certain time intervals, washed with deionized water, dried at ambient temperature under vacuum, and weighed. Determination of polymeric fragments released into the degradation media was conducted via LC-MS (The Thermo Scientific™ TSQ Quantum™ Access MAX LC-MS) analysis to reveal degradation mechanism. Flow rate was adjusted as 0.3 mL/min and methanol was used as mobile phase.

RESULTS AND DISCUSSION

Synthesis and Structural Characterization of PmPL

PmPL was synthesized according to the literature [14,15] and the route was outlined in Figure 2. Although the bromination step resulted in about 90% yield, the ring-closure reaction (intramolecular addition reaction) yielded to 15% of mPL possibly due to the intermolecular addition reaction. PmPL was obtained with high yield (about 98 %), and purified via precipitation by using diethyl ether.

FTIR spectra of α-methyl β-bromo propionic acid (Br-PA), α-methyl β-propiolactone (mPL) and poly(α-methyl β-propiolactone) (PmPL) were given in the Figure 3. The bands at 1705, 1817 and 1732 cm^{-1} belong to the carbonyl (C=O) stretching vibrations of the corresponding compound. The band at 1705 cm^{-1} belongs to the vibration of carbonyl of carboxylic acid. The wavenumber of carbonyl stretching in lactones may shift over 1800 cm^{-1} depending on the ring size. The band at about 1817 cm^{-1} is due to the carbonyl vibration in lactone ring, which shows the success of ring-closure reaction. FTIR spectrum of PmPL has a strong band at 1732 cm^{-1} showing the aliphatic ester carbonyl.

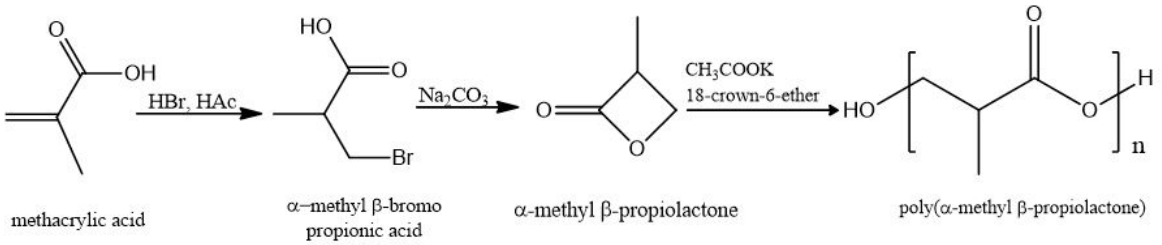


Figure 2. Synthesis route of PmPL.

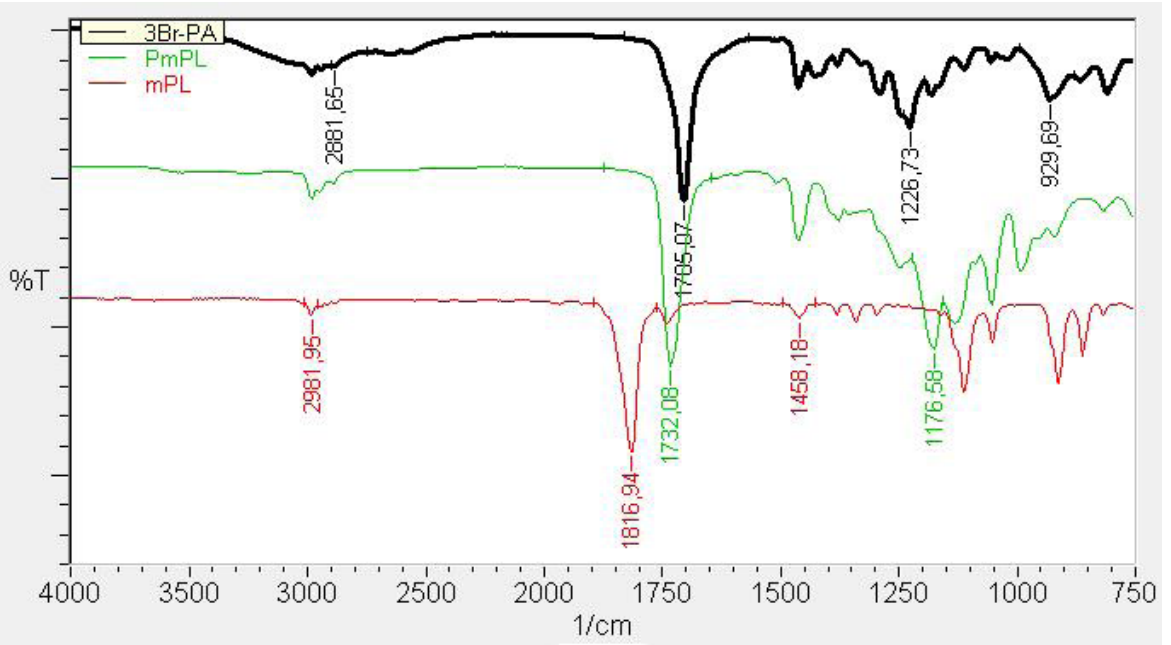


Figure 3. FTIR spectra of Br-PA, mPL and PmPL.

Structural characterization of the prepared compounds was additionally conducted by using ^{1}H-NMR spectroscopy not only to make sure the success of the synthesis but also to have information about their purities. Figure 4 gives ^{1}H-NMR spectra of Br-PA, mPL and PmPL. The signals at 1.13, 2.81 and 3.58 ppm with the intensity of 3:1:2 belong to the methyl (CH_3), methyne (CH) and methylene (CH_2) protons in the Br-PA. Moreover, the broad signal at 12.5 ppm shows the carboxylic acid proton. ^{1}H-NMR spectrum of mPL has four independent signals. The signals at 1.42 and 3.77 ppm with relative intensities of 3:1 were attributed to methyl (CH_3) and methyne (CH) protons, respectively. The triplet signals at 3.96 and 4.41 ppm belong to magnetically inequivalent methylene (CH_2) protons. The results prove the formation of mPL. The signals at 1.29, 2.79 and 4.21 ppm with the intensities of 3:1:2 were attributed to the methyl (CH_3), methyne (CH) and methylene (CH_2) protons, respectively.

MALDI-MS spectrum of PmPL was given in Figure 5. Average molar mass of the PmPL was determined as about 1700 gmol^{-1} using MALDI-MS spectrometry. Since lithium salt of trifluoroacetic acid was used as cationization agent, lithium adduct was found in each signal. The mass differences between the signals were measured as 86 Da and consistent with the structure. Two types of signals with 18 Da differences were mainly observed. The signals 1743.57, 1829.52, 1915.46 and 2001.39 were attributed to the structure I in Figure 6. The signals obey the equation M_n=(86*n)+7. n is the number of repeating unit.

The signals 1761.56, 1847.51 and 1933.44 Da were attributed to the structure II in Figure 6. The signals obey the equation M_n=(86*n)+7+18. The structures I and II are consistent with the initiation mechanisms suggested by Lecomte et al. [16].

Thermal Characterization of PmPL

Differential scanning calorimeter (DSC) and thermogravimetry (TG) curves of PmPL in Figure 7 were recorded under the same experimental conditions (nitrogen atmosphere and heating rate of 10 °C/min) to interpret the thermal transitions comparatively. Characteristic glass transition shift occurred at -36.7 °C and weak endothermic band at about 95.5 °C was attributed to either melting phenomena or evaporation of possible moisture in the sample. Strong endothermic band centered at 242.4 °C belongs to thermal decomposition, which is consistent with the results in the TG curve. Onset temperature of thermal decomposition in the TG curve was determined as 162 °C. Thermal decomposition completed at 284 °C.

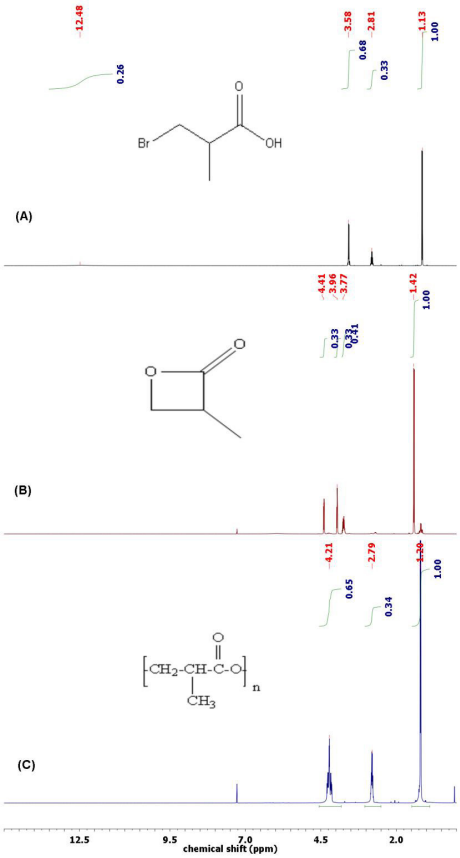


Figure 4. ^{1}H-NMR spectra of of Br-PA (A), mPL (B) and PmPL (C).

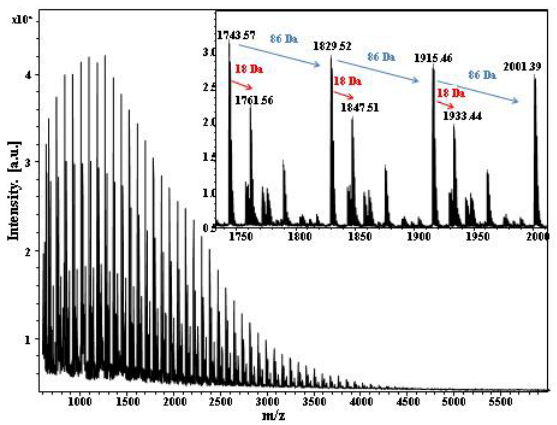
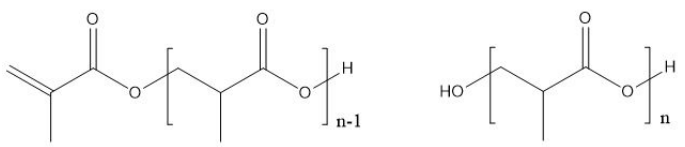


Figure 5. MALDI mass spectrum of poly(α-methyl β-propiolactone) (PmPL).



Structure I Structure II

Figure 6. Chemical structures of PmPL with different end-groups.

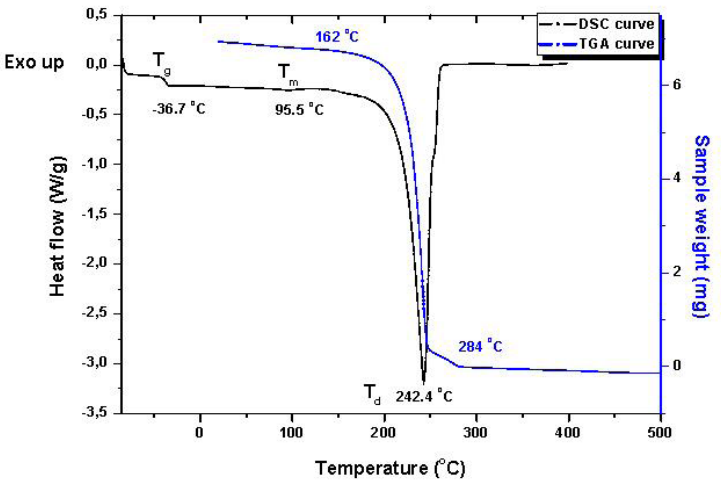


Figure 7. DSC and TG curves of PmPL.

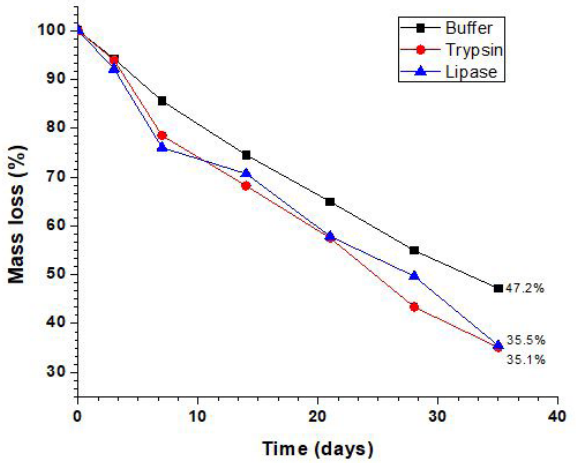


Figure 8. Time dependence of mass loss for PmPL samples in the buffer, trypsin and porcine pancreatic lipase.

Hydrolytic and Enzymatic Degradation of PmPL

Hydrolytic degradation of solution-cast films was carried out in PBS solution. Enzymatic degradation study was carried out in PBS solution in the existence of pancreatic trypsin or pancreatic lipase. Films immersed into the buffer solutions were shaken (90 rpm) at constant temperature of 37 °C for 5 weeks. The films were then removed from the solutions at definite time intervals, dried and weighed. Figure 8 shows time-dependencies of percent sample weights in the three different media.

For the samples in PBS solution, rate of mass loss decreases with time. For instance, while percentage of mass loss is about 15% at the end of the fifth day it takes 2-3 weeks to reach to 30%. This behavior may be explained by semi-crystalline structure of the PmPL. It is well-known that first stage of biodegradation undergoes at amorphous regions [17,18]. At the end of the fifth week, almost half of the samples in PBS solution became soluble in the medium. Similar degradation kinetics has been exhibited by the samples in the PBS solutions containing trypsin and lipase. Extents of mass losses in the samples treated with enzymes are 10-15% higher than that in PBS. The difference may be attributed to the catalytic activity of trypsin and lipase on the hydrolysis of ester bond.

LC-MS Analysis

Oligomeric fragments detached from the polymer bulk may give information about the degradation mechanism. LC-MS analysis of the sample taken

from the degradation media was conducted to determine the molecular mass characteristics of degraded fractions. Figure 9 gives the LC-MS spectra of solutions taken from each degradation media. Although many signals with various intensities were observed for each medium, four signals outshined with regard to intensity. Since the mass spectra were recorded with negative ion mode each signal was evaluated as anionic form (M^-). Mass of repeating unit of PmPL is about 86 Da. The signals with 87 Da were attributed to α-methyl propionic acid which may be regarded as monomeric unit. However, the signals with 55 Da observed belong to the smaller molecules formed by the secondary or tertiary transformation of monomeric units. As well-known [19-24], hydroxyl carboxylic acids are the main products released from biodegradation of polyesters. For instance, polyhydroxybutyrate (PHB) can be degraded to 3-hydroxybutyric acid by oligomer hydrolase and PHB depolymerase [25]. The signals with 109, 119 and 173 Da were also evaluated and attributed to the structures given in Table 1. Briefly, LC-MS analysis revealed that PmPL films in all media degraded to α-methyl propionic acid, its dimer and derivatives.

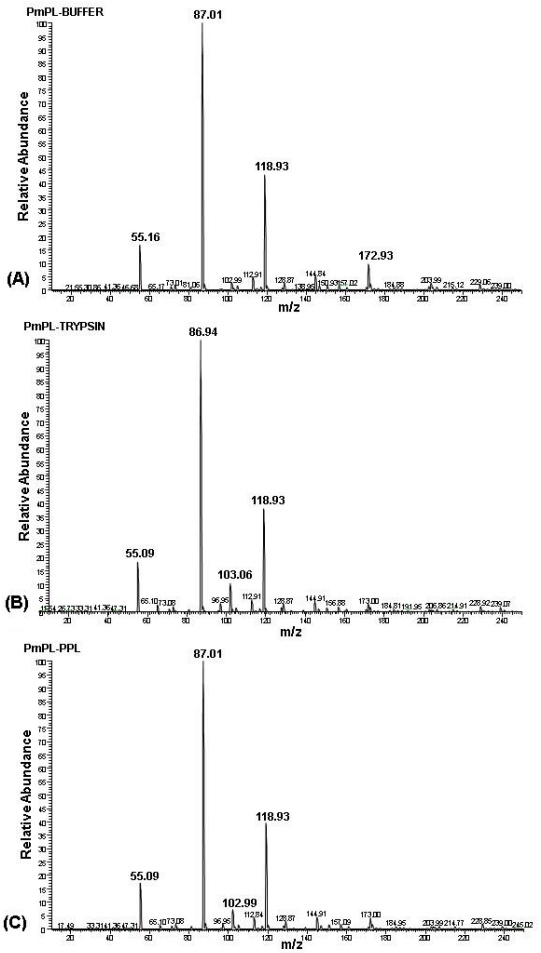
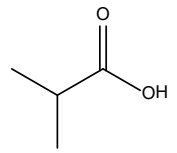
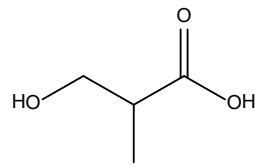
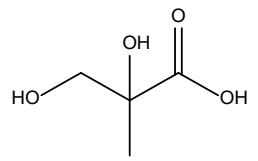
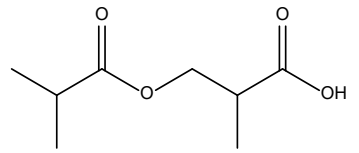


Figure 9. LC-MS spectra of residues taken from (A) buffer, (B) buffer+trypsin and (C) buffer+pancreatic lipase media

Table. 1 Suggested structures considering the LC-MS analysis.

Medium	Signal (Da)	Chemical structure
Buffer, trypsin and pancreatic lipase	87	
trypsin and pancreatic lipase	103	
Buffer, trypsin and pancreatic lipase	119	
Buffer	173	

Acknowledgments
The financial support of the Ordu University Scientific Research Projects #AR-1411 is gratefully acknowledged.

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