ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

DESIGN AND SYNTHESIS OF COMPLEX BIOMACROMOLECULES AND THEIR USE IN VARIOUS APPLICATIONS

Ph.D. THESIS

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KARMAŞIK BİYOMAKROMOLEKÜLLERİN TASARIMI, SENTEZİ VE DEĞİŞİK UYGULAMALARDA KULLANIMI

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ABBREVIATIONS

PS	**:**Polystyrene
PCL	**:**Poly(ε-Caprolactone)
CL	**:**ε-Caprolactone
NMP	**:**Nitroxide Mediated Polymerization
$Sn(Oct)_2$	**:**Stannous Octoate
Bipy	**:**Bipyridine
CuBr	**:** Copper (I) bromide
NaN_3	**:**Sodium azide
DMF	**:**Dimethyl Formamide
THF	**:**Tetrahydrofuran
PBS	**:**Phosphate Buffer Saline
GA	**:**Glycolide
LA	**:**Lactide
PLA	**:**Poly(D,L-lactide)
PLLA	**:**Poly(L-lactide)
PLLGA	**:**Poly(L-lactic-co-glycolic acid)
PLGA	**:**Poly(lactic-co-glycolic acid)
PS-*g*-PLLA	**:**Poly(styrene-*graft*-lactic acid)
PS-*g*-PLLGA	**:**Polystyrene-*graft*-(lactic-co-glycolic acid)
$PS\text{-}N_3$	**:**Polystyrene azide
PS-PLA-PEG	**:**Polystyrene-poly(lactic acid)-poly(ethylene glycol)
PBS	**:**Phosphate buffered saline
β-BL	**:**β-butyrolactone
POE	**:**Poly(ortho ester)
PA	**:**Polyanhydride
PHB	**:**Poly(3-hydroxybutyrate)
BAPO	**:**Bis(2,4,6-trimethylbenzoyl) phenylphosphine oxide
IMM	**:**Imatinib mesylate
TMC	**:**Trimethylcarbonate
FDA	**:**Food and Drug Administration
SEM	**:**Scanning electron microscopy
DSC	**:**Differential Scanning Calorimetry
^{1}H-NMR	**:**Hydrogen Nuclear Magnetic Resonance Spectroscopy
FT-IR	**:**Infrared Spectrophotometer
GPC	**:**Gel Permeation Chromatography
DP-MS	**:** Direct Pyrolysis Mass Spectrometry
HPLC	**:** High Pressure Liquid Chromatography
EE	**:**Encapsulation Efficiency
ATRP	**:**Atom Transfer Radical Polymerization
CuAAC	**:**Copper catalyzed Azide–Alkyne Cycloaddition

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DESIGN AND SYNTHESIS OF COMPLEX BIOMACROMOLECULES AND THEIR USES IN VARIOUS APPLICATIONS

SUMMARY

The first applications of a biodegradable polymers were at 1980s. They have gained much attention since then by the discovery of their feasibility to biomedical applications such as sutures, bone screws, tissue engineering scaffolds and drug delivery systems. Physical properties, hydrolytic degradation profiles, and biocompatibility of aliphatic polyesters and co-polyesters make them incomparable materials within the biomedical products. Also, biodegradable polymers are of special interest because they do not accumulate in nor harm the environment and thus can be considered "green". Polymers derived from glycolide (GA), lactide (LA), β-butyrolactone (β-BL), ε-caprolactone (ε-CL), and trimethylcarbonate (TMC) are known to be the most popular ones. The main reason of popularity is degradation products of these polymers are toxicologically safe which are eliminated by normal metabolic pathways. The potential use of these biopolymers is polymeric particles as carriers of of a wide range of drugs and therapeutic agents. Polymeric particles such as micro- or nano- spheres which have the ability of delivering drugs in specified area and sustained periods of time are being employed to avoid the inconvenient surgical insertion of large implants. The drug release properties of the biodegradable polymeric particles can be controlled by polymer degradation under physiological conditions and is found to depend on copolymer ratio and molecular weight. Drug release from polyesters, such as poly(L-lactic acid) (PLLA) and poly(lactic-co-glycolic acid) (PLGA), is generally controlled by both drug diffusion and polymer erosion. In the diffusion process, water diffuses into the polymer matrix faster than the polymer is degraded. The hydrolysable bonds in the whole polymer matrix are cleaved homogeneously. Therefore, the average molecular weight of the polymer decreases homogeneously. Release of the drug occurs by diffusion which is related to concentration of the drug. In the case of surface erosion, the diffusion rate of water into the polymer matrix is slower than the degradation rate of the macromolecules. The degradation only takes place in the thin surface layer while the molecular weight of the polymer in the bulk remains unchanged.

Despite superior properties of aliphatic polyesters, they still need to be improved for certain applications. The basic approach behind the utilization of aliphatic polyesters in biomedical field is the controllable degradation behavior of the polymers. Hydrophilicity, surface chemistry, morphology and crosslink density are major factors that influences degradation rate. The degradation rate can be controlled by the incorporation of new segments to biodegradable aliphatic polyesters. Grafting of biodegradable polymer chains, such as PLGA, onto their backbone may provide new biomaterials with interesting properties.

In the current thesis, we described the synthesis of new biodegradable polymers for various applications in biomedical field. In this respect, we focused on introducing new segments to biodegradable polymers to overcome the common problems associated with the chemical composition of the biodegradable polymers such as the control of drug release, degradation behavior, and effective treatment. Controlled release profiles and hydrolytic degradation behaviors of suitable polymers for microsphere preparation are also investigated.
There are four outcomes of thesis:

i. Preparation of a model drug loaded microspheres from various poly(lactic-co-glycolic acid) (PLGA) copolymers and *in vitro* release studies
ii. Synthesis of polystyrene-*g*-poly (lactic acid) (PS-*g*-PLLA) and polystyrene-*g*-poly (lactic-co-glycolic acid) (PS-*g*-PLLGA), degradation behavior of these polymers and *in vitro* release of the model drug from microspheres made of PS-*g*-PLLA, PS-*g*-PLLGA.
iii. Synthesis of poly(styrene-lactic acid-ethylene glycol) (PS-PLA-PEG) miktoarm star copolymer and pyrolysis studies.

We firstly aimed to prepare microspheres made of commercial biodegradable polymers at different conditions. Polymers with a different molecular weights and compositions are used for comparison. Surface characteristics and controlled release profiles of synthesized microspheres are examined. Commercial PLGA copolymers are used for fabrication of microspheres to compare after synthesis of new biopolymers. Imatinib mesylate, used as model drug for the treatment of craniopharyngiomas which is a kind of brain tumor and grows by vascularization. Microspheres were prepared by double emulsion/ solvent evaporation method. Nearly, eighty different microspheres were synthesized and optimized microspheres were chosen for investigation. Influence of process parameters on microsphere encapsulation, initial burst release and release profile from PLGA microspheres were studied. *In vitro* release studies were performed at 37˚C in phosphate buffer saline solution (PBS) at pH 7.4 and release profiles of microspheres were monitored by high pressure chromatography (HPLC). Surface morphologies of the microspheres before and during release studies were determined by scanning electron microscopy (SEM). As a result of this work optimized microspheres were chosen for *in vivo* experiments. It is found that, local delivery of model drug using biodegradable microspheres may be a promising way of treatment.

In the second part, new copolymers of commercial biopolymers are synthesized named PS-*g*-PLLA and PS-*g*-PLLGA to overcome the shortcomings of biodegradable polymers. Hydrolytic degradation behaviors, *in vitro* release studies of a model drug from microspheres made of PS-*g*-PLLA and PS-*g*-PLLGA graft copolymers and surface characterization of microspheres are investigated. The graft copolymers were synthesized through combination of nitroxide mediated free radical polymerization (NMRP) and ring opening polymerization (ROP) with copper (I)-catalyzed “Click” chemistry. First, the click component, azido-functional PS (PS-N_3) was prepared by copolymerization of styrene and chloromethyl styrene by NMRP followed by the conversion of chlorine groups of the resulting copolymer to azido groups by using NaN_3 in N, N-dimethylformamide. Propargyl functional APEs were prepared independently by ROP of lactic acid and glycolic acid using tin octoate in the presence of propargyl alcohol at 130 ˚C. Finally, PS-N_3 was coupled with the resulting polymers by click chemistry to yield graft copolymers (PS-*g*-PLLA and PS-*g*-PLLGA, respectively). The intermediates and final graft copolymers

were characterized by ^{1}H-NMR and FT-IR spectrally, and GPC chromatographic analyses. The hydrolytic degradation behavior of the obtained graft copolymers in PBS were investigated by the weight loss and molecular weight measurements. It is shown that both copolymers undergo slow hydrolytic degradation, PS-*g*-PLLGA being relatively faster due to the presence of hydrophilic glycolic acid groups in the structure. Microspheres of the graft copolymers were prepared and characterized. *In vitro* release studies showed that PS backbone of the graft copolymers slows down the degradation rate and gives opportunity to control the drug release.

In the final part, we describe the synthesis of of PS-PLA-PEG miktoarm star copolymer and thermal degradation behavior studies. An ABC type miktoarm star copolymer possessing polystyrene (PS), poly(lactic acid) (PLA) and poly(ethylene glycol) (PEG) arms was synthesized by combining Atom Transfer Radical Polymerization (ATRP) and Ring Opening Polymerization (ROP) with two click chemistries, namely thiol–ene and copper catalyzed azide–alkyne cycloaddition (CuAAC). For this purpose, a core 1-(allyloxy)-3-azidopropan-2-ol with allyl and azide functionalities was synthesized in two steps. Then, clickcable polymers, polystyrene with thiol functionality (PS-SH) and poly(ethylene glycol) with alkyne functionality (PEG-acetylene) were independently prepared. As the first step of the grafting onto process, PS-SH was thiol–ene clicked onto the core to yield PS-N_3-OH. The second arm was then incorporated on to the core by the Ring Opening Polymerization (ROP) of l-(-)-Lactide (LA) using as PS-N_3-OH initiator and tin(II) 2-ethylhexanoate as catalyst. Finally, alkyne-PEG-acetylene was bonded to the resulting PLA-PS-N_3 using CuAAC click reaction. All intermediates, related polymers at different stages and final PS-PLA-PEG miktoarm star copolymer were characterized by ^{1}H NMR, FT-IR, GPC and DP-MS analyses. Direct pyrolysis mass spectrometry, (DP-MS) analyses indicated that the decomposition of PS and PEG chains occurred almost independently. On the other hand, hydrolysis of PLA chains due to the elimination of H_2O during the thermal degradation of PEG, increasing the yields of CO_2, CO and units involving unsaturation and/or crosslinked structure was detected.

KARMAŞIK BİYOMAKROMOLEKÜLLERİN TASARIMI, SENTEZİ VE DEĞİŞİK UYGULAMALARDA KULLANIMI

ÖZET

Biyobozunur polimerlerin ilk uygulamaları 1980 yıllarında görülmüştür.Biyobozunur polimerler dikişler, doku mühendisliği ve ilaç salım sitemleri gibi biyomedikal alana uyarlabilirliğinin keşfedilmesiyle büyük önem kazanmışlardır. Fiziksel özellikleri, hidrolitik bozunma profilleri ve biyolojik uyumlulukları alifatik poliesterleri ve kopoliesterlerini biyomedikal ürünler arasında karşılaştırılamaz kadar önemli bir konuma getirmiştir. Ayrıca, bu polimeler çevreye zararlı olmadıklarından daha yakından incelenmekte olup, 'yeşil' olarak adlandırılmaktadırlar. En popüler poliesterler arasında glikolid (GA), laktid (LA), β-bütirolakton (β-BL), ε-kaprolactone (ε-CL) ve trimetilkarbonat (TMC) gibi malzemelerden türetilen polimerler yer almaktadır. Bu polimerlerin popüler olmasının en büyük sebebi, degredasyon ürünlerinin toksik olmayışı ve normal metabolik yollar ile elimine edilebilmeleridir. Bu biyopolimerlerin potansiyel uygulamaları arasında polimerik tanecikler ile ilaçların ya da tedavi edici ajanların taşınması yer almaktadır. Mikro- ya da nano- küreler gibi polimerik taşıyıcı tanecikler, zor cerrahi operasyonlar ile büyük implatasyon uygulamaları yerine, belirlenen bölgede ve belirli zaman miktarlarında istenilen ilacı salmak üzere kullanılabilirler. Polimerik taneciklerin ilaç salım özellikleri kullanılan polimerin degredasyonunun belirli fiziksel şartlar altında kontrol edilebilmektedir. Polimerlerin degrdasyonun büyük çoğunlukla kopolimer oranı ve molekül ağırlığına bağlı oldugu görülmüştür. Poli(L-laktik asit) (PLLA) ve poli(laktik-ko-glikolik asit) (PLGA) polimerleri kullanılarak gerçekleştirilen ilaç salımlarında, ilaç salımının genellikle ilacın difüzyonu ve polimer erozyonu ile gerçekleştiği görülmüştür. İlaç difüzyonu sırasında, ortamda bulunan su, polimer matriksinin içerisine polimer erozyonundan çok daha hızlı difüzlenir. Polimerin yapısında bulunan ve hidrolitik olarak kırılabilen bağlar polimer matriksinin içerisinde homojen olarak parçalanırlar. Bu nedenle polimerlerin ortalama molekül ağırlıgı homojen olarak azalır. Difüzyon yolu ile salınan ilaç sistemleri, ilacın konsantrayonuna bağlıdır. Polimerlerin yüzey erozyonu ile geçekleşen salımlarda, ortamda bulunan suyun polimer matriksi içeresine olan difüzyonu polimerin degredasyon hızından daha yavaştır. Buradaki degredasyon ince yüzey katmanında gerçekleşmektedir.

Alifatik poliesterlerin üstün özellikleri olmasına rağmen, belirli uygulamalarda kullanılabilmeleri için geliştirilmeye ihtiyaçları vardır. Bu sebeple, poliesterlerin biyolojik uygulamalarda kullanabilirliğinin artırılması degredasyon profillerinin control edilebilmesiyle gerçekleştirilebilir. Degredasyon hızını etkileyen başlıca faktörler arasında hidrofiliklik, yüzey kimyası, morfoloji ve çapraz bağlanma yüzdesi bulunmaktadır. Degredasyon hızı alifatik poliesterlerin yeni polimer üniteleri ile kombinasyonu kullanılarak ayarlabilir. PLGA gibi biyobozunur polimerlerin ana

zincirine graft olarak yeni polimer ünitelerinin eklenmesi ile çeşitli özellikler kazandırılabilir.
Bu tezde, çeşitli biyomedikal uygulamalarda kullanılmak üzere kompleks biyomakromoleküllerin dizayn ve sentezi anlatılmıştır. Bu bakış açısıyla, biyobozunur polimerlerin yeni segmentler ile geliştirilip, polimerlerin kimyasal yapısından kaynaklanan ilaç salım kontrolü, degredasyon özellikleri ve etkili tedavi ile ilgili sorunların giderilmesi amaçlanmıştır. Elde edilen polimerlerin hidrolitik degredasyon çalışmaları ayrıca mikroküre sentezine uygun olan polimerin de kontrollü salım çalışmaları gerçekleştirilmiştir. Tez çalışmasında elde edilen sonuçlar aşağıdaki gibidir:

i. Model ilaç kullanılarak çeşitli poli(laktik-ko-glikolik asit) (PLGA) kopolimerinden mikroküre sentezi ve *in vitro* salım çalışmaları
ii. Polistiren-*g*-polilaktik asit PS-*g*-PLLA ve polistiren-*g*-poli(laktik asit-ko-glikolik asit) PS-*g*-PLLGA kopolimerlerinin sentezi, degredasyon çalışmaları ve bu kopolimerlerden elde edilen mikrokürelerin kontrollü salım çalışmaları
iii. Poli(stiren-laktik asit- etilen glikol) miktoarm yıldız polimerinin sentezi ve piroliz çalışmaları

İlk bölümde, ticari olarak bulunan çeşitli özelliklerde PLGA kopolimerleri ile farklı koşullarda mikroküre sentezi yapıldı. Farklı molekül ağırlıklarında ve farklı kopolimer oranlarına sahip polimerler kullanılarak karşılaştırmalar yapıldı. Sentezlenen mikrokürelerin yüzey karakterizasyonları ve kontrollü salım çalışmaları gerçekleştirildi. Bu çalışmada, sonraki aşamalarda kullanılmak üzere metod geliştirme amaçladığından ticari olarak bulunan PLGA kopolimeri kullanıldı. Mikroküre sentezlerinde model ilaç olarak kraniofrangenom tumorünü damarlanmayı azaltarak inhibe edici özelliği bulunan imatinib mesilat kullanıldı. Mikroküreler çift emisyon/solvent uçurma yöntemi kullanılarak sentezlendi. Gerçekleştirilen çalışmada seksen farklı mikroküre sentezlendi. Kullanılan metodun ilaç enkapsülasyonu, kontrollü salım çalışmaları ve mikrokürelerin yüzeyindeki etkisi araştırıldı. Salım çalışmaları 37˚C'de fosfat tampon çözeltisinde (pH: 7.4) gerçekleştirildi. Salım çalışmaları yüksek basınç sıvı kromatografisi (HPLC) ile takip edildi. Mikrokürelerin yüzey görüntüleri ise taramalı elektron mikroskopu (SEM) ile alındı. *In vivo* deneyleri için optimize edilen mikroküreler seçildi. Gerçekleştirlen deneyler sonucunda, biyobozunur polimerlerden elde edilen mikrokürelerin tedavi amaçlı lokal salım çalışmaları için uygun olabileceği görülmüştür.

İkinci bölümde, ticari biyobozunur polimerin ile karşılaşılan sorunları gidermek amacıyla PLLA ve PLLGA içeren polistiren sentezi gerçekleştirlmiştir. Elde edilen polimerlerin hidrolitik degredasyon ve kontrollü salım çalışmaları gerçekleştirilmiştir. Sentezlenen polimerlerden elde edilen mikrokürelerin yüzey incelemeleri SEM ile gerçekleştirlmiştir. Kopolimerler nitroksit aracılıklı radikal polimerizasyonu, halka açılma polimerizasyonu ve klik reaksiyonun kombinasyonu ile sentezlenmiştir. Öncelikle klik reaksiyonu için azio fonksiyonu içeren polistiren ($PS\text{-}N_3$), stiren ve klorometil stiren ile nitroksit aracılıklı radikal polimerizasyonu kullanılarak sentezlendikten sonra NaN_3 varlığında klor grupları azid fonksiyonu ile yer değiştirmesi ile elde edilmiştir. Propargil fonksiyonlu alifatik poliester sentezi halka açılma reaksiyonu ile propargil alkolün başlatıcı olduğu reaksiyon ile 130˚C'de sentezlenmiştir. Son aşamda, $PS\text{-}N_3$ ve propargil fonksiyonlu alifatik polieseterler klik reaksiyonu kullanılarak graft kopolimerler elde edilmiştir. Elde edilen graft kopolimer ve ara basamaklarda sentezlenen polimerlerin ^{1}H-NMR, FT-IR ve GPC kullanılar karakterizasyonu yapılmıştır. Graft kopolimerlerin hidrolitik degredasyon

çalışmaları 37˚C'de fosfat tampon çözeltisinde moleküler ağırlıklarındaki azalma takip edilerek gerçekleştirilmiştir. Elde edilen sonuçlar her iki polimerinde degredasyona uğradıklarını fakat PS-*g*-PLLGA kopolimerinin içerdiği hidrofilik glikolit ünitelerinden dolayı daha hızlı degrede olduğunu göstermektedir. Kontrollü salım sonuçları ise, graft kopolimerlerinin yapısında bulunan polistiren ana zincirinin degredasyonu yavaşlatmasından dolayı daha iyi kontrol sağlandığını ve salım zamanını uzattığını göstermiştir.

Son bölümde, PS-PLA-PEG miktoarm yıldız polimerinin sentezi ve piroliz çalışmaları gerçekleştirlmiştir. Polistiren, poli(laktik asit) ve poly(etilen glikol) içeren yildiz polimer sentezi atom transfer radikal polimerizasyonu ve iki ayrı klik reaksiyonu ve halka açılma reaksiyonu kullanılarak sentezlenmiştir. Bu amaçla, polimerin çekirdek kısmı olan allil ve azid fonksiyonları içeren 1-(allyloxy)-3-azidopropan-2-ol iki aşamada sentezlenmiştir. Daha sonra kliklenebilen polimerler olan tiol ile azid fonksiyonlandırılmış poli(etilen glikol) ayrı olarak sentezlendi. İlk aşamada, tiol fonksiyonlu polistiren klik rekasiyonu ile çekirdek üzerine bağlandı. İkinci kol olan poli(laktik asit) ise elde edilen polimeri başlatıcı olarak kullanılarak halka açılma reaksiyonu ile sentezlenmiştir. Son aşamada ise azid içeren poli(etilen glikol) klik reaksiyonu ile iki kollu çekirdeğe bağlanmıştır. Elde edilen graft kopolimer ve ara basamaklarda sentezlenen polimerlerin ^{1}H-NMR, FT-IR ve GPC kullanılar karakterizasyonu yapılmıştır. Direkt piroliz kütle spektroskopsi ile termal analizleri gerçekleştirldi. PEG polimerinin degredasyonu sonunca açığa çıkan H_2O'nun PLA polimerinin degredasyonunu hızlandırdığı görülmüştür.

1. INTRODUCTION

Biodegradable polymers were first introduced in 1980s [1]. These polymers have gained much attention since the discovery of their feasibility to biomedical applications such as sutures, bone screws, tissue engineering scaffolds and drug delivery systems [2, 3]. Generally, aliphatic polyesters are known to be biodegradable polymers which derived from glycolide (GA), lactide (LA), β-butyrolactone (β-BL), ε-caprolactone (ε-CL), and trimethylcarbonate (TMC). Aliphatic polyesters can be synthesized in three major routes such as poly condensation, ring-opening polymerization and enzymatic polymerization. The most common way of synthesis is the ring-opening polymrerization which leads high molecular weight polymers. The popularity of aliphatic polyesters depends on their resorbability and good mechanical properties. Also, Food and Drug Administration (FDA) have approved the usage of these aliphatic polyesters [4]. Despite their superior properties, they are still need to be improved. Degradation is the main feature of these polymers in general applications. Degradation mechanisms of biodegradable polymers can be categorized as hydrolytic and/or microbial routes [5]. The degradation processes occur by the cleavage of the backbone ester linkages of polymers. Hydrophilicity, surface chemistry, morphology and crosslink density are major factors that influences degradation rate [6]. Although aliphatic polyesters have been used for many years for biomedical and pharmaceutical applications are not good enough for certain applications such as those involving drug delivery devices and implant systems. The basic approach behind the utilization of aliphatic polyesters in biomedical field is the controlling the degradation behavior of the polymers. Recently, many studies have focused on block and graft copolymers in biomedical applications [7]. It is well known that the modification of the chemical structure of a polymer may lead to improvements in both physico-chemical and drug release properties[8]. Grafted copolymers not only have different architectures to linear polymers, but also different chemical and physical properties.

1.1 Purpose of the Thesis

The main subject of this thesis is to synthesize new biodegradable polymers for various applications in biomedical field. Additionally, controlled release profiles and hydrolytic degradation behaviors of suitable polymers for microsphere preparation among the synthesized polymers and common biodegradable polyesters are also studied.

This thesis also focuses on introducing new segments to common biodegradable polymers to overcome the common problems associated with the chemical composition of the biodegradable polymers such as the control of drug release, degradation behavior and effective treatment.

In this respect, we firstly aimed to prepare microspheres made of commercial biodegradable polymers at different conditions. Polymers with a different molecular weights and compositions are used for comparison. Surface characteristics and controlled release profiles of synthesized microspheres are examined.

Secondly, new copolymers of commercial biopolymers are synthesized. These include polystyrene-*g*-poly (lactic acid) (PS-*g*-PLLA) and polystyrene-*g*-poly(lactic-co-glycolic acid) PS-*g*-PLLGA. Suitability of the synthesized polymers for microsphere preparation is also investigated. Controlled release studies are performed to confirm hydrolytic degradation profiles and prolonged release times.

Finally, a new ABC type miktoarm star copolymer possessing polystyrene (PS), poly(lactic acid) (PLA) and poly(ethylene glycol) (PEG) arms was synthesized. PLA and PEG segments compose biodegradable and biologically manageable parts of the polymer. Thermal degradation studies are performed in order to obtain information for future applications of the polymer in biomedical field.

2. THEORETICAL PART

2.1 Biodegradable Polymers

The use of polymeric materials for administration of pharmaceuticals as drug delivery devices generated a continuously growing amount of interest. Biodegradable polymers are of special interest because they do not accumulate in nor harm the environment and thus can be considered "green" [9]. A wide range of applications of biodegradable polymers exist including pharmacological uses, mechanical support, mechanical barrier and carriers to cells with or without targeting [10]. Biodegradable polymers are preferred as drug carriers since the need for surgical removal of the depleted device is eliminated [11]. The important properties that are required for biodegradable biomaterials can be summarized as follows [12-14]:

- Nontoxic and endotoxin - free, aiming to minimalize unwanted foreign body responses upon implantation.
- Degradation time should be matched to the regeneration or required therapy time.
- Mechanical properties must be suited to the required task.
- Degradation products should be nontoxic and readily cleared from the body.
- Material must be easily processed to allow tailoring for the required task.

Although there is variety of biodegradable polymers, only a limited number of polymers elicit the requirements for drug delivery. Biodegradable polymers can be either natural or synthetic. In general, synthetic polymers offer greater advantages over natural ones as they can be tailored to give a wider range of possibilities with a variety of properties [15]. The natural polymers consist of polysaccharides, dextran or cellulose, chitin, chitosan and proteins [16-21]. Synthetic degradable polymers include aliphatic polyesters, polyanhydrites, aliphatic polycarbonates and poly (ortho) esters [22-30]. Thermal and mechanical properties of polyesters shown in Table 2.1 [31].

Table 2. 1: Thermal and mechanical properties of biodegradable polyesters.

Polymer	M_w	T_g (°C)	T_m (°C)	Tensile Strength (MPa)	Elongation Yield(%)	Break (%)
Poly(lactic acid)						
L-PLA	50,000	54	170	28	3.7	6.0
L-PLA	100,000	58	159	50	2.6	3.3
L-PLA	300,000	59	178	48	1.8	2.0
DL-PLA	107,000	51	-	29	4.0	5.0
Poly(glycolic acid)						
PGA	50,000	35	210	NA	NA	NA
Poly(3-hydroxybutyrate)						
PHB	370,000	9	171	36	2.2	2.5
Polycaprolactone						
PCL	44,000	-62	57	16	7.0	80
Poly(trimethylene carbonate)						
PTC	48,000	-15	-	0.5	20	160

2.1.1 Aliphatic polyesters

The first report of aliphatic polyesters dates back as early as 1930 [32]. Biodegradable aliphatic polyesters, which are in principle degradable hydrolytically, are by far the most employed. Generally, polyesters with short aliphatic chains between ester linkages, poly(hydroxycarboxylic acid)s, degrade slower than expected for a biomedical use. Polymers obtained from the ring opening polymerization reaction of lactones or cyclic diesters compose the major group of poly(hydroxycarboxylic acid)s. This polymerization process has been up-graded to the industrial production of PLA and poly(ε-caprolactone) (PCL). It is worth noting that polylactide can be made available from agricultural renewable resources. Contamination of the aliphatic polyesters by potentially toxic metallic residues is a concern for many applications, particularly for biomedical applications. In order to alleviate this problem, organometallic initiators have been successfully replaced by lipases and full organic systems. Another source of poly(hydroxycarboxylic acid)s is poly(hydroxyalkonoate)s (PHA) produced by bacteria. Polyesters can be obtained by

direct condensation of alcohols or diols and acids or diacids. Polymers with a low and uncontrolled molecular weight are not suitable for biomedical applications.

Commonly, lactide, glycolide, ε-caprolactone (ε-CL), and hydroxybutyrate monomers are used to obtain homo- and copolymers for medical applications. Structures of these monomers and polymers shown in Figure 2.1.

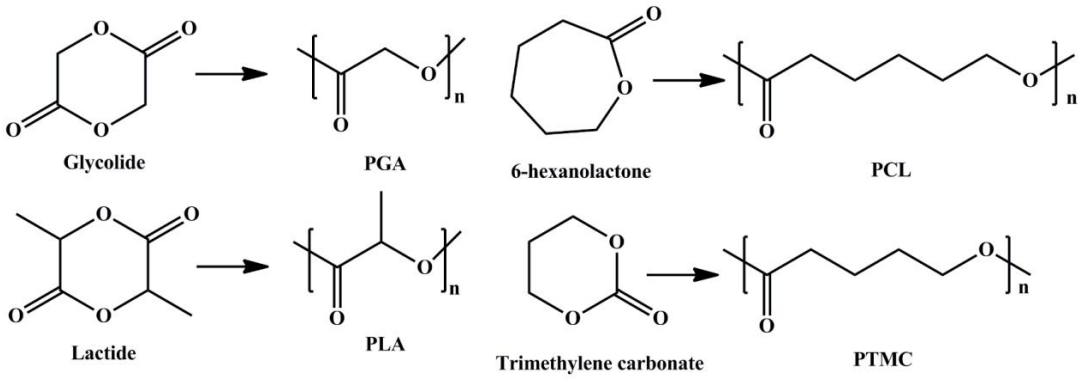


Figure 2. 1: Molecular structures of common cyclic monomers and their polymers

2.1.1.1 Polylactide, PLA

Lactic acid (chemically, 2-hydroxypropanoic acid, LA), also known as milk acid, is the most widely occurring carboxylic acid in nature. It was first isolated in 1780 by a Swedish chemist, Carl Wilhelm Scheele, but it was first produced commercially by Charles E. Avery at Littleton, Massachusetts, USA in 1881 [33]. The L-isomer constitutes the main fraction of PLA derived from renewable sources since the majority of lactic acid from biological sources exists in this form PLA belongs to the family of aliphatic polyesters derived from α-hydroxy acids. Biomedical use of PLA started at 1960s [34]. PLA gained much attention due to its good mechanical properties and non-toxic degradation products. PLA has been approved by FDA and commercially available. Lactide has two asymmetric carbons and thus exists as the optically active L- and D- forms or as the racemic D, L- form. Possible stereochemical structures of lactide are shown in Figure 2.2.

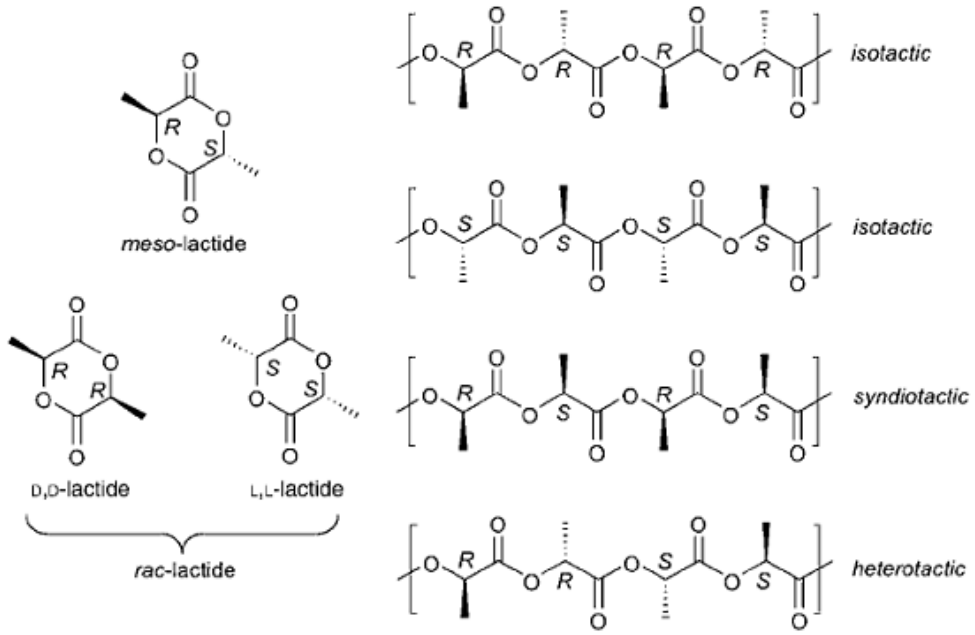


Figure 2. 2: Stereochemical possibilities observed with polylactide synthesis.

Commercial PLA are homopolymer of poly(L-lactic acid) (PLLA) or copolymer of poly(D,L-lactic acid) (PDLLA), which are produced from L-lactic acid and D,L-lactic acid [33]. PLA can be synthesized by polycondensation, azeotropic dehydrative polycondensation, and ring-opening polymerization (ROP) of lactide (Figure 2.3).

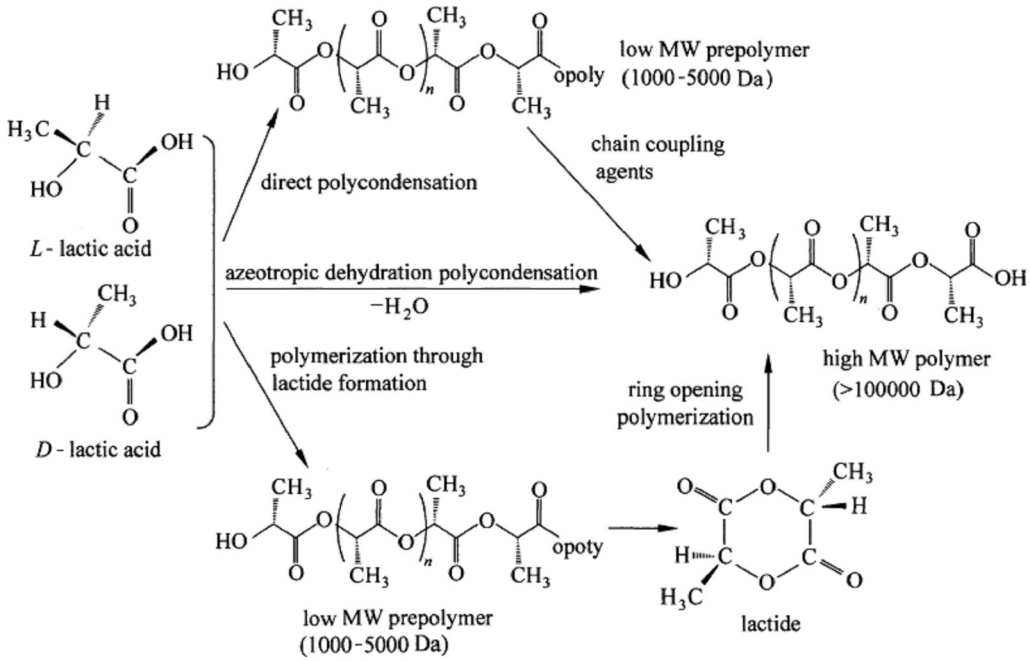


Figure 2. 3: Different preparation methods of PLA.

By and large, commercially available high-molecular-weight PLA resins are mainly produced via the ring-opening polymerization route of lactide [35]. ROP of lactide monomer can be initiated with various compounds. Reaction mechanism of ROP with different initiators is shown in Figure 2.4.

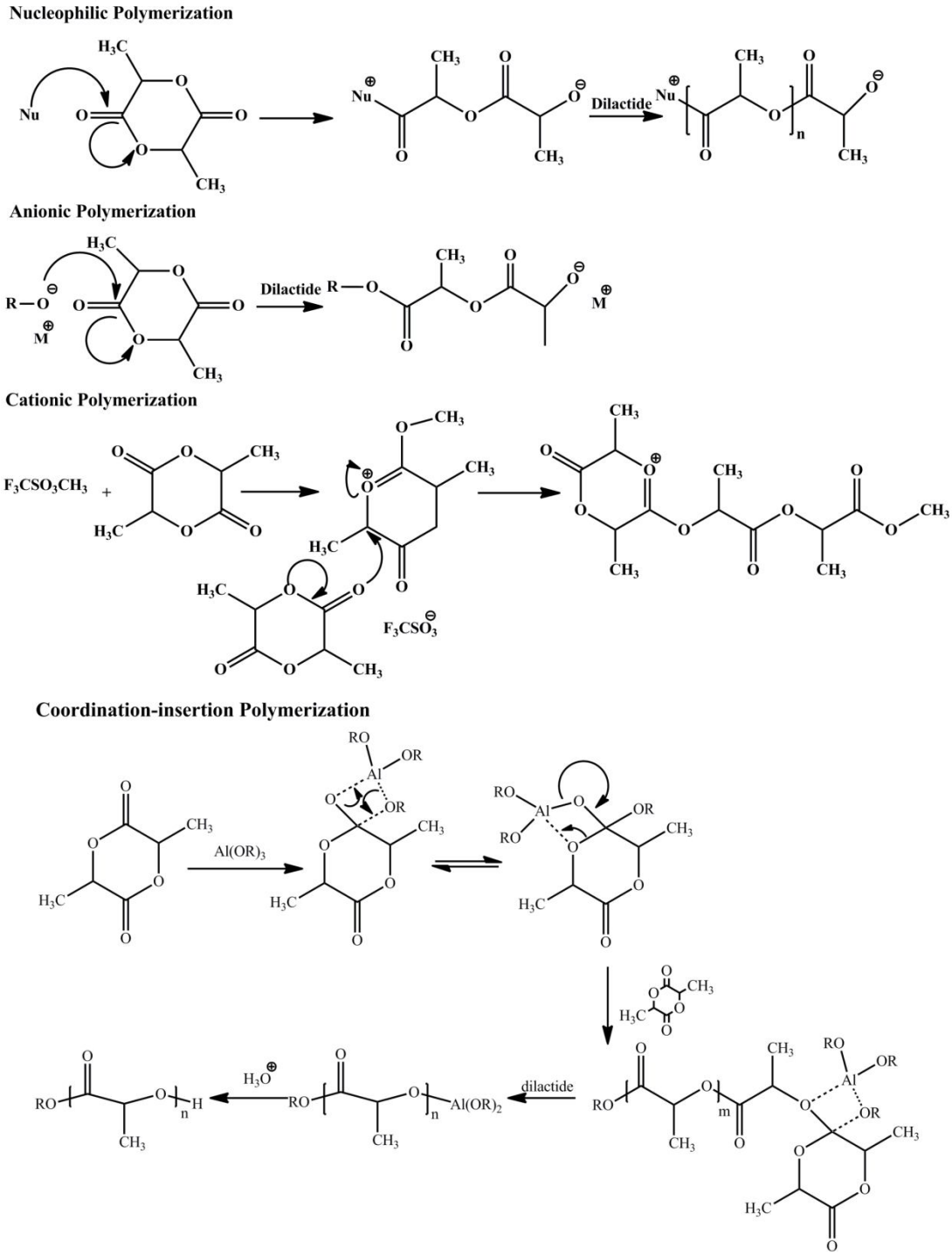


Figure 2. 4: Reaction mechanism of ROP with different initiators.

The most common used catalyst in synthesis of polylactide is aluminum isopropoxide. It is less reactive than stannous (II) octaoate ($Sn(Oct)_2$) and also there are some suspects about the relation between aluminum ions and Alzheimer's disease [36]. Stannous (II) octaoate is a commonly used catalyst in polymerization of cyclic lactones such as glycolide, ε-caprolactone or trimethylene carbonate [37]. Reaction mechanism differs between lactones. The mechanism of ROP of lactide in presence of stannous octaoate catalyst is shown in Figure 2.5.

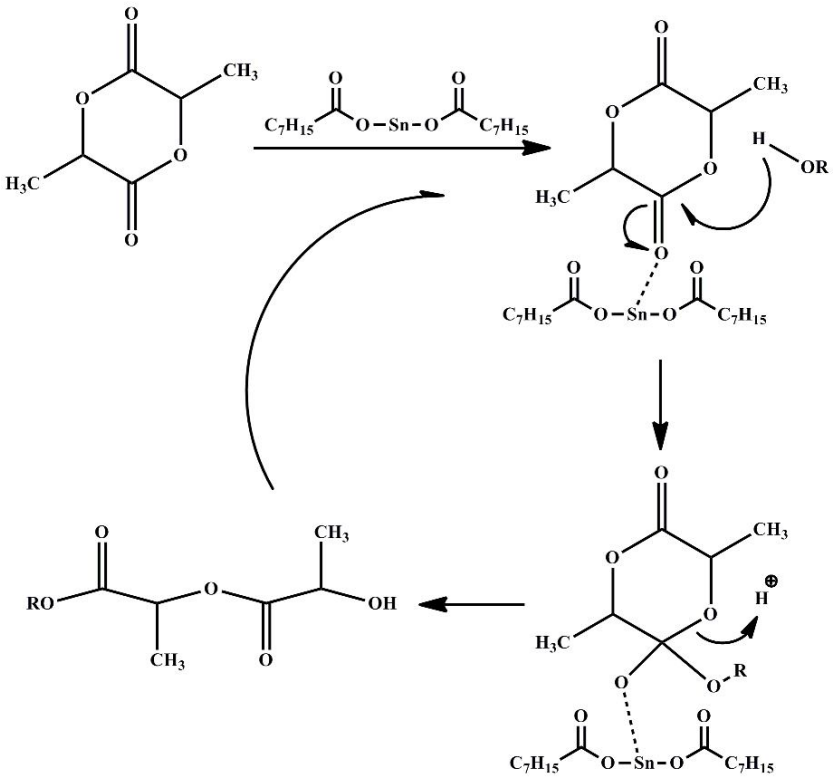


Figure 2.5: Hypothetical mechanism of ring-opening polymerization of lactide using $Sn(Oct)_2$ as catalyst.

Also, there are eco-friendly processes catalyzed by enzymes to produce polyesters [38]. Enzyme catalyzed reactions has benefits like elimination of side reactions and regio- and steriospecificticy [39].

PLLA has crystalline melting temperature (T_c) range between 170-180 ˚C due to its sterioregular structure and glass transition temperature (T_g) of PLLA is about 60-67˚C [40]. Racemic PLA is purely amorphous instead of PLLA which affects the biodegradation of polymers. Crystallinity is an important factor on biodegradation. However, unitary structure and poor properties of PLLA such as the inherent brittleness, poor melt strength, low heat deflection temperature, narrow processing window and low thermal stability have posed considerable scientific challenges and

limited their large-scale applications. The most common strategy to overcome this limit is to prepare the copolymers. Copolymerization of lactic acid (or lactide) with hydroxyl acid, amino acid or polymers such as PEG, starch, etc., can markedly improve the strength, toughness, hydrophilic and controlled degradable properties of PLA, and at the same time numerous new copolymers in different macromolecular architectures (linear, branch, star and dendron) can be obtained [41]. These materials are attracting considerable interest in materials science research. In the past several decades, extensive work has been carried out to synthesize the desired PLA copolymers for drug delivery, hydrogels, nanoparticles, microparticles, implants, micelles, etc. PLA copolymers can be synthesized by several kinds of well-known polymerization techniques, including polycondensation, anionic, cationic, and coordination-insertion ring-opening polymerization [42]. Copolymers of PLA with different cyclic esters such as glycolide, ε- caprolactone, ε- decaloactone or and blends of PLA with various polymers like PEG, alginate or chitin are highly noteworthy materials especially for drug delivery applications [42, 43]. PLA has been used in systematic delivery of several therapeutic agents such as contraceptive steroids, narcotic antagonists, antimalarial agents etc. [44-46]. Delivery of proteins from microspheres prepared from PLLA and PDLLA have also been reported [47].

2.1.1.2 Polyglycolide, PGA

PGA is a linear, biodegradable and thermoplastic polymer. It is mostly prepared by ring-opening polymerization of glycolide. Owing to crystalline structure, PGA has Tm about 225 ˚C and T_g of 36 ˚C [48]. Its hydrolytic instability and insolubility in common organic solvents have limited the usage of the polymer. However, PGA has an excellent fiber-forming property that is commercialized as the first absorbable suture in 1970 [48]. Copolymers of PGA are generally preferred for drug delivery applications depending on the deficiencies of the polymer that it cannot be made into films, capsules or microspheres.

2.1.1.3 Poly(lactide-co-glycolide), PLGA

Copolymers of lactide/glycolide monomers have received the most attention in recent years. PLGA ows its popularity to its, degradation profile, non-toxic degradation products, ease of preparation and commercially avalibity. Composition of LA and GA units determine the hydrophiliciy and crystallinity of the copolymer.

As GA units increase in the polymer composition, hydrophilicity of the polymer increases. Increasing number of GA units lead higher degradation rates. Linear lactide- and glycolide-based polymers are most commonly synthesized by ring-opening melt polymerization of lactide and glycolide at 140–180°C for 2–10 h using a catalyst [49]. Extensive research has been devoted to the use of these polymers as carriers for controlled drug delivery of a wide range of bioactive agents for human and animal use [50].

2.1.1.4 Poly(ε-caprolactone), PCL

The successful use of lactide and glycolide polymers in absorbable drug delivery systems and medical devices and absorbable sutures encouraged the evaluation of other polyesters for this purpose. The most studied polymers in this category are the PCLs. PCL is also a biodegradable and harmless material. PCL is a semi-crystalline and hydrophobic polymer with fine non-polar methylene groups in its repeating unit [51]. Different routes can be followed to synthesize PCL such as anionic, cationic, coordination, radical and enzyme catalyzed polymerizations shown in Figure 2.6 [11].

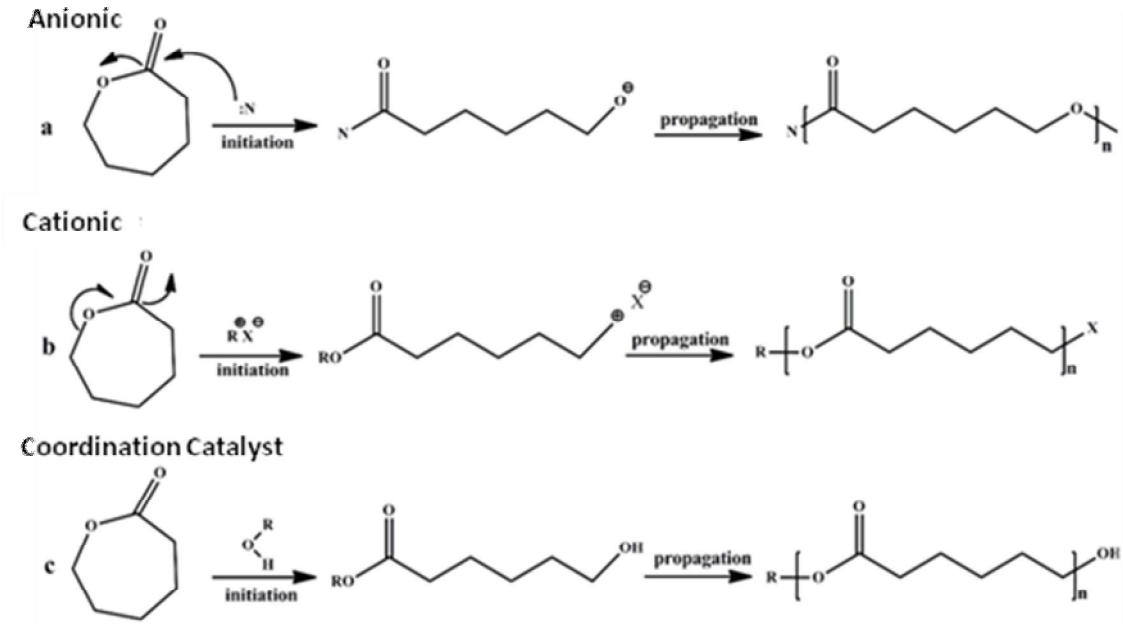


Figure 2. 6: Polymerization of ε-caprolactone using (a) anionic, (b) cationic, (c) coordination catalysts.

Most common way of synthesizing PCL is ring-opening polymerization of ε-caprolactone monomer shown in Figure 2.7 [52].

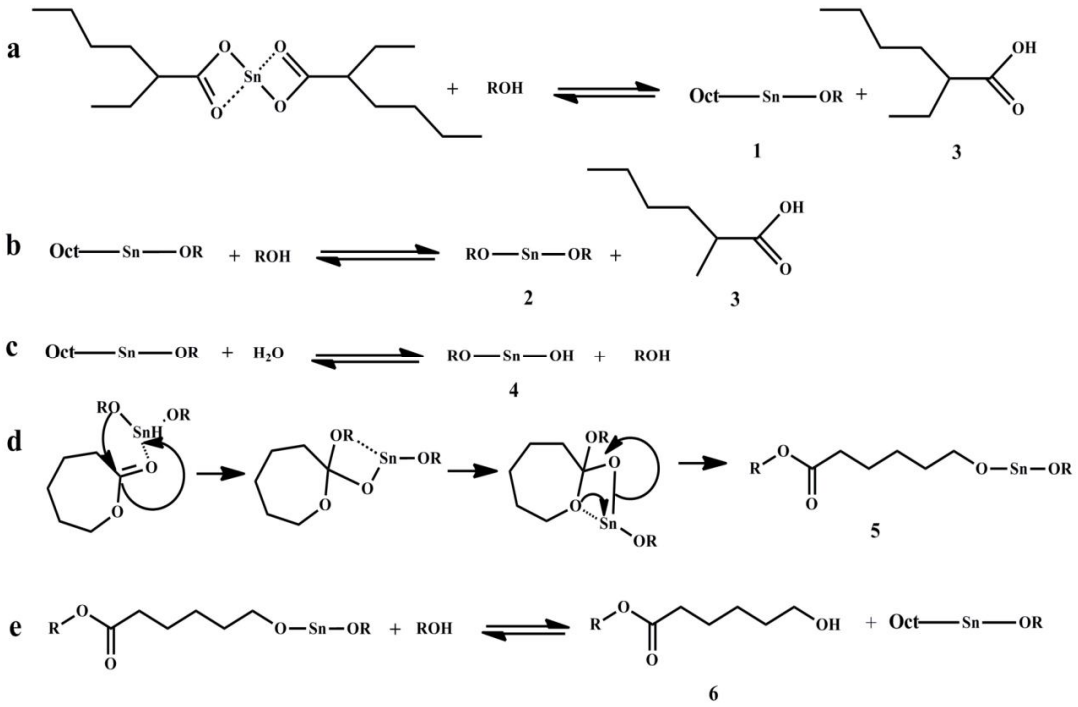


Figure 2.7: Synthesis of PCL in the presence of stannous octaoate; (a,b) formation of stannous alkoxide, (c) deactivation of the catalyst by reaction of water, (d) coordination/insertion of monomer into the stannous alkoxide bond generating dimer, (e) chain transfer of the active polymerizing center.

The homopolymer melts at 59–64°C with a T_g of -60°C [53]. PCL has slower degradation rate than PLA and PLGA. Degradation of PCL and its copolymers occurs both *in vivo* and *in vitro* conditions by bulk hydrolysis like lactide polymers. Degradation of PCL proceeds through hydrolysis of ester linkages at the backbone of the polymer which yields 6-hydroycaproic acid that enters the citric acid cycle and completely metabolized [11]. Degradation rate of PCL can be adjusted by copolymerization of lactide or glycolide monomers. Also, addition of oleic acid or tertiary amines which catalyses the chain hydrolysis may increase the degradation rate of PCLs.

There are many approaches to combine properties of PCL with other biodegradable polymers to provide desired needs in biomedical field [54]. Copolymers of PCL with glycolides or lactides and blends with PEG, polyhydroxybutyrates (PHB) or poly(mandelic acid) have been prepared to fabricate microspheres for drug delivery systems [55].

2.1.1.5 Poly(3-hydroxybutyrate), (PHB)

The most common polymer derived from bacteria is poly(3-hydroxybutyrate). PHB first isolated and characterized in 1925 by Indian microbiologist Abhilash Singh [56]. PHB produced by controlled bacterial fermentation. An optically active polymer of 3-hydroxybutyrate has been produced from propionic acid or pentanoic acid by *Alcaligeneseutrophus* [57]. Molecular structure of 3-hydroxybutyrate is shown in Figure 2.8.

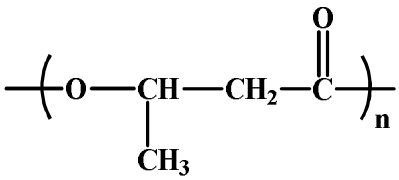


Figure 2. 8: 3-Hydroxy butyrate.

PHB has attracted much attention because they are produced biosynthetically from renewable sources and biocompatible. Copolymers of PHB with different monomers have been demonstrated but poor resistance to acids and bases limits modification of the polymer [56].

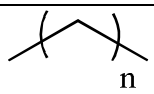
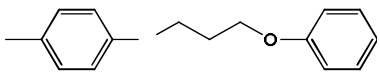
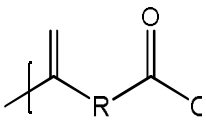
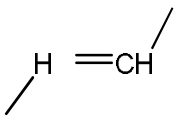
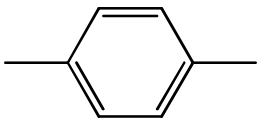
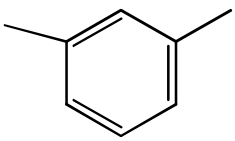
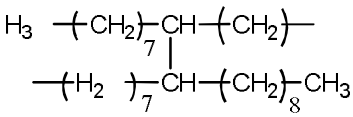
PHB can replace synthetic polymers due to similar properties with an advantage of being completely biodegradable. Mechanical and thermoplastic properties of PHB are similar to polypropylene. PHB is non-toxic material with properties like good oxygen permeability and ultra-violet resistance but poor resistance to acids and bases. PHB is characterized as having a high molecular weight with a narrow polydispersity and a crystallinity of around 50%. Depending on the crystallinity, PHB has the melting point of 171°C with a T_g at 9°C. PHBs are water insoluble and relatively resistant to hydrolytic degradation [58]. In vitro hydrolytic degradation of microspheres made of PHB degradated about 20-40% after 5 months at 85˚C which is very long time for a biodegradable polymer [59]. The factors affect the biodegradability of PHB depend on the characteristics of PHB such as stereospecificity, crystallinity, molecular weight and monomeric composition. Stereospecificity has influence on degradation because only monomers are in R configuration are hydrolyzed by depolymerases. High degree of crystallinity and high molecular weights of the polymers result in lower degradation rates.

PHBs are getting popular with biodegradability, mechanical and physical properties. The major disadvantage of the PHB is high production cost. Researches on production of PHB from cheap raw materials are still in progress.

2.1.2 Polyanhydrides, (PA)s

Polyanhydrides were synthesized by Bucher and Slade in 1909 but first utilization of PAs for controlled drug delivery was in 1980 [60]. But today, PAs have been used in various forms to deliver diverse active agents and other biomedical applications. PAs can be synthesized with different specific properties such as crystallinity, degradation behavior, crosslinking or branching etc. A broad variety of PAs has been synthesized over years, based on one or a combination of aliphatic, aromatic, and heterocyclic monomers [61]. There are various kinds of polyanhyrides depending on the monomer type. Some examples of PAs are shown in Table 2.2.

Table 2. 2: Molecular structures of common polyanhydrides.

Main Structure	R Group	Structure of R Group
	n = 4, adipic acid n = 6, suberic acid	
	p-Carboxyphenoxypropan	
	Fumaric acid (FA)	
	Terepthalic acid (TA)	
	Isopthalic acid (IPA)	
	Fatty acid dimer (FAD)	

Several techniques exist in literature to synthesize polyanhydrides such as melt condensation, ROP, interfacial condensation, dehydrochlorination, and dehydrative Coupling agents shown in Figure 2.9 [62].

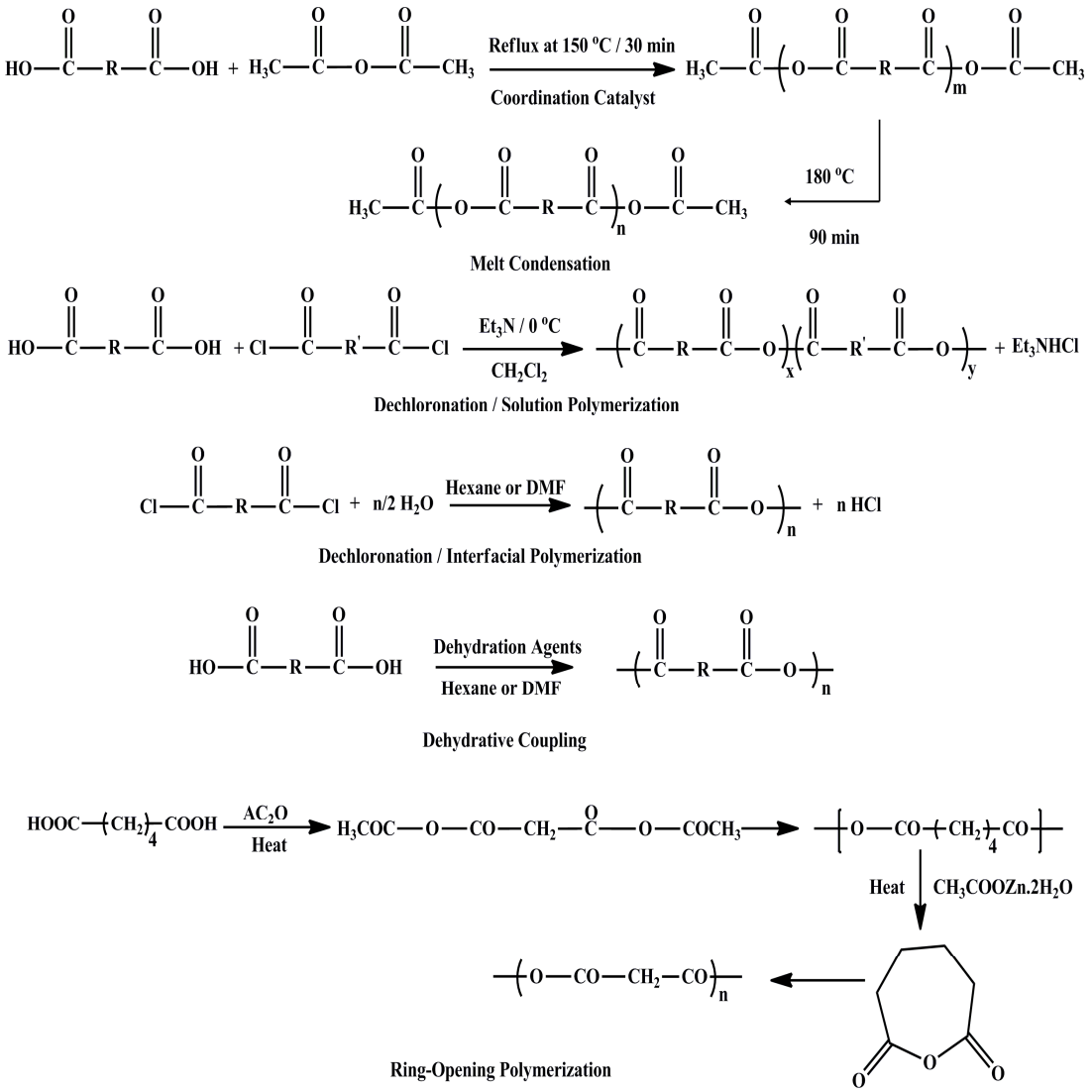


Figure 2. 9: General synthesis schemes of polyanhydrides.

The common way of synthesizing PAs is melt polycondensation. A dicaboxylic acid is refluxed with an excess of acetic acid anhydride yielding a solution of mixed anhydride. The excess acetic anhydride is removed in vacuum at high temperatures, resulting in high polyanhydrides [63]. Also, photo initiated thiol-ene chemistry can successfully be used to obtain linear and crosslinked polyanhyrides [64]. Many kinds of catalysts can be used in PA synthesis varying by the type of the techniques.

Many polyanhyrides have low melting points and are soluble in common organic solvents. Some PAs are nontoxic and degrade finally into diacids which are either

excreted as such in feces/urine or undergo extensive metabolism to form carbon dioxide and water in the body.

Surface erosion is an important issue to control over release. Expectation from a surface eroding polymeric carrier is releasing drug at a constant rate which is proportional to surface eroding rate of the polymer. The polymer should have water labile linkages but should be hydrophobic. PAs are believed to predominantly undergo surface erosion due to the high water lability of the anhydride bonds on the surface and hydrophobicity, which restricts water penetration into the bulk [65]. pH of the surrounding media is important on hydrolytic degradation of polyanhydrides due to nature of base catalyzed anhydride bond. High hydrolytic reactivity of the anhydride linkage provides an intrinsic advantage in versatility and control of degradation rates. By varying the type of monomer and their ratios, surface - eroding polymers with degradation times of 1 week to several years can be designed and synthesized.

2.1.3 Aliphatic polycarbonates

Aliphatic polycarbonates gained much attention owing to their restorability and they are used as polymeric additives for plasticizing properties [66]. Polycarbonates are linear thermoplastic polymers of carbonic acid and aliphatic dihydroxy compounds. Aliphatic polycarbonates can be synthesized from the reaction of dihydroxy compounds with phosgene or bischloroformates of aliphatic dihydroxy compounds by transesterificaiton and ring-opening polymerization of cyclic carbonates, commonly 5 or 6 membered ring monomers [66]. Suggesting that the carbonate bond may be labile to hydrolysis a biodegradation study is performed [67]. According to *in vivo* studies of poly(ethylene carbonate) and poly(propylene carbonate) a weight loss of polymer pellets is monitored. But *in vitro* studies showed that of poly(ethylene carbonate) completely depredated in 15 days while poly(propylene carbonate) pellets did not degradade even after an incubation of 40 days in PBS with pH 7.4 at 37˚C. Monitoring a weight loss as a result of degradation of poly(propylene carbonate) pellets in *in vivo* studies while there is no change in *in vitro* researches indicate that, the polymer pellet was degraded by enzymes [67].

Copolymer of aliphatic polycarbonates and lactides showed excellent mechanical properties and biocompability. Especially, poly(trimethylene carbonate), (PTMC),

had been investigated in biomedical applications shown in Figure 1. PTMC has low T_g of -17 ˚C and it stays rubbery at room temperature. Degradation of PTMC occurs by both hydrolysis and enzymatically [68]. Polycarbonate bond is more sensitive than polyester linkage but PTMC hydrolyses very slowly due to lack of an autocatalytic process. Total degradation of PTMC may take years [69]. Degradation products of PTMC are carbon dioxide and diols. Copolymers of PTMC with various polymers have been reported to be useful matrix for biomedical applications [70].

2.1.4 Poly(orthoester)s, POE

Poly(orthoester)s developed to meet the needs for an ideal biodegradable polymer such as surface erosion, non-toxic degradation products with low molecular weight and controllable degradation rate. Erosion on the surface should be faster than interior part of the particle to control the degradation rate by surface erosion. Degradation rate slowly increases as the pH of the surrounding medium decreases due to the orthoester linkage. Polymeric carriers made of POEs which has a sensitive bond to acids may be successful if the interior part of the carrier buffered by basic salts. Poly(orthoesters) are divided into four different classes shown in Fig. 2.10.

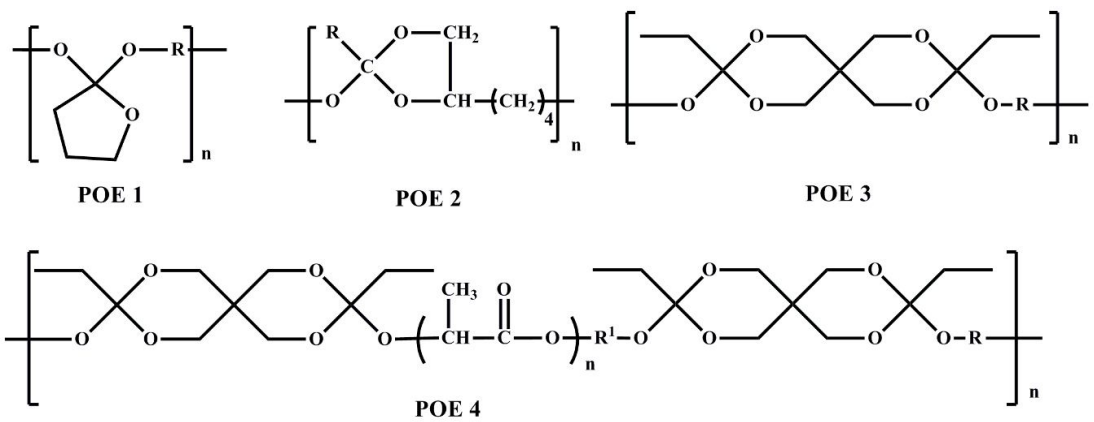


Figure 2. 10: Classes of poly(orthoester)s

POE 1 was first synthesized by ALZA Corporation in 1970s [71]. POE 1 undergoes an autocatalytzed and uncontrollable hydrolysis as a consequence of acid labile natures. First hydrolysis product of POE1 is butyrolactone which finally hydrolysis into butyric acid shown in Figure 2.11.

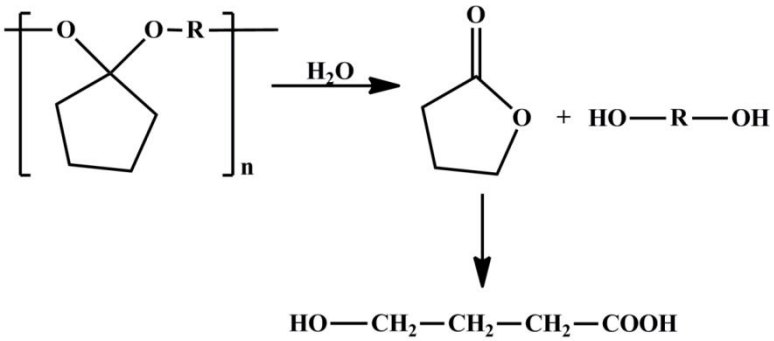


Figure 2. 11: Hydrolysis of POE 1.

Prevention of POE 1 from autocatalyzed hydrolysis a base must be added to stabilize the polymer. Poor mechanical properties, difficulties in synthesis and uncontrollable hydrolysis of POE 1, blocked the commercialization of the polymer and therefore POE 1 is no longer under investigation.

Researches on POE 2 showed that the polymer has high biocompability with great drug release profile [72]. Nevertheless, being unrepeatable in synthesis and difficulties in scaling up the reaction also limited the commercialization of the polymer [72].

In spite of problems faced in POE 1 and POE 2, the other two structures POE 3 and POE 4 are quite successful. For instance, POE 4 has completed Phase 2 and Phase 3 clinical trials on delivery of granisetron and mepivacaine, respectively. [73]. Extensive researches on POE 3 are still ongoing [73].

POE 3

POE 3 can be obtained by the reaction of diketene acetal with 3,9 diethylidene-2,4,8,10 - tetraoxaspiro undecane as shown in Figure 2.12 [73].

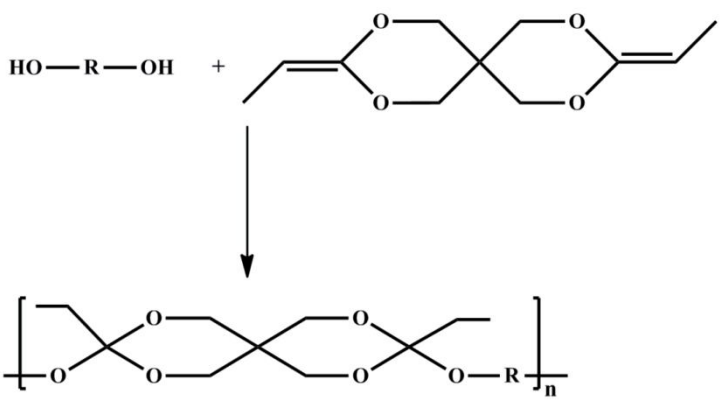


Figure 2. 12: Synthesis of POE 3.

But the 3, 9 diethylidene-2, 4,8,10 - tetraoxaspiro undecane is not commercially available. It must be synthesized individually by the rearrangement of diallyl pentaerytritol as shown in Figure 2.13 [73].

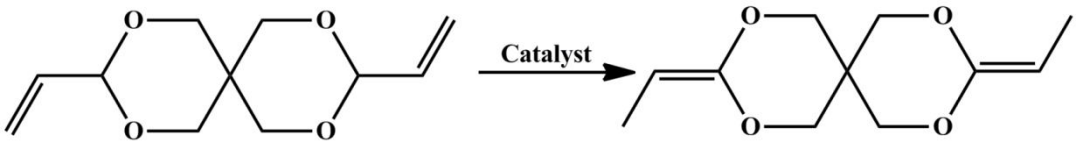


Figure 2.13: Synthesis of 3, 9 diethylidene-2, 4,8,10 - tetraoxaspiro undecane (DETOSU).

Instead being very labile in aqueous environment, the nature of the polymer is hydrophobic. Because of this reason hydophilicity of the polymer must be adjusted to control the surface erosion rate of the polymer.

POE 4

Development of POE 4 is consequence of uncontrollable surface erosion of POE 3. POE 4 can be obtained from the reaction of the diketene acetal, 9 diethylidene-2, 4,8,10-tetraoxaspiro undecane, a diol or mixture of diols with latent acid diol as shown in Figure 2.14 [73].

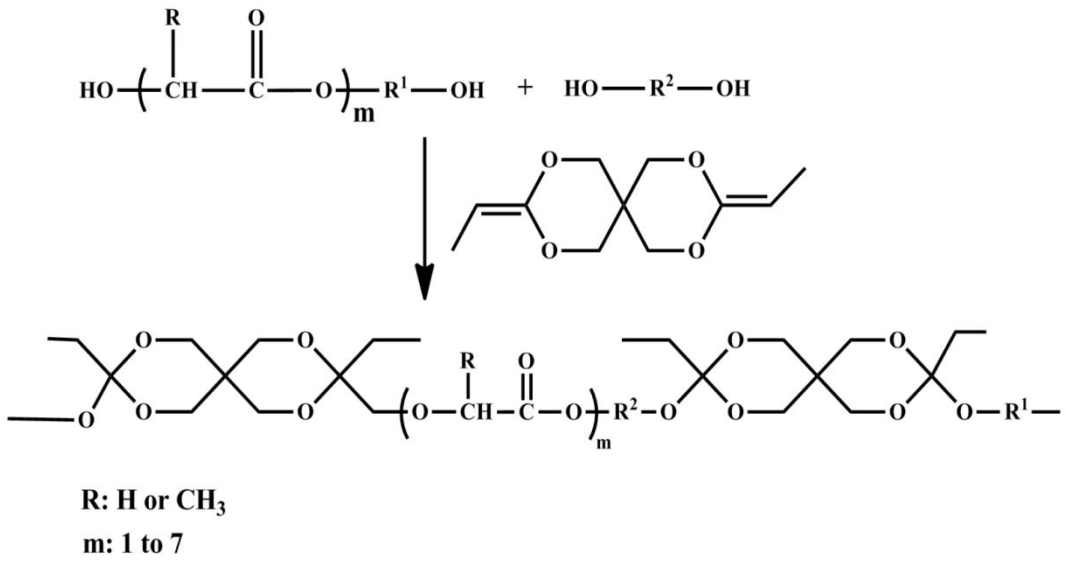


Figure 2. 14: Synthesis of POE 4.

Latent acid is used to control the surface erosion rate by α-hydroxy acid segments. Latent acid is synthesized from the reaction between a diol and ether lactide or glycolide as shown in Figure 2.15 [73]

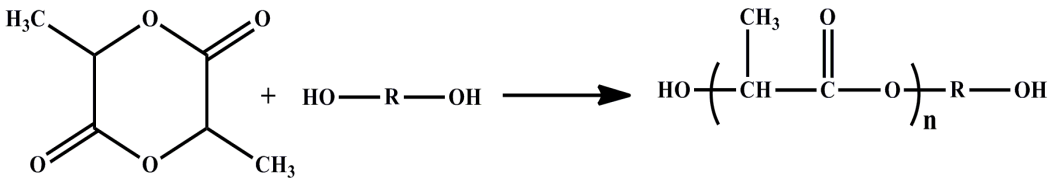


Figure 2. 15: Synthesis of latent acid based on lactide.

Having various diols at preparation stage, gives the opportunity to synthesize polymers with a wide range of different physical properties. A mixture of rigid diol and flexible diol can be used to control the mechanical properties of the polymer.

2.1.5 Poly(ethylene glycol), PEG

Poly(ethylene glycol) contains ether linkages in repeating units of the polymer which has many application fields differ from industrial manufacturing to medicine. PEG is a hydrophilic, non-ionic polymer with excellent biocompability which also approved by FDA. It has also been known as poly(ethylene oxide) or poly(oxy ethylene) depending on its molecular weight. The trade name for PEG is Carbowax [74]. The polymers with a molecular weight below 20.000g/mol is called as PEG where polymers with a molecular weight above 20.000g/mol is called as poly(ethylene oxide) (POE). Poly(oxy ethylene) is used for any molecular weight. PEGs are synthesized from the reaction of ethylene oxide with water, ethylene glycol or ethylene glycol oligomers and commercially available over a wide range of molecular weights [75]. Ethylene glycol or ethylene glycol oligomers are preferable against water because the capability of producing polymers with low polydispersity. The reaction is generally catalyzed by acidic or basic reactants. Figure 2.16 shows the molecular structure of PEG [76].

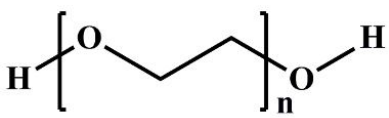


Figure 2. 16: Poly(ethylene glycol).

The catalyst type determines the kind of polymerization that can be either anionic or cationic. Anionic type of polymerization leads producing PEG with low polydispersity. POEs are produced by suspension polymerization where magnesium-aluminum-, or calcium-organoelement compounds used as catalyst [77]. Chelating

additives such as dimethylglyoxime are used to prevent coagulation of polymer chain from solution.

PEG and POE are liquids or low-melting materials. The chemical properties of these polymers are nearly identical. Applications of PEG and POE change due to different physical properties due to chain length effects. PEG and POE can used as non-ionic surfactants, coupling agent, humectants, lubricants in cosmetics, antifoams, seal lubricants, plasticizer to increase the lubricity, cryogenic fluids, anti-dusting agents in agricultural formulations, dye carriers in paint and inks, softener and anti-static agent for textiles, and etc. Many different forms of PEG exist dependent on the initiator used in polymerization [78]. The most common example is monofunctional PEG which is called methoxypoly(ethylene glycol) (mPEG).

PEG with a molecular weight about 1000g/mol is a crystalline, linear polymer. The crystalline melting point of the polymer increases with molecular weight. PEGs and copolymers with high PEG content are generally water soluble.

PEG has variety of usage areas in biomedical applications according to non-toxicity and solubility in water. For instance, when PEG is attached to protein medications the polymer slows the clearance of the carried protein from the blood [79]. It allows longer dosing intervals. Another commercialized example of the polymer is PEG-interferon alpha which is used to treat hepatitis C. Also, PEG-filgrastim is used to treat neutropenia. The most common usage of PEG is fusing B-cells with myeloma cells in monoclonal antibody production [80]. Copolymers and blends of PEG are used in fabrication of microspheres for controlled drug delivery applications where PEG is used to control the release rate of the drugs by the degradation of polymers [81].

2.2 Polymer Degradation and Erosion

Degradation of the biodegradable polymers is as important as their synthesis and properties, in biomedical applications. The polymer must perform its duty while degradation occurs in a predictable way. Degradation products of the polymer must be non-toxic and must have no harm to the other organs or organ functions. Polymer degradation is a change in the properties of polymer like tensile strength, color, shape etc. by chemical reactions resulting in a cleavage of main chain bonds. Generally,

polymer degradation begins with hydrolytic and/or enzymatic attack which results in shorter oligomers, monomers and low molecular weight products [11]. If the degradation occurs via environmental action, bacteria or enzymes it is called biodegradation or biotic degradation and polymer is considered as biodegradable polymer [11-82].

Bulk and surface erosion are two different mechanisms discussed in the literature for biodegradation [83]. In the bulk degradation process, water diffuses into the polymer matrix faster than the polymer is degraded. The hydrolysable bonds in the whole polymer matrix are cleaved homogeneously. Therefore, the average molecular weight of the polymer decreases homogeneously. Release of the drug occurs by diffusion which is related to concentration of the drug. The bulk degradation process is shown in Figure 2.17. In the case of surface erosion, the diffusion rate of water into the polymer matrix is slower than the degradation rate of the macromolecules. The degradation only takes place in the thin surface layer while the molecular weight of the polymer in the bulk remains unchanged. Surface erosion is a heterogeneous process, with a rate strongly dependent on the shape of the test sample [84]. The surface erosion is shown in Figure 2.18. But achieving surface erosion is difficult. Generally, biodegradable polymers are not hydrophobic enough to keep water from penetrating into material to let the surface layer to erode [85]. Release of the drug occurs in zero-order fashion by surface erosion.

Figure 2. 17: Bulk erosion of a degradable polymer.

Figure 2. 18: Surface erosion of a degradable polymer.

Erosion rates can be determined *in vitro* and *in vivo* [84]. For *in vitro* experiments, the polymers are exposed to an aqueous solution, in which ionic strength, pH-value, and temperature can be varied. Additionally, polymers can be tested on cell and tissue cultures. Determination of the degradation behavior of polymers under suitable

conditions for *in vivo* experiments gives information on the compatibility of the partially degraded polymer samples and their degradation products.

Chemical structure, molecular weight and polydispersity, crytallinity, water solubility and permeability, mechanism of hydrolysis, method of chain scission, morphology of the polymeric carrier, porosity, method of sterilization and site of application are known as main factors that affect degradation and the life time of biopolymers [11]. The most important parameter of the degradation is chemical structure and hyrophilicity/hyrdphobicity of the polymer [85]. Hydrophilic polymers are known as bulk-degrading polymers where surrounding aqueous media diffuses into polymer matrix. Polymers made of hydrophobic backbone with hydrolytically non-labile linkages are very slow degradating polymers but polymers synthesized from hydrophobic monomers having labile bonds which do not allow the penetration of water but erodes from the surface are called surface-degrading polymers. Mass loss of surface-degrading polymers does not change significantly because the monomer is so hydrophobic that they do not diffuse into the surrounding medium. Natural polymers are generally hydrophilic and undergo bulk degradation and enzymatic degradation [86]. A few synthetic polymers undergo enzymatic degradation such as polyesters. Instead of hyrophlicity of the polymers, solubility of the degradation product is an important issue, too. Lower molecular weight of the degradation products with higher aqueous solubility result in faster degradation and easier evacuation from the body. Some of the by-products of degradation are hydrophobic, as in the case of polyanhydrides, make the degradation slow and accumulate on the polymer matrix [86]. Another situation caused by the degradation products is auto catalytic effect. The catalytic effect usually occurs by the pH change of the surrounding medium because of the degradation products. Free radical production during degradation sometimes causes chain scission in some polymers.

Having high number of molecular weight distribution increases the degradation rate where there are more groups to accelerate the chain scission that lead faster degradation of the polymer. A few polymers exist with completely crystalline structure as they degrade compared to their amorphous analogues. Molecular arrangement of crystalline polymers does not allow water to penetrate into polymer matrix due to higher lattice energy [86].

Glass transition temperature has an influence on degradation of amorphous structures. Polymers with higher T_g, degredates slower in the same class of polymers due to increasing molecular weight. Also, T_g is the proof of that, the molecule is stable in particular molecular configuration which may cause the hydrophobic interaction resulting in slower degradation rate.

As the size of the polymeric carrier increases, the time is required for complete degradation increases. The effect of increase in the size of device is more clearly seen in surface-degrading polymers than bulk-degrading polymers as the degradation takes place throughout the matrix [87].

Porosity changes the surface-to-volume ratio which affect the degradation rate by the increase in the surface area inturn exposes more surfaces to the hydrolytic media results in faster degradation rate [88].

Drug containing additives, may change the degradation rate of the polymer by affecting the polymer due to the hydrophilicity/hydrophobicity of the drug [89].

Processing the polymer during sterilization or modification, physical or chemical structure may be affected. Any change in chemical structure of the polymer affects further degradation profile [90].

Location or site of the drug affects degradation by blood flow, movement of the device or loading of the device. If the environment where the polymeric carrier exists causes faster removal of the drug, degradation of the polymer increases if degradation occurs via surface eroding.

2.3 Controlled Drug Delivery

Several routes of administration of drugs exist such as tablets, injections etc. Entire dose of drug administers by this way which plasma concentration of drug sometimes close to toxic [91]. The therapeutic effect cannot be achieved by only administration of drug just once. Prolonged drug delivery system is needed to control the plasma concentration of drugs to overcome this problem. Releasing the drug in controlled fashion should provide the drug only where and when it is needed for long periods of time. Figure 2.19 shows the therapeutic effect as the plasma concentration of drug as a function of time after administration of drug.

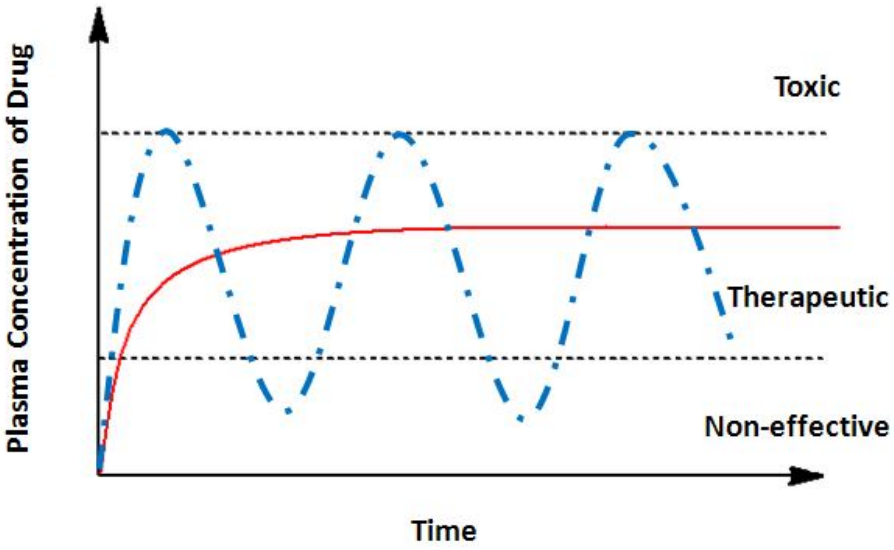


Figure 2. 19: (. _ . _) traditional delivery system, (____) prolonged delivery system.

Controlled release systems must be able to carry required amount of drug needed to treat the tumor, deliver the drug within a specific period of time and must be biocompatible. Controlled release systems vary by administration route and drug release mechanism. For instance diffusion controlled membranes which a reservoir encapsulates all drug and released by rate controlling polymer matrix. Another example of controlled release system is cross-linked hydrophilic polymers, hydrogels; drug is released by diffusion from network which is insoluble in surrounding medium [92]. Commonly, polymeric carriers are preferred in controlled release systems. Polymeric carriers are biodegradable or water soluble polymer matrices which encapsulate drugs without any chemical bonding. The drug is released by degradation and erosion of the polymeric structures. Controlled release fashion helps to protect therapeutic agents from physiological degradation that peptides, proteins, enzymes and some drugs lose their activity before reaching targeted area. Depending on the needs, polymeric carriers can be placed to the targeted tissue by surgery to improve efficiency of therapeutic agents.

2.3.1 Release of therapeutic agents

Release of therapeutic agents depends on the erosion of the polymer, dissolution of drug in the surrounding tissue and diffusion of the drug from polymeric carrier [93]. Release rates of therapeutic agents are related to hydrophilicity, flexibility, crystallinity, degradation rate, molecular weight of the polymers used as carrier.

Also, size, shape and the porosity of the carrier affect the release mechanism. Properties of the drugs such as size of drug molecule, solubility in water, and molecular weight have influence on release kinetics, too. The relationship between time and the amount of drug released is proportional to the square root of time. But, desired drug release based on zero-order kinetics which is almost unattainable.

2.4 Microspheres

Microspheres have received significant amount of interest after adaptation into biomedical applications for regeneration of tissue, targeted delivery of drugs, peptides, proteins, enzymes, etc. [94, 95]. Microspheres are small particles in a range between 1µm and 1000µm. Microspheres which are aimed to use in biomedical applications should be easy to fabricate, biocompatible and biodegradable [96]. Figure 2.20 shows the microparticle types as drug carriers made of biodegradable polymers.

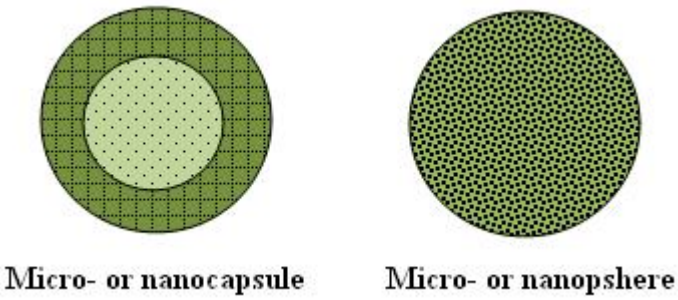


Figure 2. 20: Polymeric micro- or nano- capsules or spheres.

Microspheres can be prepared from natural or synthetic polymers. For instance polystyrene microspheres are used in cell sorting and immunio precipitation that, proteins and ligands adsorb onto polystyrene make it suitable for medical applications [97]. The most common used microspheres are made from aliphatic polyesters such as PLGA, PLA, PCL, PHB, etc.

Therapeutic agent, carriers are divided in to three groups as first-, second- and third-generation of carriers. The first generation of carriers are microspheres/microcapsules which able to deliver active substances to the targeted area. But they have to be implanted as closely as to the site of action. These systems are generally used for controlled release of proteins or peptides. The second generation of carriers are not only release the active products to the indented area but

also of carrying it there after administration of general route. This group is called as passive carriers and includes liposomes, nanocapsules or nanaospheres. But rapidly removal of colloidal carriers by phagocytic cells exist in the liver and spleen limit their utilization. The third generation carries are capable of specific recognition of the target that monoclonal antibodies belong to this group.

Table 2. 3: Definition of Different Carrier Systems.

Generation	Size	Definition	Examples
First	>1 µm	Systems able to release a drug at the target site but in a particular type of administration	Microspheres and microcapsules for controlled release of proteins and peptides
Second	<1 µm	Carriers able to transport a drug to the target site by general type of administration	*Passive Carriers:* Liposomes, nanoparticles, erythrocytes *Active Carriers:* Temperature or pH sensitive liposomes, magnetic nanoparticles
Third	<1 µm	Carriers able to recognize the target spesifically	Monoclonal antibodies, second-generation carriers

Therapeutic agent release from micropheres, such as PLLA and PLGA, is generally controlled by both drug diffusion and polymer erosion. In the diffusion process, water diffuses into the polymer matrix faster than the polymer is degraded. The hydrolysable bonds in the whole polymer matrix are cleaved homogeneously. Therefore, the average molecular weight of the polymer decreases homogeneously. Release of the drug occurs by diffusion which is related to concentration of the drug. In the case of surface erosion, the diffusion rate of water into the polymer matrix is slower than the degradation rate of the macromolecules. The degradation only takes place in the thin surface layer while the molecular weight of the polymer in the bulk remains unchanged.

2.4.1 Preparation methods of microspheres

There are several techniques exist for fabrication of microspheres which affect characteristics of microspheres. An encapsulation technique should provide the following requirements [98].

- Microsphere production should not have any influence on stability and the biological activity of the drug.
- Size of the fabricated microspheres must be <125 µm with high encapsulation efficiency.
- Fabrication of microspheres and release of drug must be repeatable.
- The microspheres should not exhibit aggregation or adherence.

The choice of the microencapsulation technique depends on the nature of the polymer, usage area and the time required for the therapy. Generally solvent evaporation, spray dry and phase separation methods are used in preparation of microspheres. Solvent evaporation method is the most common technique among these approaches.

2.4.1.1 Single emulsion method

The single emulsion technique requires the emulsification of an oil phase in a continuous aqueous phase (o/w) or an oil phase (o/o). The o/w method needs an emulsifier to obtain o/w emulsion. Poly(vinyl alcohol) (PVA) and dichloromethane (DCM) are relatively the most common used emulsifier and organic solvent for fabrication of microspheres. The emulsion (o/w) stirring speed should be reduced and let it at reduced pressure or atmospheric pressure for evaporation of organic solvent to harden the oil droplets [99]. Then, the solid microspheres obtained via filtration and should be dried under vacuum. There are many facts that influence the structure of microspheres as:

a) structure and solubility of the drug ,
b) concentration and molecular weight of the polymer,
c) drug/polymer ratio,
d) choice of organic solvent,
e) concentration and the type of surfactant,
f) temperature,
g) stirring speed,
h) viscosities and volume ratios of continuous phase.

Solvent evaporation process is similar to the extraction method where the solvent must first diffuse out into the external aqueous dispersion medium before it could be removed by evaporation. The rate of the solvent removal strongly affects the chacteristics of final microspheres which depends on the temperature, pressure

and the solubility parameters of the polymer. Very rapid solvent evaporation may be resulted in local explosion of inside the droplets and increased porosity on the surface of the microspheres [100].

A disadvantage of the singe emulsion method is poor encapsulation efficiencies of hydrophilic drugs. The drug would diffuse out to the continuous phase from oil phase. As a result, hydrophilic drugs get deposit on the microspheres which finally affect the release studies, initial bursts and release time of the drug.

Steroids are generally preffered drugs for single emulsion method due to lipid soluble nature.

Oil in oil technique was developed to increase encapsulation efficiencies of hydrophilic drugs. Oil in oil processes tend to be complex techniques requiring the removal of oil from final product. This method can be used to encapsulate hydrophobic drugs in the first oil phase. Acetonitrile is use to solubilise the hydrophilic drug which also solubilize the polymers intended to use. The choice of acetonitrile depends on the miscibility of the solvent in water. The solution consists of the polymer and the drug is dispersed in an oil phase such as light mineral oil in the presence of a surfactant [101]. Then, the microspheres obtained by evaporation or extraction of oil phase. At the final step the oil is removed from microspheres by hexane. Sometimes this method is called as water-in-oil (w/o) emulsification technique.

2.4.1.2 Double emulsion method

The double emulsion technique requires the emulsification of an aqueous solution in an oil phase (w/o) which is than emulsified in a continuous aqueous phase (w/o/w) [102]. A schematic diagram of double emulsion technique is shown in Figure 2.21. Double emulsion method is a proper way for encapsulation of peptides, drugs, proteins and vaccines which are soluble in water.

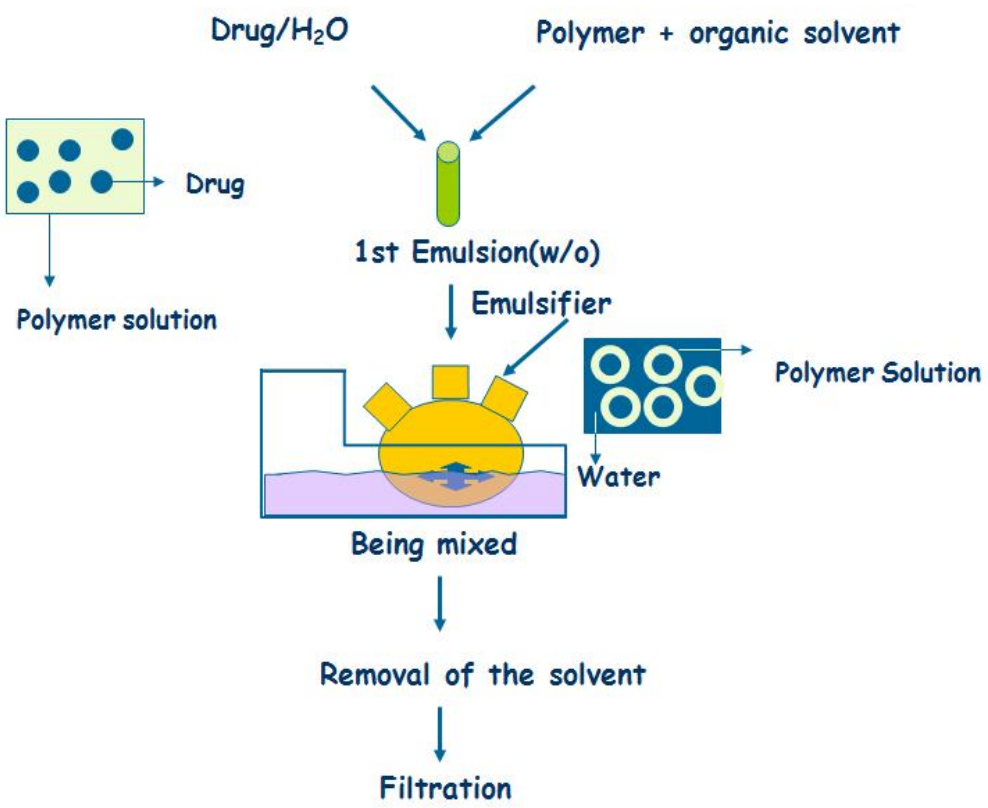


Figure 2. 21: A diagram of double emulsion technique.

An aqueous or buffered solution of the drug is added to an organic phase containing polymer with vigorous stirring to form the first emulsion (w/o). This emulsion is added to large volume water containing an emulsifier to obtain w/o/w emulsion. Then evaporation or extraction processes take place to remove organic phase. The solid phase is washed and collected by filtration, sieving or centrifugation. Ethyl acetate, dichloromethane and hydrophilic stabilizers may be used in double emulsion method. Peptides, leuprolide acetate [103], a luteinizing hormone-releasing hormone agonist [104], vaccines, protein/peptides and conventional molecules are successfully encapsulated by w/o/w method. This method is popular among other preparation techniques owing to ease of preparation but low entrapment efficiency and high initial burst releases are major problems of the method. This situation may be attributed to water soluble drug diffusion during hardening phase of microspheres [105].

2.4.1.3 Phase separation (Coacervation) method

This method is carried out by reducing the solubility of polymer as consequence of adding the third component, such as salts or changing pH, to the organic phase which is a colloid phenomenon. The process yields two liquid phases: the polymer containing phase (coacervate) and the supernatant phase depleted in polymer. The drug is dissolved in the polymer solution and coated by the coacervate. The coacervation process includes three steps:

1. Phase separation of the coating polymer solution
2. Adsorption of the coacervate around drug particles
3. Solidification of microspheres

The polymer intended to use first dissolved in an organic solution than the drug is added to the organic phase if the drug is hydrophilic than aqueous solution of the drug is added (w/o emulsion). An organic non-solvent is added to the system with stirring resulting in extraction of the polymer solvent. Then, a phase separation occurs and soft coacervate droplets form which entrap the drug. Hardening of the microspheres is done by transferring the system to a large quantity of organic non-solvent. The final microspheres are collected by filtration, sieving or centrifugation [106]. This method is suitable for both hydrophilic and hydrophobic drugs unlike o/w process. But, generally this method is used to encapsulate hydrophilic drugs such as peptides, proteins and vaccines.

The concentration of the polymer is important where higher concentrations cause rapid phase separation and non-uniform microspheres. Agglomeration is a common problem in these systems due to the absence of emulsion stabilizer. The coacervate droplets adhere each other before complete phase separation. Adjustment of stirring rate, temperature and addition of an additive is enough to overcome this problem [106].

Requirements of solvents are easier in phase separation technique compared to solvent evaporation methods. The nonsolvents affect the hardening and phase separation stages. The polymer and the drug should be non-soluble and also miscible in nonsolvent. The second nonsolvents should be easy to remove. Silicone oil, vegetable oils, low molecular weight polybutadienes can be used as first nonsolvents in phase separation method [106]. Aliphatic hydrocarbons such as hexane, heptane and petroleum ether can be used as second nonsolvents. [106].

2.4.1.4 Spray dry method

The spray dry technique has been widely employed for the fabrication of micro particle systems. Injectible microspheres made of biodegradable polymers can be synthesized by the spray dry method. This method can be applied to heat resistant, heat sensitive, water-soluble and water insoluble drugs, as well as to hydrophobic and hydrophilic polymers [106]. The phase separation method has difficulties such as mass production, requiring large quantities of organic solvents, removal of residual solvents. Also, double emulsion method requires many steps with low entrapment efficiencies and high burst releases. In contrast, spray drying method is rapid and convenient. The technique can be adapted to industrial scale procedures. The solubility of the drug and the polymer is less dependable in this method. The method is done by spraying polymer containing solution through heated chamber which lead polymer precipitation [107].

There only may be a loss of product during spray-drying caused by the adhesion of the microspheres to the spray drier apparatus. A double-nozzle spray drying technique is developed to overcome this problem which includes mannitol as an anti-adherent.

2.4.2 Controlled release applications

Acceleration on the formulation of drug delivery devices has increased rapidly, since injectable microspheres are used in treatments. Injectable microspheres are administrated when insertion of large implants avoided. Microspheres larger than 6μm are not used due to acute toxicity reaction associated with capillary blockage in pulmonary circulation. Microspheres are used in delivery of peptides, vaccines and variety of drugs. Especially, targeted drug delivery is the focus point of the studies. Targeted delivery of drugs can be summed up in three subjects such as oral and brain delivery and cancer therapy.

An example of oral delivery of polymeric carriers is the uptake of microspheres by the Peyers Patches in gut associated lymphoid tissue. Peyer Patches are determined by M cells that overlay the lymphoid tissue. The aim is to bind the polymeric carriers to the apical membrane of the M cells. There have been only two published phase I clinical trials on oral uptake of polymeric carriers. Another application of microspheres is the mucosal delivery of cancer vaccines. For this system,

microspheres are designed to increase the uptake of antigens by the M cells to enhance the mucosal immune response and to induce long and slow antigen release. This approach is validated by showing oral immunization of mice with phosphorylcholine. The aim of cancer vaccines are not only target and kill tumor cells in a specific manner, but also to alert immune system, so the residual tumor kept in check.

Brain delivery of microspheres is a challenging issue because of restricting effect of the blood brain barrier. The most achieved work in this area is delivering drugs via receptor-mediated transcytosis without damaging blood brain barrier by pegylated immunoliposomes [108]. Copolymers of PEG with different functionalities had been synthesized for this purpose [109]. Administration of the polymeric carriers which are not able access through blood brain barrier should be done by surgery.

The most targeting application of polymeric carriers is against cancerous cells and tumor targeting with incorporation of chemotherapeutic agents. Tumors can be exploited by the polymeric carriers via overexperssion of specific antigens on cancer cells.

2.5 Applications of copolymers of biodegradable polymers

Biodegradable aliphatic polyesters have an extensive family of polymers which some of them are commercially available and have been widely studied for biomedical purposes. Copolymers of these biodegradable polyesters have the potential to tune up the properties for specific medical requirements. In this view of significance, some examples of copolymers of aliphatic polyesters on biological applications are given below.

Triblock copolymers of PLGA and PEG show thermoreversible characteristics. ABA and BAB type of copolymers where A and B represents PEG and PLGA respectively, are good candidates for slowly dissolving implants and drug delivery systems [110, 111]. Jeong and coworkers has extensively studied on synthesis, characterization and use of PEG-PLGA-PEG copolymers as drug delivery systems [112,113]. If the polymer presents as a solution with a concentration below critical gel concentration, the solution would exist as a sol. But if the solution is heated above the critical gel concentration then the solution undergoes a phase transition to

a non-flowing gel [112, 113]. This property gives the opportunity for injection of the material as a liquid. The polymer turns into a semi-solid gel upon heating in the body. This phase transition property of brings convenience for many applications.

BAB type copolymer is commercialized under trade name ReGel and has been used for the release of insulin, porcine growth hormone, recombinant hepatisis B surface antigen and granulocyte colony stimulating factor [111, 114, and 115]. Nowadays, formulation of ReGel with paclitaxel under trade name OncoGel is under investigation for treatment of breast [111], pancreatic[116] and primary brain cancers [116].

Copolymers of polyanhdyrides can given as an another example of a biodegrdable polymers. Derivatives of polyanhydrides have also been used for delivery of proteins and peptides [117]. According to a study of Hanes group, poly(anhyride-co-imides) degrades by erosion which gives an opportunity to control release of drugs [118].

Poly(ε-caprolactone-co-glycolic acid) is a good example of biodegradable copolymers. The copolymer has variety of usage areas such as sutures, dermal tissue repair and controlled release applications. It is repeorted that, sutures coated with poly(ε-caprolactone-co-glycolic acid) copolymer is less stiff than sutures coated with other materials [119]. The copolymer has used as a wound-healing matrix in chronic wounds for dermal tissue repair [120]. Mostly synthetic and animal derived materials are used in this manner. A similar approach is developed by Yannas and Burke [121]. They used completely synthetic material instead of animal derived materials [121]. Poly(ε-caprolactone-co-glycolic acid) copolymer is still need to be improved for long term uses.

Bio-stable polyurethanes have been widely investigated as drug delivery devices. Having excellent biocompability in the body brought the need for biodegradable polyurethanes with it [122]. Biodegradable polyurethanes are good candidates for bone regenerative medicines. PLA, PGA and PCL are widely used as soft segments of polyurethanes [123]. Additionally, biodegradable polyurethanes are investigated as drug carrier system after administration of PLA, PGA or PCL segments [124].

In conclusion, copolymerization of common biodegradable polymers is a necessity. Improving the properties of the biodegradable polymers by copolymerization gives a great advantage on medical applications. Adjustment of controlled release of drugs, degradation profiles, biocompability, and strength of the tissues are just a few

examples of the benefits provided by copolymerized biopolymers. But still, having superior properties by copolymerization is needed to be improved for better results.

3. EXPERIMENTAL PART

3.1 Materials and Chemicals

3.1.1 Monomers

Styrene (S, 99%, Aldrich):

It was passed through a basic alumina column to remove the inhibitor before use.

4-vinyl benzyl chloride (CMS, 90%, Acros):

It was passed through a basic alumina column to remove the inhibitor before use.

L(-)-Lactide(LA, Polysciences):

It was crystallized from dry benzene.

Glycolide (GA, 99,9 %, Polysciences):

It was crystallized from dry benzene.

ε- Caprolactone (CL, %97 Aldrich):

It was distillated under vacumm to remove the inhibitor before use.

3.1.2 Polymers

Poly(DL-lactic-co-glycolic acid) (PLGA, 50:50, inherent viscosity (0.55-0.75), Lactel):

It was used as received.

Poly(DL-lactic-co-glycolic acid) (PLGA, 50:50, inherent viscosity (0.75-0.96), Lactel):

It was used as received.

Poly(DL-lactic-co-glycolic acid) (PLGA, 50:50, inherent viscosity (0.96-1.20), Lactel):

It was used as received.

Poly(DL-lactic-co-glycolic acid) (PLGA, 75:25, inherent viscosity (0.55-0.75), Lactel):

It was used as received.

Poly(DL-lactic-co-glycolic acid) (PLGA, 85:15, inherent viscosity (0.55-0.75), Lactel):

It was used as received.

Poly(vinyl alcohol) (PVA, 88% hydrolized, Mw: 25000g/mol, Polysciences):

It was used as received.

Poly(ethylene glycol) (PEG, M_w:2000, Polysciences):

It was used as received.

Poly(ethylene glycol) (PEG, M_w:750, Sigma-Aldrich):

It was used as received.

3.1.3 Solvents

Dichloromethane (J.T. Baker):

It was dried with calcium chloride and distilled over P_2O_5. It was stored over molecular sieves for use as a solvent in the photopolymerization experiments.

Methanol (Technical):

It was used for the precipitation of polymers without further purification.

Tetrahydrofuran (THF, 99.8%, J.T.Baker):

(a) It was used as eluent for chromatography as received (High Performance Liquid Chromatography Grade).

(b) For use in the chemical reactions, it was dried and distilled over benzophenone/sodium.

N,N-Dimethyl formamide (DMF, 99%, J. T. Baker):

It was used as recieved.

3.1.4 Other chemicals

Dicyclohexylcarbodiimide (DCC, Sigma-Aldrich):

It was used as recieved.

4-(N,N'-dimethyl)amino pyridine (DMAP, Sigma-Aldrich):

It was used as recieved.

Sodium azide (NaN_3, 99% , Aldrich):

It was used without further purification.

Copper(I) bromide (CuBr, 98%, Acros):

It was used as recieved.

Propargyl alcohol (C_3H_4O, 99%, Aldrich):

It was used without further purification.

2,2-dipyridyl (99%, Acros Organics):

It was used as recieved.

Imatinib Mesylate

It was kindly provided by Novartis.

Na_2HPO_4 (99.95%, Sigma Aldrich):

It was used as recieved.

KH_2PO_4 (99%, Merck):

It was used as recieved.

KCl (99.99%, Sigma Aldrich):

It was used as recieved.

NaCl (99%, Merck):

It was used as recieved.

Tin octoate ($Sn(Oct)_2$, Sigma Corp.):

It was used as recieved.

3.2 Equipments

3.2.1 Nuclear magnetic resonance spectroscopy (NMR)

(a) 1H NMR measurements were recorded in $CDCl_3$ with $Si(CH_3)_4$ as internal standard, using a Bruker AC250 (250.133 MHz) instrument.

(b) ^{1}H NMR and ^{13}C NMR measurements were recorded in $CDCl_3$ with $Si(CH_3)_4$ as internal standard, using Bruker Spectrospin Avance DPX-400 (400 MHz) spectrometer.

3.2.2 Infrared spectrophotometer (FT-IR)

FT-IR spectra were recorded on a Perkin Elmer FTIR Spectrum One B spectrometer.

3.2.3 Gel permeation chromatography (GPC)

(a) Gel permeation chromatography (GPC) analyses were performed with a set up consisting of a Waters 410 Differential Refractometer, a Waters 515 HPLC Pump and an apparatus equipped with three Waters ultrastyragel columns (HR series 4, 3, 2 narrow bore), with THF as the eluent at a flow rate 0.3 mL/min. Molecular weights

were calculated on the basis of a calibration curve recorded with mono disperse polystyrene standards.

(b) Gel permeation chromatography (GPC) measurements were obtained from a Viscotek GPCmax Autosampler system consisting of a pump, a Viscotek UV detector and Viscotek a differential refractive index (RI) detector. Three ViscoGEL GPC columns (G2000HHR, G3000HHR and G4000HHR), (7.8 mm internal diameter, 300 mm length) were used in series. The effective molecular weight ranges were 456–42800, 1050–107000, and 10200–2890000, respectively. THF was used as an eluent at flow rate of 1.0 mL min-1 at 30°C. Both detectors were calibrated with PS standards having narrow molecular weight distribution. Data were analyzed using Viscotek OmniSEC Omni-01 software. Molecular weights were calculated with the aid of polystyrene standards.

(c) The GPC set-up (TD-GPC) with an Agilent 1200 model isocratic pump, four Waters Styragel columns (guard, HR 5E, HR 4, HR 3, and HR 2), and a Viscotek TDA 302 triple detector (RI, dual laser light scattering (LS) (λ = 670 nm, 90° and 7°) and a differential pressure viscometer was conducted to measure the absolute molecular weights in THF with a flow rate of 0.5 mL/min at 35 °C. All three detectors were calibrated with a PS standard having narrow molecular weight distribution (M_n = 115,000 g/mol, M_w/M_n = 1.02, [n] = 0.519 dL/g at 35 °C in THF, dn/dc = 0.185 mL/g) provided by Viscotek company. Data were collected by using Omni-Sec version 4.5 software from Viscotek Company.

3.2.6 High pressure liquid chromatography (HPLC)

HPLC assays were performed on a a Nucleosil 100-5- C18 AB (Macherey-Nagel, Duren, Germany) column equipped with a guard column (8 mm x 4mm i.d.) filled with the same packing material. The imatinib mesylate concentration in aqueous phase was determined using HPLC (Shimadzu LC-10Ai, Japan). Imatinib mesylate was isolated isocratically at flow rate of 0.8 mL/min using mobile phase consisting of methanol:water (70:30) with UV detection at 261 nm.

3.2.5 Scanning electron microscopy (SEM)

The surface morphology of the microspheres was determined by scanning electron microscopy (JEOL JSM-633 5F, Oxford Instruments). Microspheres were mounted

on metal stubs with conductive silver paint and then sputtered with a 150-Å-thick layer of gold for SEM analysis.

3.2.6 Particle size analyzer

Mean particle sizes were determined using a Mastersizer X (Malvern Inst.).

3.2.7 Shaker

Innova 4230 ıncubator used as a shaker bath (37˚C) at 200 rpm.

3.2.8 Homogenizer

Laboratory Mixer-L4R (Silverson) used as homogenizer.

3.3 Preparation Methods

3.3.1 Preparation of imatinib mesylate loaded microspheres from various poly(lactic-co-glycolic acid) (PLGA) copolymers

Controlled release behavior of microspheres made of PLGA with different LA:GA ratios and molecular weights are studied. Microspheres were prepared by double emulsion solvent evaporation method (W/O/W). Briefly 0.5g of polymer was dissolved in 3 mL methylene chloride (DCM). 250 µl of aqueous drug solution was emulsified into the organic phase by vortex. This emulsion was added to 400mL aqueous phase containing 1% (w/v) PVA by drop wise and stirred at 1000 rpm for 5 min. The resulting solution was kept stirring at 500 rpm for 3 hours to allow solvent evaporation and hardening microspheres. The microspheres were then filtered and dried under vacuum. Final products were stored at -20 ºC.

3.3.1.2 Calculating the encapsulation efficiency of imatinib mesylate loaded PLGA microspheres

Encapsulated amount of drug was measured by suspending 10 mg PLGA microspheres in 1.5 mL dichloromethane. The samples were placed in a shaker maintained at 200 rpm for 2 hours. Then 2 mL of PBS buffer pH 7.4 is added and the samples placed back in the shaker for another 2 hours. The samples were taken out of shaker and centrifuged at 10.000 rpm for 5 minutes and 0.6 mL of supertant was

taken for analysis. The drug concentration in aqueous phase was determined by HPLC at 261 nm.

Actual drug loading and drug encapsulation efficiency (EE) were calculated using the following equations:

Theoretical drug loading = drug (tot.)/ (drug (tot.) +polymer)

Actual drug loading = drug (enc.) / (drug (tot.)+polymer)

Encapsulation Efficiency = actual drug loading/ theoretical drug loading x 100%.

3.3.1.3 *In vitro* release studies of imatinib mesylate loaded PLGA microspheres

In vitro release of imatinib mesylate from microspheres was measured by suspending 10 mg microspheres in 1 mL PBS buffer pH 7.4. The samples were placed in a shaker maintained at 37˚C and shaken at 200 rpm. At predetermined time intervals the samples were taken out of shaker and centrifuged at 10.000 rpm for 5 minutes and 0.6 mL of supernatant was taken for analysis and fresh medium of equal volume was added in the meantime. The precipitated microspheres was resuspended in the medium and placed back in the shaker. The imatinib mesylate concentration in aqueous phase was determined by HPLC at 261 nm.

3.3.2 Synthesis of polystyrene-*g*-poly (lactic acid) (PS-*g*-PLLA) and polystyrene-*g*-poly (lactic-co-glycolic acid) (PS-*g*-PLLGA)

PS-*g*-PLLA and PS-*g*-PLLGA are synthesized through combination of Nitroxide Mediated Free Radical Polymerization (NMRP) and Ring Opening Polymerization (ROP) with copper (I)-catalyzed "Click" chemistry. First, the click component, azido-functional PS (PS-N_3) was prepared by copolymerization of styrene and chloromethyl styrene by NMRP followed by the conversion of chlorine groups of the resulting copolymer to azido groups by using NaN_3 in N, N-dimethylformamide. Propargyl functional APEs were prepared independently by ROP of lactic acid and glycolic acid using tin octoate in the presence of propargyl alcohol at 125 ˚C. Finally, PS-N_3 was coupled with the resulting polymers by click chemistry to yield graft copolymers (PS-*g*-PLLA and PS-*g*-PLLGA, respectively).

3.3.2.1 Synthesis of poly(styrene-*co*-chloromethylstyrene), P(S-co-CMS) and polystyrene-azide PS-N_3

Poly(styrene-*co*-chloromethylstyrene), P(S-co-CMS), prepared via nitroxide-mediated radical polymerization (NMRP) of styrene (S) and chloromethylstyrene

(CMS) at 125 ˚C for 18 h. The obtained polymer precipitated from methanol and dried under vacuum. Chlorine units of the P(S-co-CMS) polymer successfully converted to azide groups. P(S-co-CMS) (1.00 g, 1.24 x 10^{-4} mol) dissolved in DMF and reacted with NaN_3 (8.85 x 10^{-3} g, 1.36 x 10^{-4} mol) 1.1 times excess in mol of Cl units of P(S-co-CMS) polymer at room temperature for 24 h. The resulting polymer was filtered and precipitated from methanol and finally dried under vacuum. The synthesis of P(S-co-CMS) and PS-N_3 is shown in Figure 3.1.

^{1}H-NMR ($CDCl_3$) δ (TMS, ppm) for P(S-*co*-CMS) : 7.04 -6.54 (aromatic protons), 4.5 (C*H*$_2$-Cl), 1.82-1.42 (aliphatic protons)

^{1}H-NMR ($CDCl_3$) δ (TMS, ppm) for PS-N_3: 7.04 -6.54 (aromatic protons), 4.25 (C*H*$_2$-N_3), 1.82-1.42 (aliphatic protons)

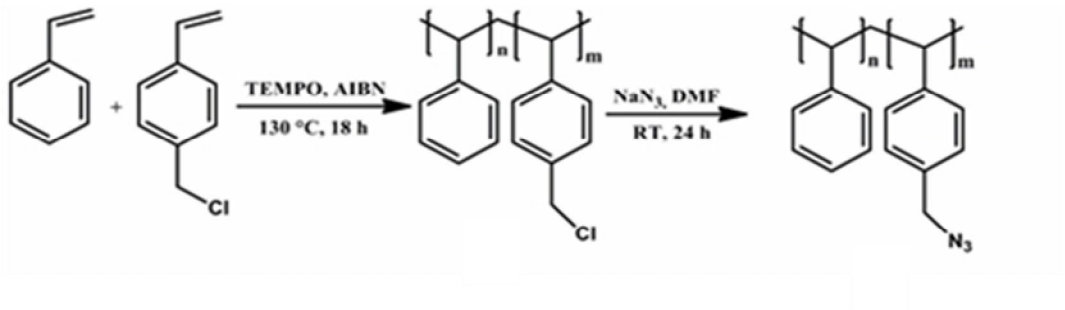


Figure 3. 1: Synthesis of P(S-co-CMS) and PS-N_3.

3.3.2.2 Synthesis of acetylene terminated PLLA and PLLGA

Acetylene terminated PLLA synthesis is conducted by ring opening polymerization of monomer. L-lactide (2.00g, 13.80 mmol) and propargyl alcohol (15.50 mg, 0.27 mmol) reacted in the presence of $Sn(Oct)_2$ (catalytic amount) at 125˚C under N_2 for 24 hours. The resulting polymer was diluted by dichloromethane and precipitated from cold methanol and finally dried under vacuum. The synthesis of acetylene terminated PLLA and PLLGA are shown in Figure 3.2.

^{1}H-NMR ($CDCl_3$) δ (TMS, ppm): 5.16-5.13 (C*H*-CH_3), 4.32 (C*H*$_2$-O), 2.50 (C≡C*H*), 1.58 (CH-C*H*$_3$).

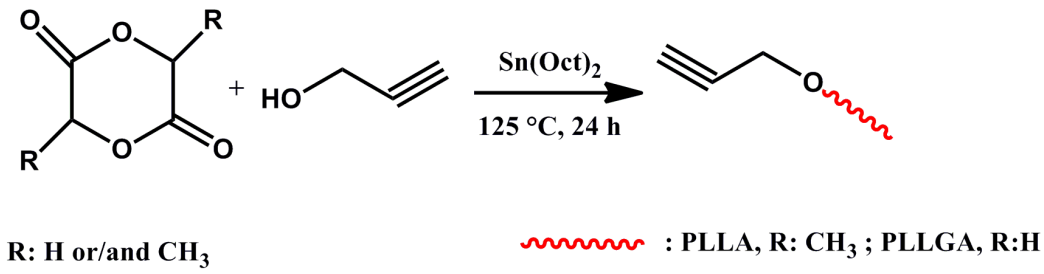


Figure 3. 2: Synthesis of acetylene terminated PLLA and PLLGA.

The same procedure using L-lactide-and glycolide with 3:1 mole ratios, respectively is followed for the synthesis of corresponding PLLGA.

^{1}H-NMR ($CDCl_3$)δ (TMS, ppm): 5.16-5.13 (C*H*-CH_3), 4.81 (CH_2-C=O), 4.32 (CH_2-O), 2.50 (C≡C*H*), 1.58 (CH-CH_3).

3.3.2.3 Click reaction of PS-N_3 and PLLA and/or PLLGA

To a roud bottom flask equipped with a magnetic stirring bar, PS-N_3 (1.00 g, 1.96 mmol), PLLA (1.94 g, 0.12 mmol), ligand (2, 2-dipyridyl, 0.24 mmol), catalyst (CuBr, 0.12 mmol) were dissolved in 7 mL of dry THF and stirred at room temperature under N_2. After the reaction, the mixture was diluted with THF and then passed through a column of neutral alumina to remove metal salt. The reaction mixture was concentrated and precipitated from cold methanol. The final product was washed with methanol and dried for 24 h in a vacuum oven at room temperature. The synthesis of PS-*g*-PLLA and PS-*g*-PLLGA are shown in Figure 3.3.

^{1}H-NMR ($CDCl_3$) δ (TMS, ppm): 7.04 -6.54 (aromatic protons), 5.16-5.13 (C*H*-CH_3), 4.20 (CH_2-triazole ring), 3.48 (CH_2-O), 1.58 (CH-CH_3).

Same procedure is followed for the PLLGA.

^{1}H-NMR ($CDCl_3$) δ (TMS, ppm): 7.04 -6.54 (aromatic protons), 5.16-5.13 (C*H*-CH_3), 4.81 (CH_2-C=O), 4.20 (CH_2-triazole ring), 3.48 (CH_2-O), 1.58 (CH-CH_3).

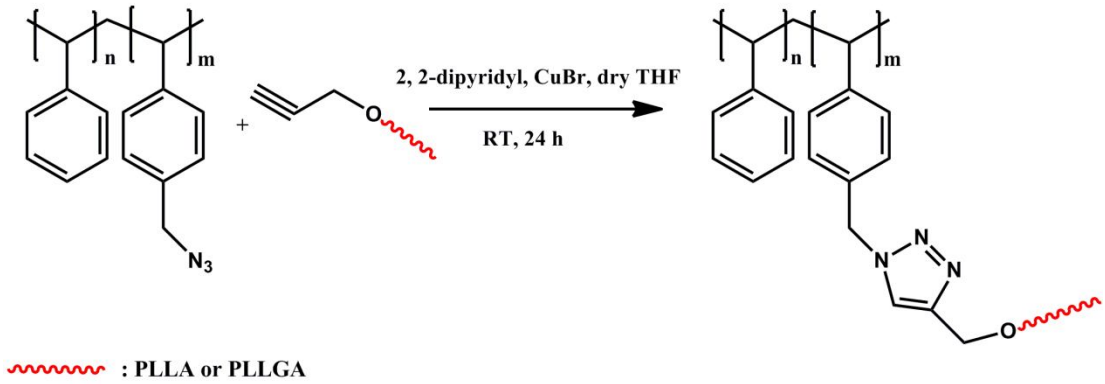


Figure 3. 3: Synthesis of PS-*g*-PLLA and PS-*g*-PLLGA.

3.3.2.4 Degradation studies of PS-*g*-PLLA and PS-*g*-PLLGA

The *in vitro* degradation studies of PS-*g*-PLLA and PS-*g*-PLLGA were performed at 37˚C in phosphate buffer saline (PBS) (contains sodium chloride, sodium phosphate) at pH: 7.4. Graft copolymer films, prepared by solvent casting were placed in vials, each containing 15 mL PBS. The vials were incubated at 37˚C for various periods of time during 6 weeks. The buffer solution was replaced every 48 h. The films were subsequently dried at 25ºC in vacuum until a constant weight was reached. The % change in mass for each film was calculated as follows:

% Change in mass = $m_i - m_f / m_i \times 100$

Where m_f is the final mass and mi is the inital mass.

3.3.2.5 Preparation of imatinib mesylate loaded PS-*g*-PLLA and PS-*g*-PLLGA microspheres

Controlled release behavior of microspheres made of PLGA with different compositions and molecular weights, PS-*g*-PLLA and PS-*g*-PLLGA are studied. Microspheres were prepared by solvent evaporation method (W/O/W). Briefly 0.5g of polymer was dissolved in 3 mL methylene chloride (DCM). 250 µl of aqueous drug solution was emulsified into the organic phase by vortex. This emulsion was added to 400mL aqueous phase containing 1% (w/v) PVA by drop wise and stirred at 1000 rpm for 5 min. The resulting solution was kept stirring at 500 rpm for 3 hours to allow solvent evaporation and hardening microspheres. The microspheres were then filtered and dried under vacuum. Final products were stored at -20 ºC.

3.3.2.6 Calculating the encapsulation efficiency of imatnib mesylate loaded PS-*g*-PLLA and PS-*g*-PLLGA microspheres

Encapsulation efficiencies of imatnib mesylate loaded PS-*g*-PLLA and PS-*g*-PLLGA microspheres were calculated as in section 3.3.1.2.

3.3.2.7 *In vitro* release studies imatinib mesylate loaded of PS-*g*-PLLA and PS-*g*-PLLGA microspheres

In vitro release of imatinib mesylate loaded PS-*g*-PLLA and PS-*g*-PLLGA microspheres were determined as in section 3.3.1.3.

3.3.3 Synthesis of ABC type miktoarm star PS-PLA-PEG copolymer

An ABC type miktoarm star copolymer possessing PS, PLA and PEG arms was synthesized by combining Atom Transfer Radical Polymerization (ATRP) and ROP with two click chemistries, namely thiol–ene and copper catalyzed azide–alkyne cycloaddition (CuAAC). For this purpose, a core 1-(allyloxy)-3-azidopropan-2-ol with allyl and azide functionalities was synthesized in two steps. Then, clickcable polymers, polystyrene with thiol functionality (PS-SH) and poly(ethylene glycol) with alkyne functionality (PEG-acetylene) were independently prepared. As the first step of the grafting onto process, PS-SH was thiol–ene clicked onto the core to yield PS-N_3-OH. The second arm was then incorporated on to the core by the Ring Opening Polymerization (ROP) of l-(-)-Lactide (LA) using as PS-N_3-OH initiator and tin(II) 2-ethylhexanoate as catalyst. Finally, alkyne-PEG-acetylene was bonded to the resulting PLA-PS-N_3 using CuAAC click reaction.

3.3.3.1 Synthesis of the core compound, 1-(allyloxy)-3-azidopropan-2-ol [125]

Allyl glycidyl ether (5.0 mL, 1eq) was dissolved in 150 mL ethanol in a 500 mL round bottom flask equipped with a stirrer. Then, sodium azide (12 g, 3 eq) in 120 mL water was simply added to the solution and the reaction mixture was left stirring for overnight. The solution was successively extracted with CH_2Cl_2 and the organic phase was dried with $MgSO_4$, filtrated and evaporated under vacuum to give viscous yellow liquid (Yield: 96%). The synthesis of 1-(allyloxy)-3-azidopropan-2-ol is shown in Figure 3.4.

^{1}H NMR ($CDCl_3$, 250 MHz): δ = 5.83 (m, 1H, -CH=), 5.24 (m, 1H, *trans* CH_2=), 5.16 (m, 1H, *cis* CH_2=) 3.94 (d, 2H, CH_2=CH-CH_2-O-), 3.40 (p, 1H, -CH-OH), 3.88 (d, 2H, -O-CH_2-), 3.30 (d, 2H, -CH_2-N_3) 3.13 (s, 1H, -OH); FT-IR (ATR): 3420, 2883, 1703, 1584, 1438, 1395, 1256, 1175, 1082, 873, 762 cm^{-1}.

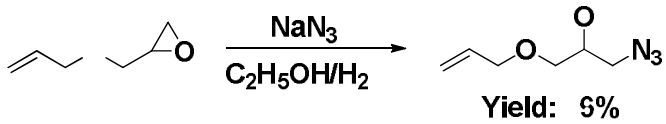


Figure 3. 4: Synthesis of the core compound, 1-(allyloxy)-3-azidopropan-2-ol.

3.3.3.2 Synthesis of polystyrene with ω-xanthate functionality (PS-Xant) [126]

Into a two necked flask equipped with a stirrer, PS-Br (0.9 g, 1eq), potassium xanthate (0.4 g, 5 eq) and 8 mL DMF was added under N_2 atmosphere. The reaction mixture was stirred for 12 h at room temperature and precipitated in 10 fold excess of methanol to yield PS-Xant. ($M_{n,GPC}$ = 2200 g/mol, M_w/M_n = 1.20)

3.3.3.3 Synthesis of polystyrene with ω-thiol functionality (PS-SH) [125]

Into a two necked flask equipped with a stirrer, DMF (8 mL), PS-Xant (0.7 g, 1eq) and metallic zinc (60 mg, 3 eq) were added under N_2 atmosphere. Next, 1,2-ethandithiol (0.3 mL, 10 eq) was added to the reaction media by a syringe. The reaction mixture was stirred for 3 h and precipitated into slightly acidic 10 fold excess methanol/water mixture (10:1, vol/vol) to give a white powder solid. (Yield: 95%) $M_{n,NMR}$ = 1980, $M_{n,GPC}$ = 1950 g/mol, M_w/M_n= 1.18. The synthesis of polystyrene with ω-thiol functionality (PS-SH)is shown in Figure 3.5.

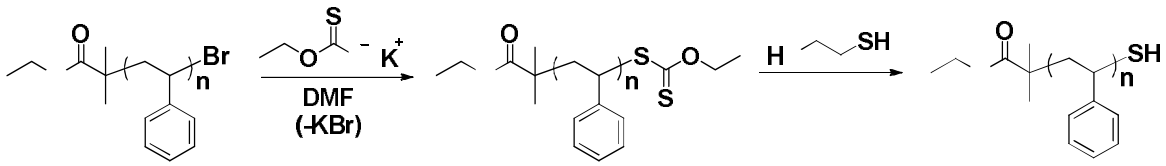


Figure 3. 5: Synthesis of polystyrene with ω-thiol functionality (PS-SH).

3.3.3.4 Synthesis of PS-core

Into a two necked flask equipped with a stirrer, toluene (5 mL), the core (0.24 g, 5 eq), BAPO (0.63 g, 5eq), and PS-SH (0.6 g, 1eq) were added in that order under N_2 atmosphere. The reaction flask was irradiated with visible light (400-500 nm) for 4 hours and then precipitated into methanol. The obtained polymer was collected by

filtration and dried under vacuum. (Yield: 94%) $M_{n,NMR}$ = 2270, $M_{n,GPC}$ = 2250 g/mol, M_w/M_n= 1.18. The synthesis of PS-core is shown in Figure 3.6.

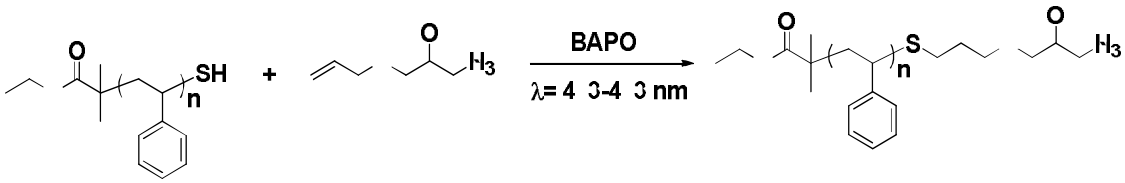


Figure 3. 6: Synthesis of PS-core.

3.3.3.5 Synthesis of PS-PLA-N_3

Synthesis of PLA-PS-N_3 is performed by the ring opening polymerization of L-lactide monomer where polystyrene with –OH functionality emerging from the core was used as initiator. l-(-)-Lactide (0.5 g, 34.5 mmol) and PS-core (0.3 g, 0.15 mmol) reacted in the presence of $Sn(Oct)_2$ (catalytic amount) at 125˚C under N_2 for 24 hours. The resulting polymer was diluted by CH_2Cl_2 and precipitated from cold methanol. Precipitated polymer dried under vacuum. $M_{n,NMR}$ = 3480, $M_{n,GPC}$ = 2860 g/mol, M_w/M_n= 1.32. The synthesis of PS-PLA-N_3 is shown in Figure 3.7.

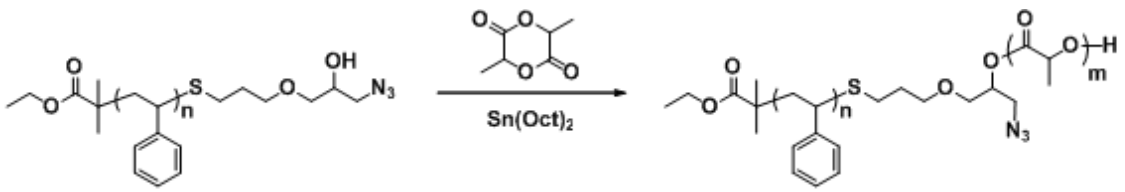


Figure 3. 7: Synthesis of PS-PLA-N_3.

3.3.3.6 Synthesis of PEG-acetylene

Me-PEG (M_n = 2000 g/mol) (2 g, 1 mmol) was dissolved in 50 mL of CH_2Cl_2. 4-Pentynoic acid (0.294 g, 3.00 mmol), DMAP (0.12 g, 1.0 mmol) and DCC (0.62 g, 3.0 mmol) in 5 mL of dichloromethane were added to the solution in that order. The reaction mixture was stirred overnight at room temperature. It was filtered and evaporated, and the remaining product was purified by column chromatography over silica gel eluting first with CH_2Cl_2/ethylacetate (1:1), and then with methanol/ CH_2Cl_2 (1:10). Finally, the organic phase was evaporated to give PEG-acetylene.

3.3.3.7 Click reaction of PLA-PS-N_3 with PEG-acetylene

To a Schlenk tube equipped with a magnetic stirring bar, PLA-PS-N_3 (0.24 g, 1 eq), PEG-acetylene (0.22 g, 1.5 eq), PMDETA (22 µL, 1.5 eq), and CuBr (15 mg, 1.5 eq) were added and dissolved in 3 mL of DMF. The tube was degassed by three freeze-pump-thaw cycles, left under vacuum, and placed in a thermostatted oil bath at 60 ^{0}C. After 24 h, the reaction mixture was diluted with THF and then passed through a column of neutral alumina to remove metal salts. The reaction mixture was concentrated and precipitated into methanol. The resulting polymer (PS-PLA-PEG) was dried in vacuum at room temperature. (Yield: 94%, $M_{n,NMR}$= 5940 g/mol, $M_{n,GPC}$= 3010 g/mol, M_w/M_n= 1.21) The synthesis of PS-PLA-PEG is shown in Figure 3.8.

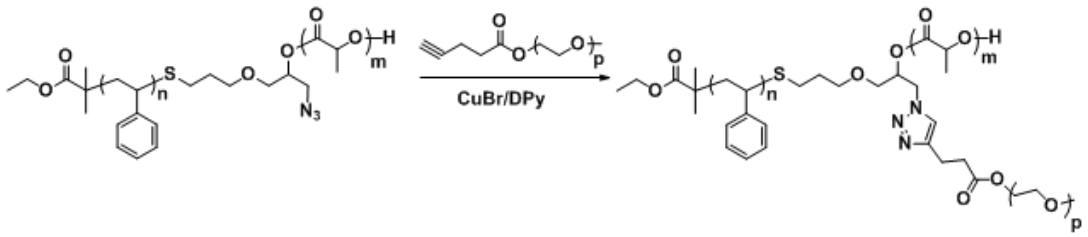


Figure 3. 8: Synthesis of PS-PLA-PEG.

4. RESULTS AND DISCUSSION

The main objective of this thesis is to synthesize new biodegradable polymers for various biomedical applications. The first part consist of controlled drug delivery studies of microspheres made of PLGA copolymers with different properties. Afterwards new biodegradable polymers were synthesized. Hydrolytic degradation profiles were studied for the polymers which are intented to use in controlled release applications. Controlled release studies of proper polymers were also investigated. Novel synthesis of a new star polymer consists of poly(styrene), poly(lactic acid) and poly(ethylene glycol) was synthesized. Pyrolysis of the polymer was investigated for future applications. In the final work, a graft copolymer of poly(*p*-phenylene) (PP) and PEG segments was synthesized to take the advantage of fluorescence property gained by the conjugation of the polymer for biomedical applications.

4.1 Controlled Release Studies of various PLGA copolymers

Commercial PLGA copolymers are used for fabrication of microspheres to compare after synthesis of new biopolymers. Imatinib mesylate, used as a hydrophilic and an effective drug for the treatment of craniopharyngiomas which grow by vascularization. Chemical structure of imatinib mesylate is shown in Figure 4.1. Craniopharyngioma is a type of brain tumor derived from pituitary gland embryonic tissue, that occurs most commonly in children but also in men and women in their 50s and 60s. We aimed to synthesize and characterize different imatinib mesylate containing PLGA microspheres by modifying certain parameters to optimize the drug release characteristics. Microspheres were prepared by double emulsion/ solvent evaporation method. Nearly, eighty different microspheres were synthesized and optimized microspheres were chosen for investigation. Influence of process parameters on microsphere encapsulation, initial burst release and release profile from PLGA microspheres were studied. Properties of the microspheres are listed in the Table 4.1.

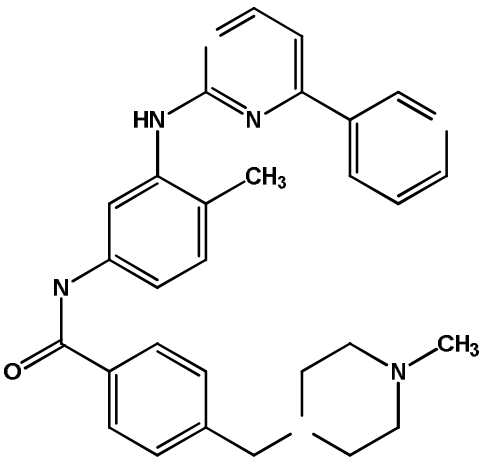


Figure 4. 1: Chemical structure of imatinib mesylate.

Table 4. 1: Characteristics of imatinib mesylate loaded PLGA microsphere prepared at different conditions

Variables	Parameters	Actual Drug Loading (%)	EE (%)	Burst Release (%)
Polymer Nature	50:50	0.36	36	30,28
	75:25	0.213	21,39	14,6
	85:15	0.187	18,75	18,5
Internal Phase Volume	125 uL	0,356	35,6	20,53
	250 uL	0,36	36	30,28
Continuous Phase Volume	100 mL	0,285	28,53	9,8
	200 mL	0,347	34,73	13,87
	400 mL	0,36	36	30,28
PVA Concentration	0,1 %	0,797	79,79	5,63
	0,5 %	0,537	53,78	14,5
	1 %	0,36	36	30,28
PLGA Concentration	10 %	0,552	55,29	30,28
	16,6 %	0,36	36	16,58
	25 %	0,46	46,71	30,14
Homogenization Speed	1000 rpm	0,36	36	30,28
	2000 rpm	0,292	29,2	8,67
	3000 rpm	0,229	22,92	40,19
Theoretical Drug Loading	10 %	0,36	36	30,28
	20 %	0,445	44,52	27,44
	40 %	0,19	19	6,11
Polymer Mw	0,55-0,75	0,36	36	30,28
	0,75-0,96	0,343	34,37	12,58
	0,96-1,2	0,718	78,18	4,83
PEG Content	25 %	0,236	27,36	7,84
	50 %	0,194	19,44	43,53

4.1.1 Influence of polymer nature

The influence of PLGA polymers with the different lactic acid/glycolic acid ratios (50/50, 75/25, 85/15) with the same molecular weight on drug release is investigated. As it can be seen from the Table 4.1, EE % decreased for the microspheres prepared from 50/50 to 85/15. This behavior can be explained by the lactic acid ratio. Due to the hydrophobic behavior of lactic acid units encapsulation of drug which is hydrophilic decreases. Also drug bursts of these microspheres relatively decreases as the lactic acid ratio increases.

In vitro release of imatinib mesylate from different microspheres is shown in Fig.4.1. It has been reported that the water uptake and degradation rate is higher for hydrophilic polymers [127].

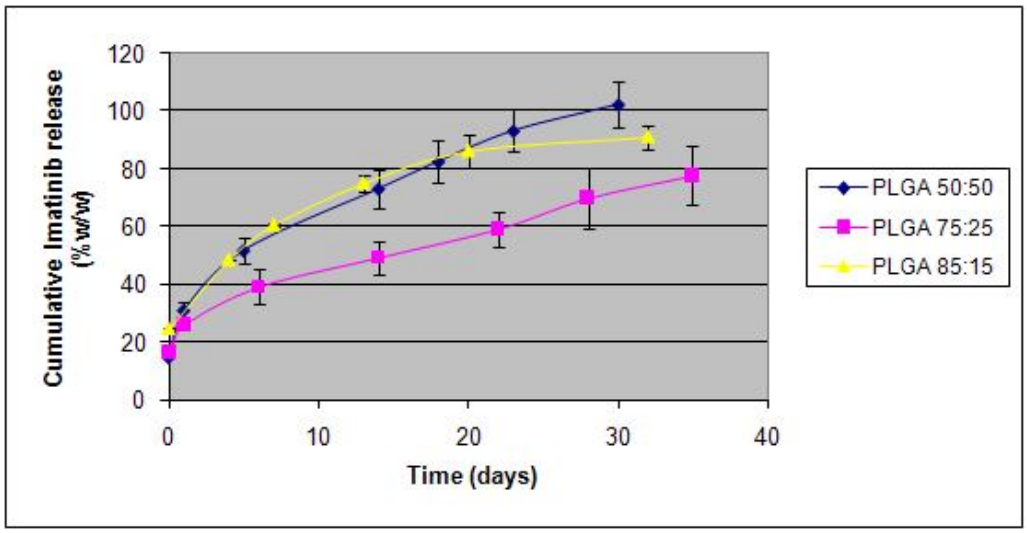


Figure 4. 2 : Influence of LA:GA ratio on the release of imatinib mesylate from PLGA microspheres.

It is expected that the release from the polymer with the ratio 50/50 would be the faster than other two. The slower release of the drug should belong to the microspheres made from the polymer with the ratio of 85/15 but experiments showed that difference between 75/25 and 85/15 is not significant as we expected. Figure 4.3 shows the surface morphology of microspheres prepared from PLGA polymers with the different lactic acid/glycolic acid ratios.

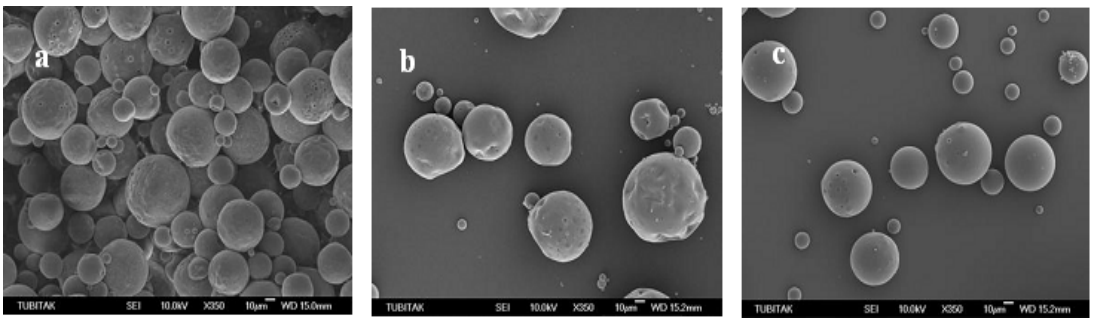


Figure 4. 3 : Surface morphology of microspheres prepared from PLGA polymers with the different lactic acid/glycolic acid ratios 50/50 (a), 75/25 (b) and 85/15 (c).

4.1.2 Influence of internal aqueous phase (W1) volume /continuous phase (W2) volume

Keeping the other conditions constant, effect of internal phase volume on properties of microspheres was investigated (Table 4.1). The EE and drug loading nearly did not change when W1 volume increased from 125 to 250 µL. The initial drug bursts were 17.5 and 18.76% at W1 volume 125 and 250 µL, respectively. Low volumes of W1 phase resulted in a minimal micro porosity of the polymer matrix, whereas high W1 volumes caused an increasing number of micropores and channels thereby facilitating the rapid initial diffusion of encapsulated drug [128]. On the other hand small internal phase is surrounded by a thicker organic phase and the diffusion rate of imatinib mesylate into the continuous phase decreased in the solvent evaporation step.

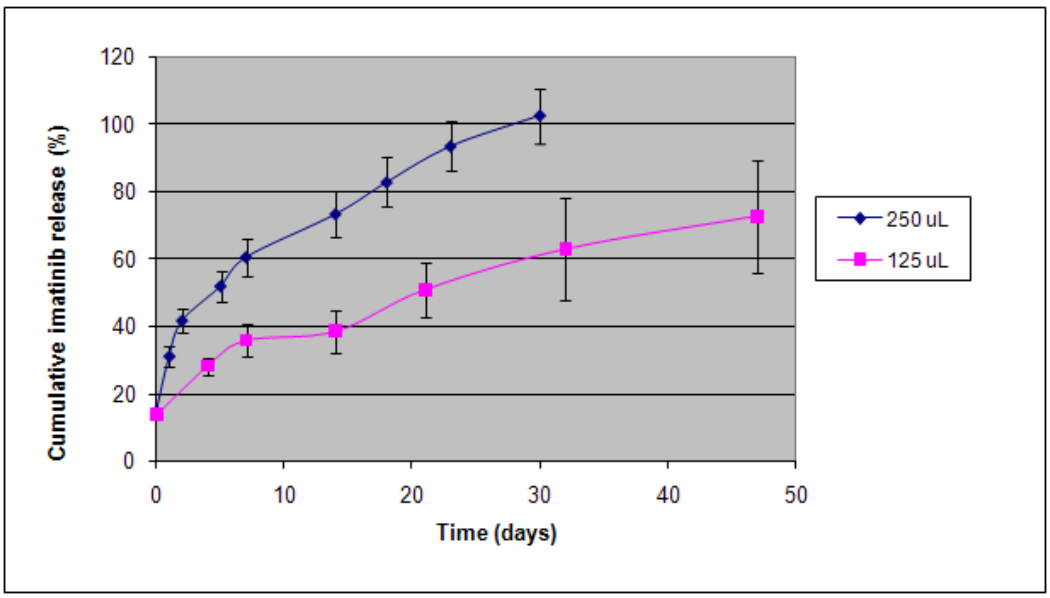


Figure 4. 4 : Influence of internal phase volume on the release of imatinib mesylate from PLGA microspheres.

The effect of internal phase volume on the *in vitro* release rate of imatinib mesylate is shown in Fig.4.4. The release rate of the drug increases as the W1 volume increases from 125 to 250µL. This is in an agreement with the previous results about encapsulation efficiency which is related with the high W1 volumes can cause microporisty. And the release rate increases with increasing W1 volume.

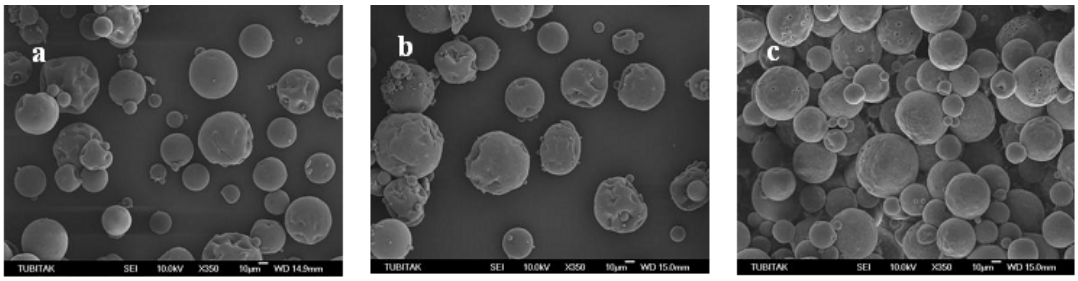


Figure 4. 5 : Surface morphology of microspheres prepared at different continuous phase volume 100mL (a), 200 mL (b), 400 mL (c).

Influence of the continuous phase volume (W2) on properties of microspheres also studied (Table 4.1). As it can be seen from the table EE slightly increased with the increasing W2. In addition to that there is a remarkable change in initial burst release. This can probably explained by the fact that the solubility of DCM in water is about 2% (v/v) and due to some loss of DCM in the first emulsion step, 100mL W2 volume might be sufficient for DCM to distribute into aqueous phase immediately. Further increase in W2 will adversely cause drug loss or drug redistribution on the microsphere surface in the hardening stage due to the increased concentration gradient. This explanation is in good agreement with initial drug burst data [129].

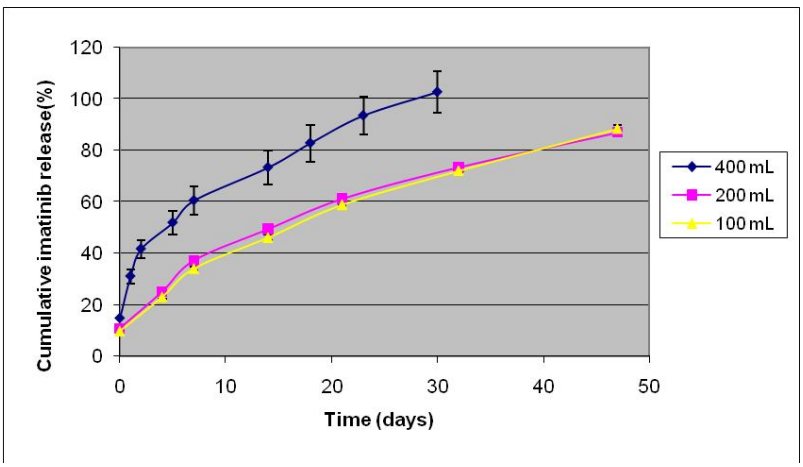


Figure 4. 6 : Influence of external phase volume on the release of imatinib mesylate from PLGA microspheres.

The effect of external phase volume on *in vitro* release of imatinib mesylate is shown in Fig. 4.6. Microspheres with the W2 200 and 100 mL nearly followed the same path with each other. But the microsphere with the W2 volume 400 mL has higher release rate. This can be explained by the same reasons with initial burst releases and also different drug distributions in microspheres could cause higher initial drug bursts when the hydrophilic drug substance is mainly located close to the surface of the microspheres [130].

4.1.3 Influence of polyvinyl alcohol concentration in the external phase

PVA concentrations in the external phase are main factors on influencing the surface and the particle size of microspheres. The effect of PVA concentrations in external water phase on microspheres is examined (Table 4.1). It is known that PVA concentration is an important factor on the particle size of microspheres. The EE and drug loading decreased when PVA concentration increased from 0, 1 to 1%. It is known that increase in the PVA concentration leads to decrease in the size of microspheres which causes faster release of the drug. Influence of polyvinyl alcohol concentration in the external water phase on the release of imatinib mesylate from PLGA microspheres is shown in Fig.4.7.

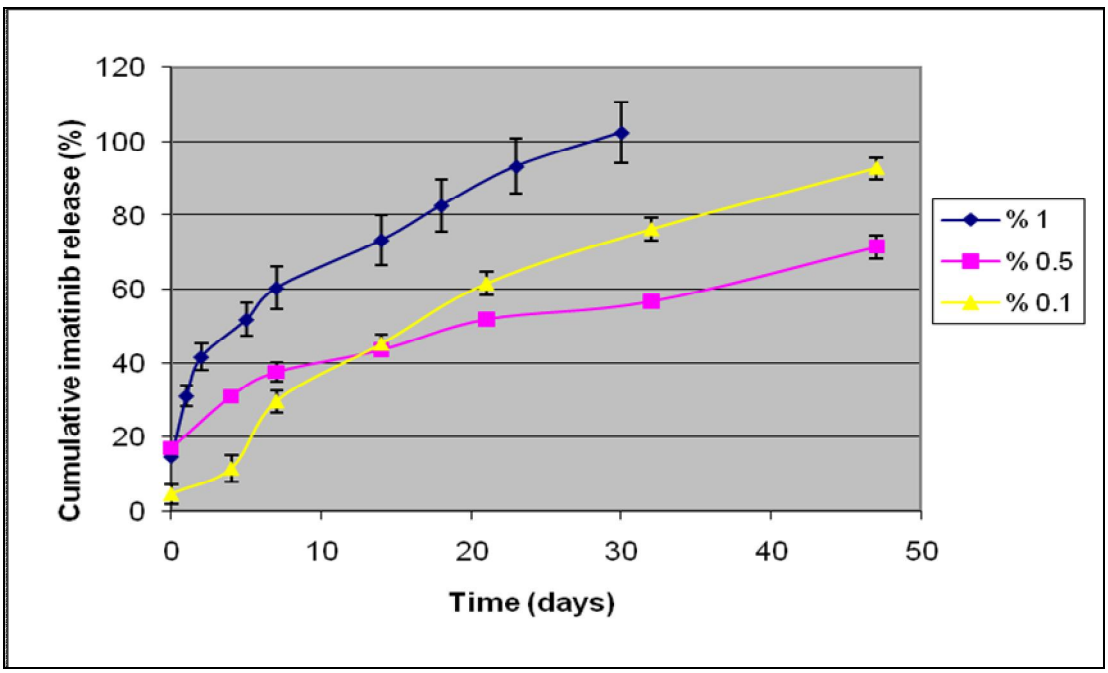


Figure 4. 7: Influence of polyvinyl alcohol concentration in the external water phase on the release of imatinib mesylate from PLGA microspheres.

Based on our experimental data the initial drug bursts were 5.63, 14.5 and 30.28 % at PVA concentrations 0.1, 0.5 and 1% respectively. Release profile of microspheres which made from external phase containing %1 of PVA compatible with the literature. In contrast the other two microspheres showed different release profiles. Surface morphology of microspheres prepared at different PVA concentrations is shown in Figure 4.8. As it can be seen from the surface structure of the microspheres low concentrations of PVA causes higher internal porosity and also external porosity. However, drug distribution of the microspheres at higher concentration of PVA is more uniform due to the dominant stabilization effect at high concentrations.

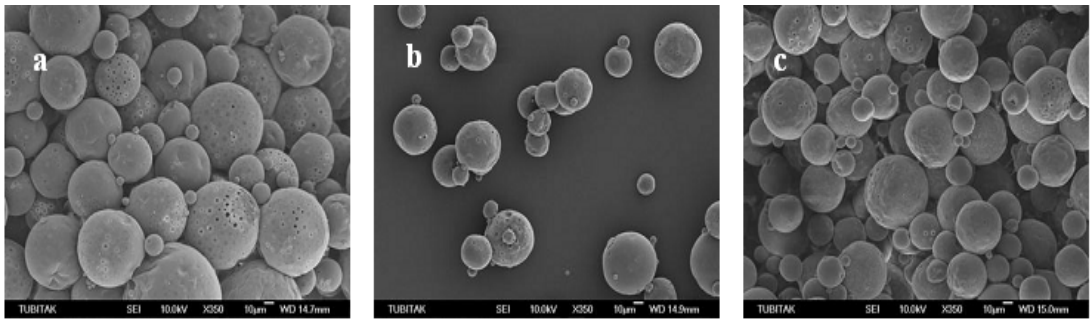


Figure 4. 8: Surface morphology of microspheres prepared at different PVA concentrations 0.1 % (a), 0.5% (b) and 1% (c).

4.1.4 Influence of polymer concentration

EE and actual drug loading decreased while the concentration of polymer increasing as shown in Table 4.1. It can be agreed upon increasing viscosity of organic phase. Also initial burst decreased as concentration of polymer increasing due to surface morphology of microspheres (Figure 4.9). Because it is reported that, increasing viscosity of organic phase causes less external pores which results in low initial burst [131]. Also the high viscosity might restrict the diffusion of the drug in the matrix and compromise the contribution of external channels.

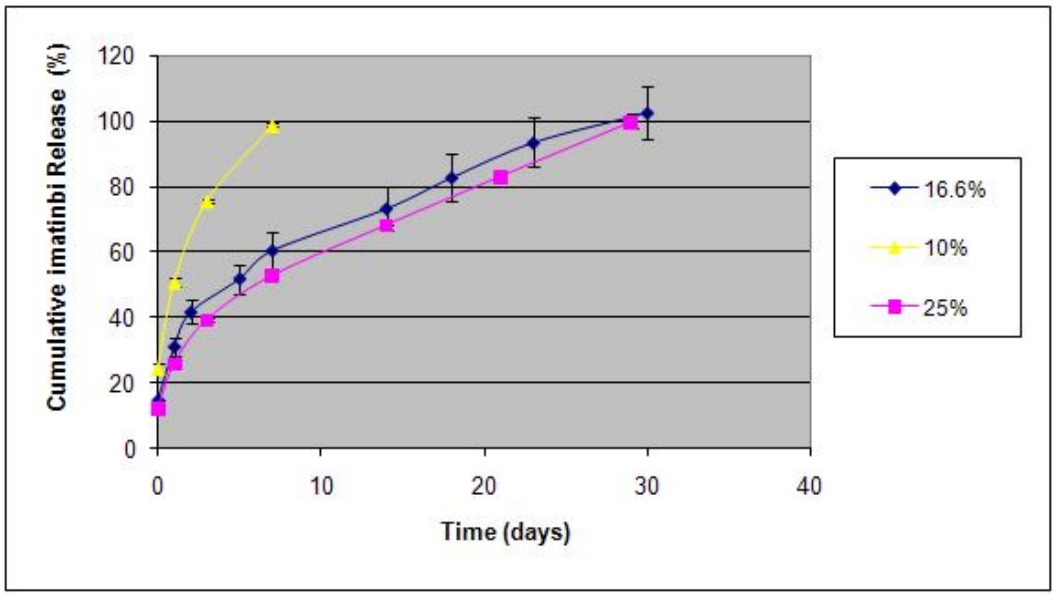


Figure 4. 9 : Influence of polymer concentration on the release of imatinib mesylate from PLGA microspheres.

Release studies are in agreement with the literature that as the concentration of the organic phase increases the release of drug decreases [132]. On the other hand surface area/volume ratio with increasing size could be another cause for the decrease in release.

As it can seen from Figure 4.10, size of microspheres increases with the increasing polymer concentration. This may be explained as it is more difficult for polymer solution to be broken into smaller oil droplets during the second emulsion.

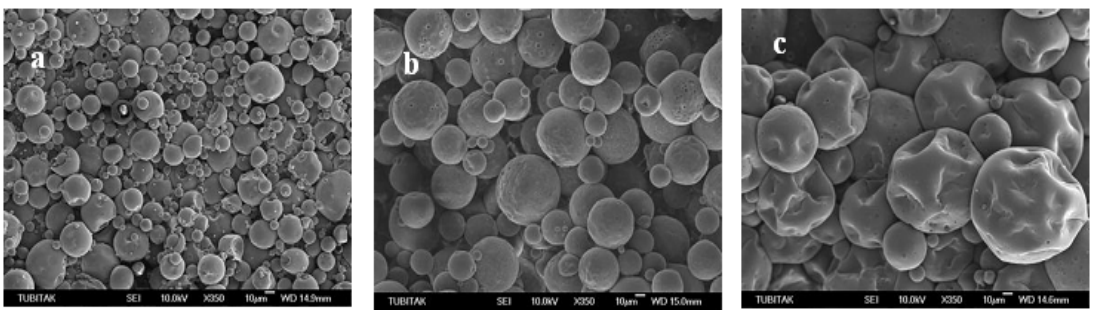


Figure 4. 10 : Surface morphology of microspheres prepared from different PLGA concentrations at 10% (a), 16.6% (b) and 25% (c).

Also, water droplets trapped at interior of microspheres that evaporates and leave empty spaces after drying. The situation is can clearly be seen from the SEM photo of the microsphere prepared at 25% polymer concentration (Figure 4.10 (c)).

4.1.5 Influence of homogenization speed

Keeping the other conditions constant, effect of homogenization speed on properties of microspheres was investigated (Table 4.1).The size of the microspheres is affected by viscosity of the polymer, internal water phase, homogenization speed, PVA concentration at external phase and molecular weight of the polymer. But the major factor beside these is the homogenization speed because it provides the energy to disperse the oil phase in water. According to our experimental data, EE decreases with the increasing stirring speed while release of imatinib mesylate increases (Figure 4.11). This may be explained by the decrease in the size of microspheres. Our previous studies indicate that, decrease in the size of microspheres result in rapid imatinib mesylate release.

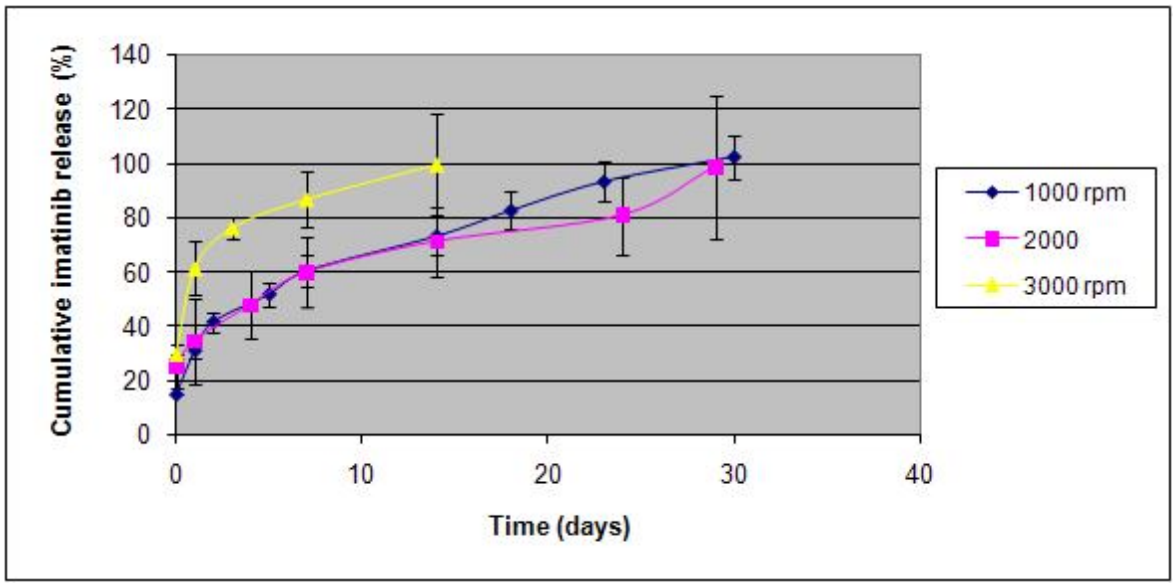


Figure 4. 11: Influence of homogenization speed on the release of imatinib mesylate from PLGA microspheres.

Also our results are in agreement with the literature that high stirring speed causes smaller microspheres as it is shown in Figure 4.12.

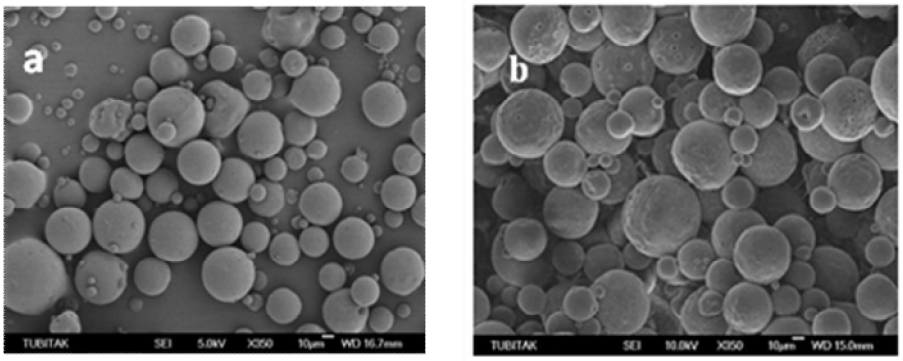


Figure 4. 12: Surface morphology of microspheres prepared at different homogenization speeds, (a) 2000rpm, (b) 1000 rpm.

4.1.6 Influence of theoretical drug loading

Keeping the other conditions constant, properties of microspheres prepared with different theoretical drug loading are described in Table 4.1. According to our experimental data EE and actual drug loading did not change at theoretical drug loading 10 and 20%. However, EE decreased at increased theoretical drug loading 40%.The initial drug loading decreased with the increasing theoretical drug loading. Since it is assumed that burst release is normally caused by the drug near the surface of microspheres, this implies that most of the drug substance was distributed in the internal matrix of the polymer instead of the surface.

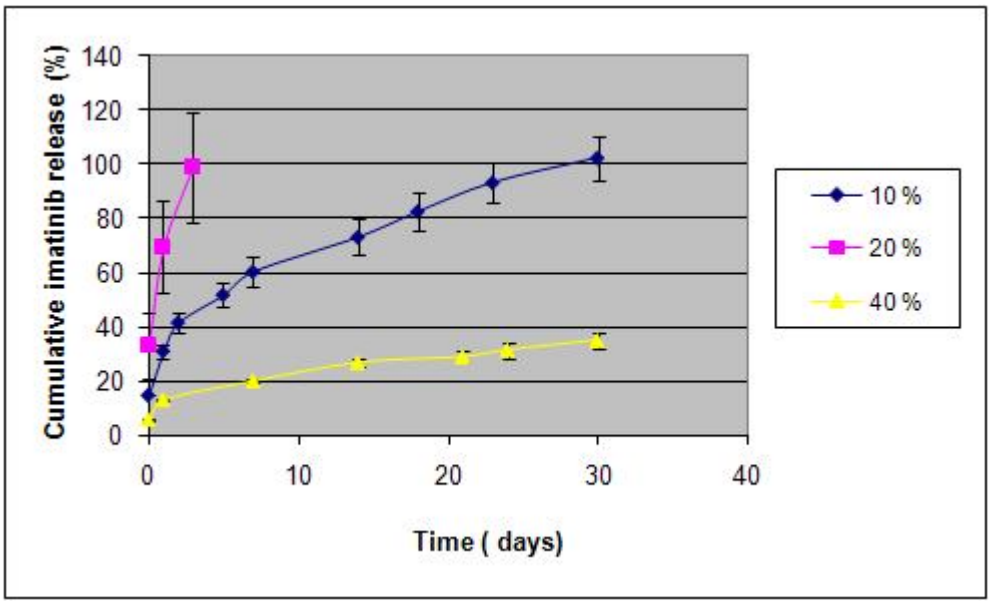


Figure 4. 13 : Influence of theoretical drug loading on the release of imatinib mesylate from PLGA microspheres.

Influence of theoretical drug loading on the release of imatinib mesylate from PLGA microspheres showed in Fig. 4.13. Remarkable change is seen in the release profile

of microspheres which prepared with theoretical drug loading 20% in early stages of release but other two microspheres did not influenced by drug loading.

4.1.7 Influence of M_w of PLGA polymers

Influence of Mw of PLGA polymers on properties of microspheres shown in Table 4.1. EE and actual drug loading increased with Mw of PLGA polymers significantly due to the increasing viscosity of the organic phase, which prevented out-flow of drug during hardening phase.

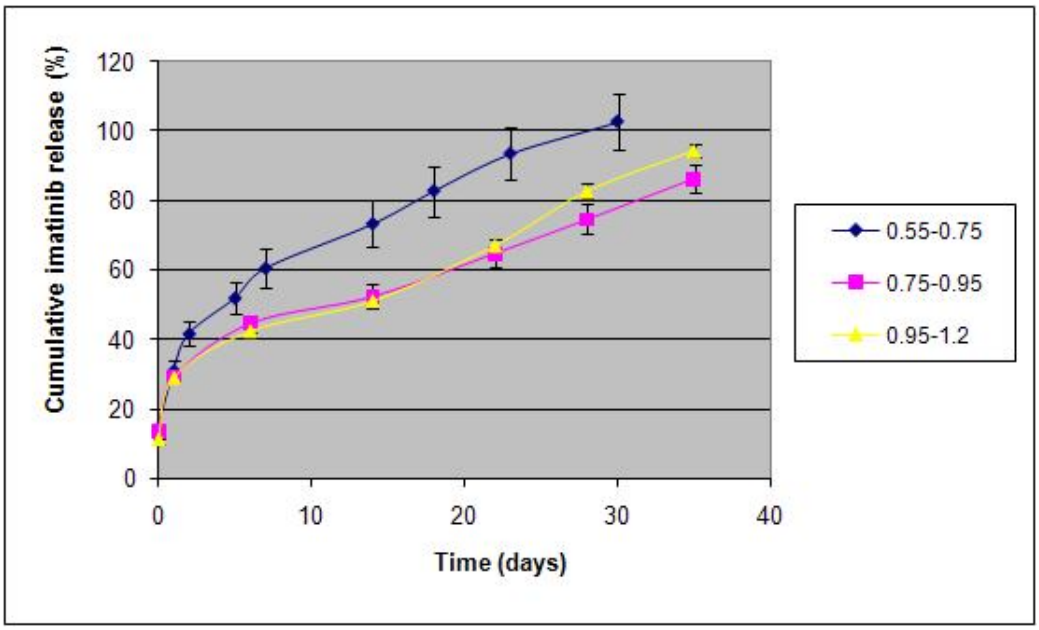


Figure 4. 14: Influence of M_w of PLGA polymer on the release of imatinib mesylate from PLGA microspheres.

Influence of M_w of PLGA polymer on the release of imatinib mesylate from microspheres is shown in Figure 4.14 and the results are compatible with the literature. As the M_w of the polymers increases, the release of the drug decreases due to the decreasing hydrolytic degradation of PLGA polymers. Viscosity of the oil phase increases with the higher M_w of the polymers that similar effects are seen with comparison at polymer concentration. Influence of M_w of PLGA polymer on surface morphologies of microspheres is shown in Figure 4.15.

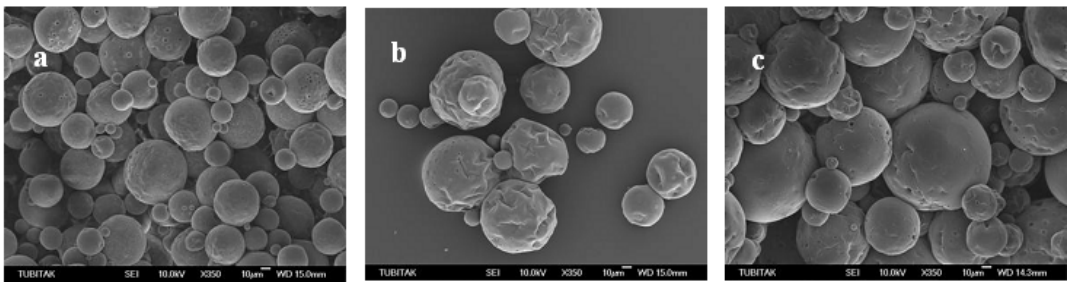


Figure 4. 15 : Surface morphology of microspheres prepared from PLGA polymers inherent viscosities at 0.55-0.75 (dL/g) (a), 0.75-0.95 (dL/g) (b) and 0.95-1.2 (dL/g) (c).

Empty spaces caused by the evaporation of entrapped water as a result of higher concentration of the oil phase are again seen due to the increasing viscosity with the increasing molecular weight. Also, size of the microspheres increased with the increasing molecular weight of the polymer due to the thicker inner shell.

4.1.8 Influence of PEG content in organic phase

PEG (M_w:2000g/mol) polymer is blended with PLGA to see the effect of increased hydrophilicity in oil phase. The influence of PEG content in organic phase is shown in Table 1. EE and actual drug loading decreased with the increasing content of PEG in organic phase due to hydrophilic behavior of PEG. Also, initial drug burst increased as PEG content increases. Hydrophilic nature and degradation rate of PEG results in fast release of drug. The release profiles of PEG containing microspheres are shown in Fig.4.16. The influence of PEG on surface of microspheres is shown in Figure 4.17. The results indicate that increased hydrophilicity of the polymer matrix causes porosity on the external surface of the microspheres.

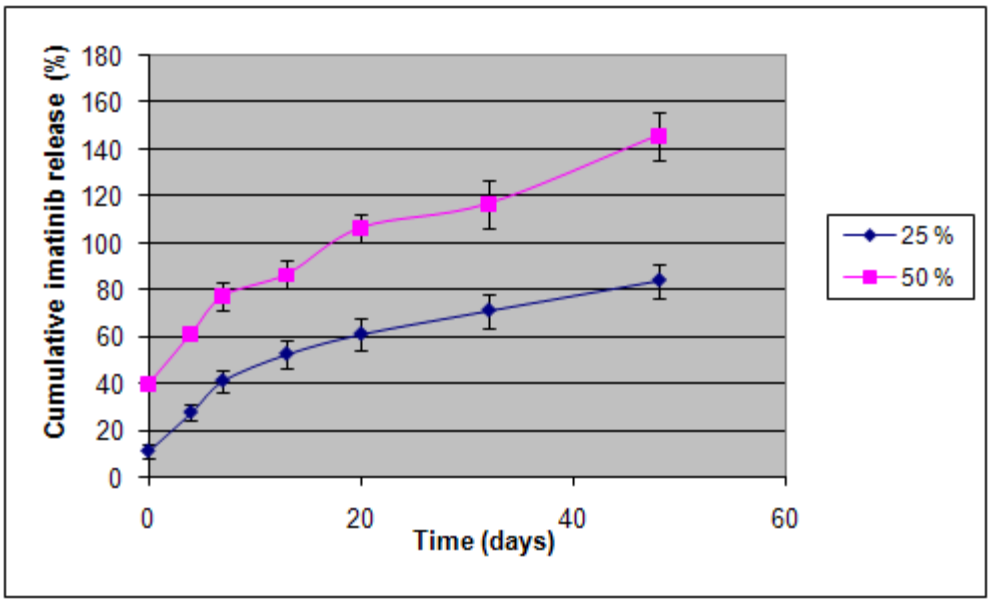


Figure 4. 16: Influence of PEG content in organic phase on the release of imatinib mesylate from PLGA microspheres.

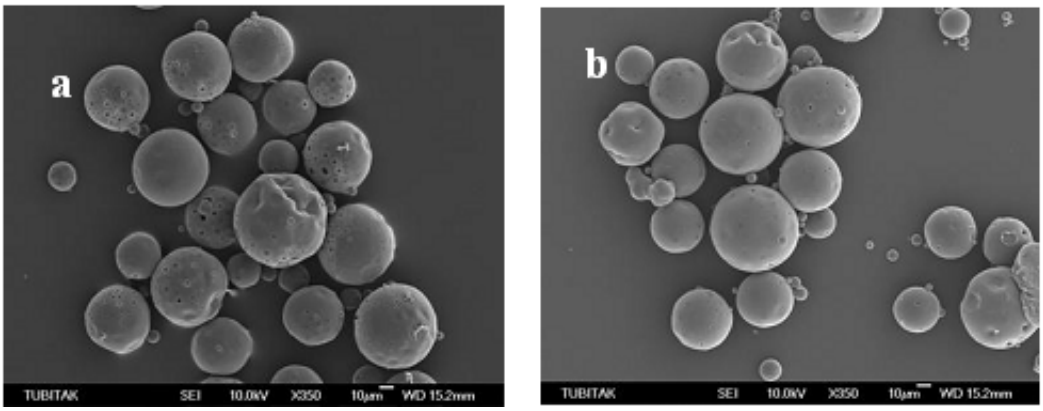


Figure 4. 17: Surface morphology of microspheres prepared with PEG in organic phase 25% (a) and 50% (b).

4.1.9 Influence of polymer blends

Influence of PLGA blends with ratios LA:GA of 50:50 and 75:25 on the release of imatinib mesylate from microspheres is investigated. The influence of PLGA blends on actual drug loading, encapsulation efficiency and burst release is shown in Table 4.2.

Table 4. 2: Influence of LA:GA ratio on PLGA blends.

PLGA Blends	Actual Drug Loading (%)	EE (%)	Burst Release (%)
50:50 (2) /75:25 (1)	0,27	27,41	50,9
50:50 (1) /75:25 (2)	0,22	22,96	14,35
50:50 (1) /75:25 (1)	0,35	35,96	42,90
50:50 (1) /75:25 (3)	1,3	103,65	47,84
50:50 (3) /75:25 (1)	0,83	83,74	55,62
50:50 (4) /75:25 (1)	0,6	60,25	88,615
50:50 (1) /75:25 (4)	0,64	64,8	78,605

The influence of PLGA polymer in organic phase with LA ratio of 75% on imatinib mesylate release is shown in Figure 4.18.

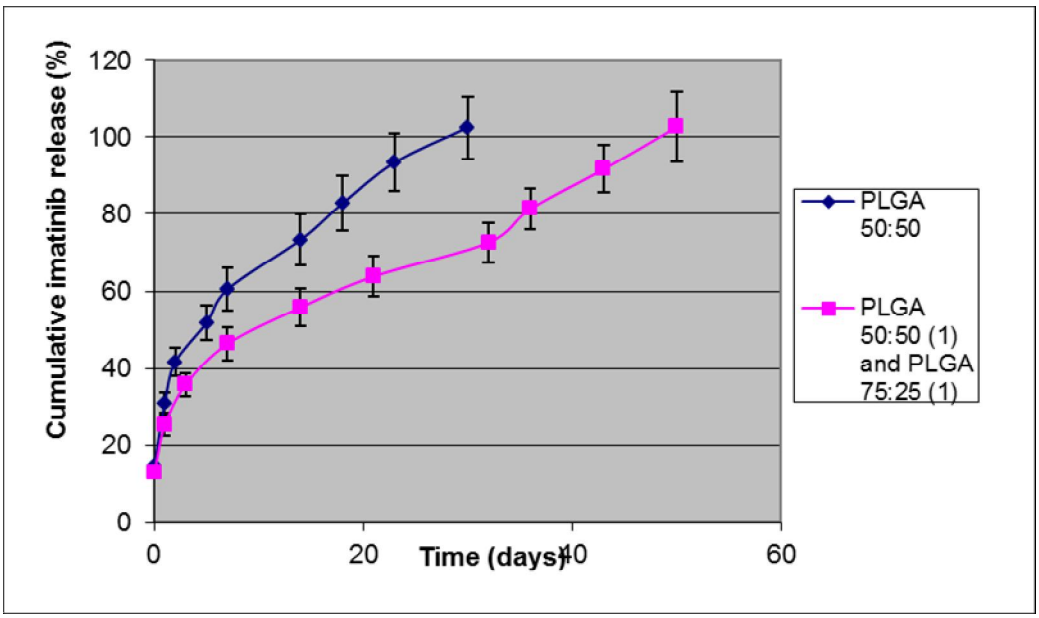


Figure 4. 18: The influence of PLGA polymer in organic phase with LA ratio of 75% on imatinib mesylate release.

As it can be seen from the Figure 4.18, when it is compared with previous microspheres, blending the PLGA polymer bearing LA ratio 50% with the polymer

bearing LA ratio 75% cause a decrease in release rates due to slower degradation rate of the PLGA polymer with LA ratio 75%.

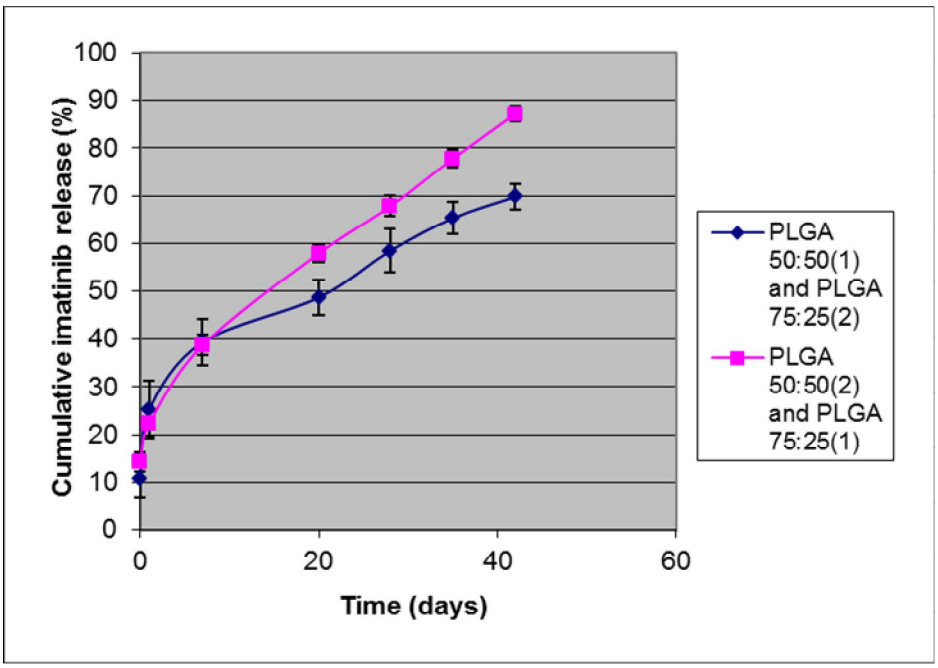


Figure 4. 19: Influence of mole ratios of PLGA polymers with different LA:GA content on imatinib mesylate release.

Increment in mole of PLGA polymer with LA ratio of 75% increases the encapsulation efficiency and decreases the release rate of drug release as shown in Table 4.2. This situation can be explained by the hydrophilic nature of LA content in the polymer composition. Influence of increment in mole of PLGA polymer with LA ratio of 75 % on cumulative release of drug is shown in Figure 4.19 An increase of PLGA polymer with LA ratio 50% in blend causes faster release. Influence of increased mole ratio of PLGA (LA:GA, 50:50) polymer is shown in Figure 4.19. Also, increase of PLGA polymer with LA ratio 50% causes a decrease in encapsulation efficiency where burst release of microspheres increased.

The mole ratio of the PLGA with 50% LA content increased 3 fold of PLGA with LA content 75%. The influence of change in the mole ratio is shown in Figure 4.20. As it is expected an increase in the mole ratio of PLGA polymer with 50% of LA content result in higher release profile.

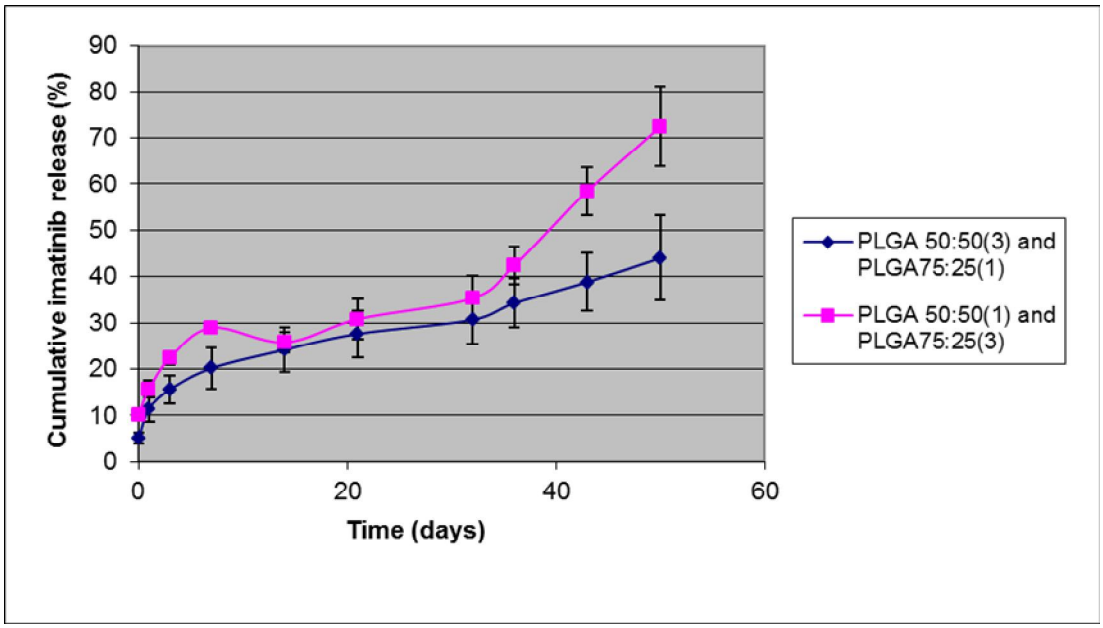


Figure 4. 20: Influence of mole ratios of PLGA polymers with different LA:GA content on imatinib mesylate release.

PLGA of 50% LA content with mole ratio 4 fold of PLGA with 75% followed similar release profile with Figure 4.20 as it shown in Figure 4.21. Encapsulation efficiencies of microspheres prepared from blends containing higher mole ratio of PLGA with LA content 75% is increased according to higher LA content in organic phase.

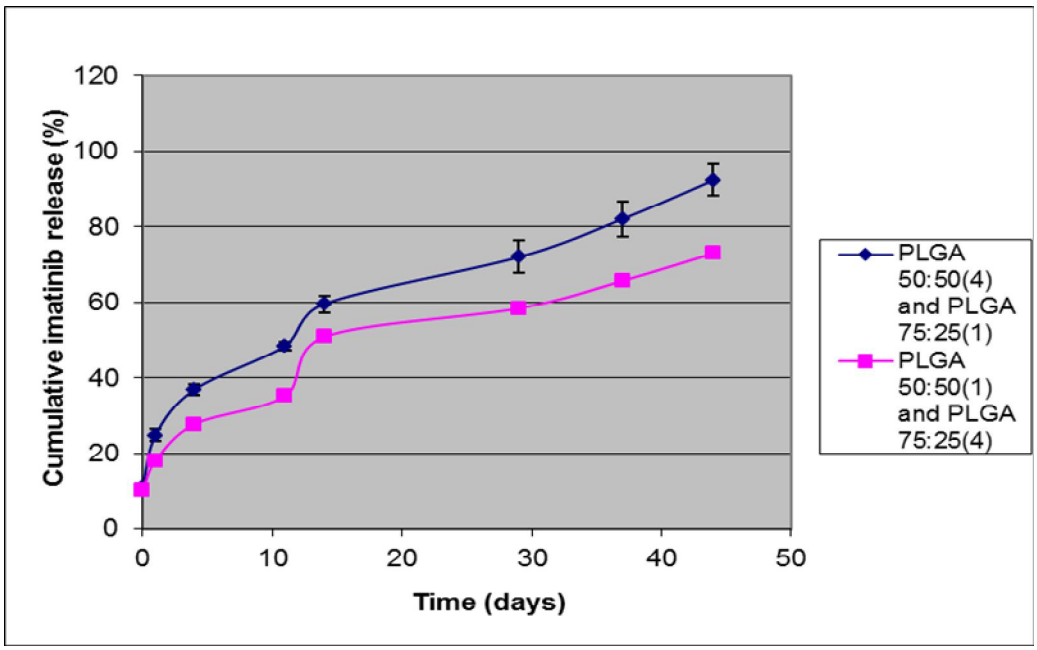


Figure 4. 21: Influence of mole ratios of PLGA polymers with different LA:GA content on imatinib mesylate release.

As a result of this work, 80 PLGA microspheres with different characteristics were synthesized by changing different parameters to optimize the encapsulation and release profiles. Local delivery of imatinib mesylate to the post-surgical tumoral cavity using biodegradable microspheres may be a promising biologically selective approach to prevent the recurrence of craniopharyngiomas via inhibition of neovascularization. Prof.Dr.Tuker Kilic's group at Marmara University tested whether controlled release of imatinib mesylate can suppress the cranipharyngioma-induced neovascularization in an *in vivo* rat cornea angiogenesis model.

In this study, we present 3 groups of PLGA microspheres with the ratios 50:50, 75:25 and 85:15 with different LA: GA contents that successfully inhibit tumor induced neovascularization in an *in vivo* angiogenesis model. The physicochemical features of the PLGA- imatinib mesylate microspheres were shown in Table 4.3.

Table 4. 3: The physicochemical characterization of imatinib mesylate loaded PLGA microspheres.

Polymer	Drug Loading (gr)	Lactic acid (LA) and Glycolic Acid (GA) Ratio	Size (µm)	Encapsulation Efficiency (%)
PLGA	0,05	50:50	58,82	36
PLGA	0,05	75:25	76,64	21,39
PLGA	0,05	85:15	61,23	18,75

After sterilization under UV for 30 min, each of 3 imatinib mesylate containing microspheres and imatinib mesylate-free control microsphere were implanted together with tumor sample and microsphere-free recurrent craniopharyngioma sample was also implanted to the other eye of the rat as a negative control. These microspheres were formulated to release their contents within a time frame of 4-5 weeks which is the maximum technically possible time window for corneal assay. For data collection, each corneas were photographed on days 5, 10, 15, 20, 25 and 30 after implantation using a digital video camera attached to a biomicroscope, and vessel counting was performed by a researcher who was blinded to the study. Those rats with signs of ocular infection in the eye were excluded and replaced with healthy animals (with the same procedure and tissue sample).

30 days follow-up in cornea angiogenesis assay showed that PLGA microspheres with 50:50, 75:25 and 85:15 LA: GA ratios, irrespective of their imatinib mesylate

contents, inhibited tumor induced neovascularization on an avascular cornea shown in Figure 4.22. These 30-35 μg imatinib mesylate loaded 3 microspheres release at first day efficiently inhibited the tumor neovascularization.

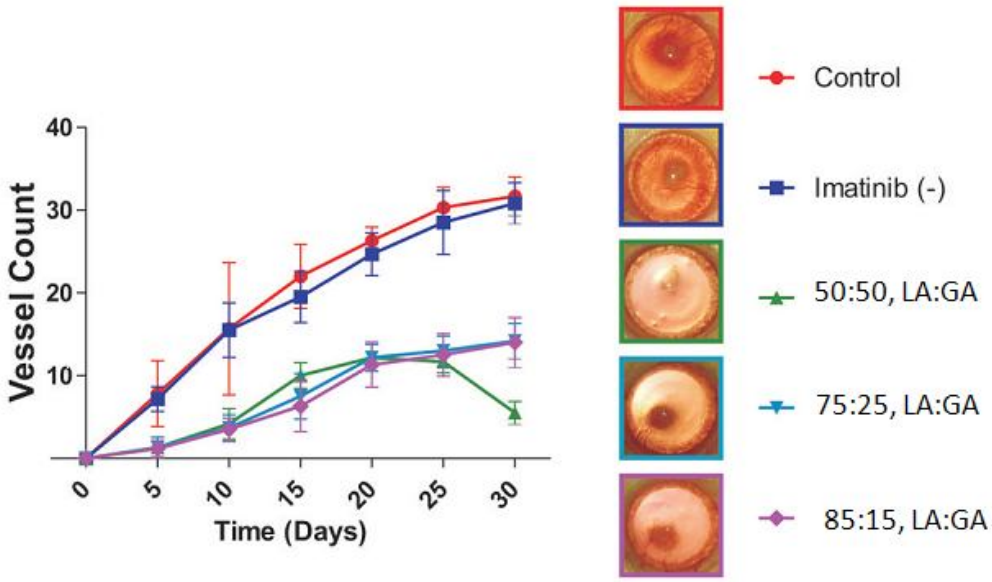


Figure 4. 22: Cornea angiogenesis assay results of PLGA with LA:GA ratios 50:50, 75:25 and 85:15 microspheres.

The critical feature of these 3 microsphere groups is their initial burst release rates. Our optimization trials showed that high initial release burst is necessary for proper inhibition of tumor-induced neovascularization.

In conclusion, there is urgent need for novel therapy modalities for craniopharyngiomas. Controlled drug delivery is an alternative to radical surgery and post-operative radiotherapy. Our results demonstrate that,during surgery, implantation of imatinib mesylate containing biodegradable PLGA microspheres into the surgical cavity may be a good approach to suppress the recurrence of the tumor.

4.2 Synthesis, Characterization and Hydrolytic Degradation of Graft Copolymers of Polystyrene and Aliphatic Polyesters

In this study, a new class of biodegradable graft copolymers of PS with aliphatic polyester side chains were synthesized through combination of Nitroxide Mediated Free Radical Polymerization (NMRP) and ROP with copper (I)-catalyzed "Click" chemistry. First, the click component, azido-functional PS (PS-N_3) was prepared by copolymerization of styrene and chloromethyl styrene by NMRP followed by the conversion of chlorine groups of the resulting copolymer to azido groups by using

NaN_3 in N, N-dimethylformamide. Propargyl functional aliphatic polyesters were prepared independently by ROP of lactic acid and glycolic acid using tin octoate in the presence of propargyl alcohol at 130 ˚C. Finally, PS-N_3 was coupled with the resulting polymers by click chemistry to yield graft copolymers (PS-*g*-PLLA and PS-*g*-PLLGA, respectively). The intermediates and final graft copolymers were characterized by ^{1}H-NMR and FT-IR spectral, and GPC chromatographic analyses. The hydrolytic degradation behavior of the obtained graft copolymers in PBS were investigated by the weight loss and molecular weight measurements.

4.2.1 Synthesis and characterization of PS-*g*-PLLA and PS-*g*-PLLGA graft copolymers

For the synthesis of parent azide functionalized polymer, we first prepared poly(styrene-*co*-chloromethylstyrene), P(S-*co*-CMS), via NMRP of styrene (S) and chloromethylstyrene (CMS) at 125 °C according to the procedure. NMRP was delibaretly selected as the controlled radical polymerization method for its suitability for the polymerization of S based monomers and tolerance to halide functional groups. The composition of copolymer was determined using ^{1}H NMR spectroscopy. The mole fractions of CMS and S were calculated from the ratio of the peak areas around 4.5 ppm, corresponding to two methylene protons of in the side chain of CMS to the total area between 6.3 and 7.4 ppm, which was attributed to the total aromatic protons (Figure 4.23). P(S-*co*-CMS) with M_n(GPC) 7.690 and 16 mol % chloromethyl groups was then quantitatively converted into polystyrene-azide (PS-N_3) in the presence of NaN_3/DMF at room temperature. From the ^{1}H NMR spectrum of PS-N_3 shown in Figure 4.23, it was observed that while the signal at 4.5 ppm corresponding to CH_2-Cl protons of the precursor P(S-*co*-CMS) completely disappeared, and a new signal appeared at 4.25 ppm due to CH_2 linked to azide groups. The structure of PS-N_3 was further supported by the observation from FT-IR spectrum of the azide stretching band at 2094 cm^{-1} (*vide infra*).

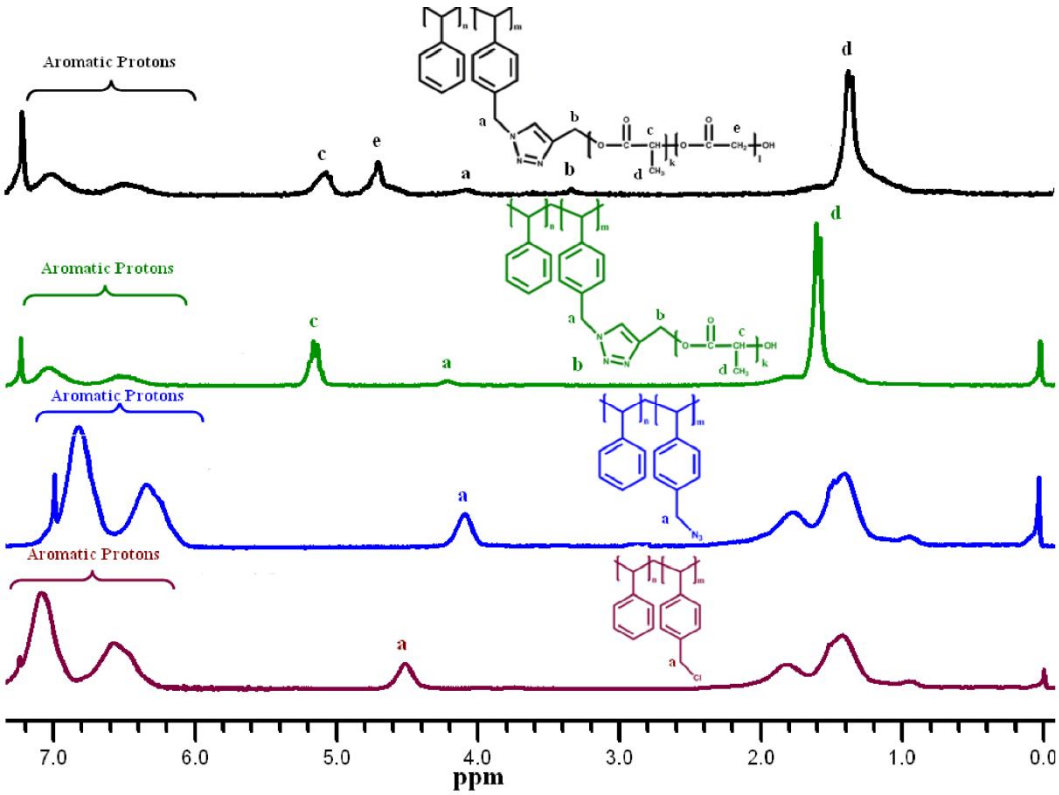


Figure 4. 23 : ^{1}H NMR spectra of P(S-co-CMS), PS-N_3, PS-*g*-PLLA and PS-*g*-PLLGA.

The second step in the synthesis of PS with biodegradable side chains was to obtain alkyne functionalized polymers (PLLA or PLLGA). To obtain the desired functionalities, combined with proper molecular weights and polydispersities, polymers were synthesized by ROP of L-lactide and glycolide, respectively by using propargyl alcohol as a functional initiator, in the presence of stannous octoate. In both cases the GPC traces are unimodal and narrow, indicating that no important side reactions occurred. Moreover, as these polymers were intended to be used in the subsequent click reactions, efforts were directed toward obtaining low molecular weights, combined with convenient yields. Some characteristics are presented in Table 4.4. Finally, the obtained PLLA and PLLGA containing acetylene groups were clicked to PS-N_3 to yield PS-*g*-PLLA and PS-*g*-PLLGA, respectively by copper catalyzed cyclo addition reaction (see Figure 3.1). After the click reaction, new peaks appeared at around 1.58, 3.48 and 5.13 ppm for PS-*g*-PLLA copolymer, and 1.58, 3.48, 4.81 and 5.13 ppm for PS-*g*-PLLGA copolymer which correspond to PLLA and PLLGA segments of the graft copolymers, respectively.

Table 4. 4: The yields of the reactions and molecular weight characteristics of the products at different modification stages.

Polymer	Yield (%)	$M_{n,\ NMR}$[a]	$M_{n,\ GPC}$[b]	M_w/M_n[b]
P(S-co-CMS)	85[c]	-	7690	1,21
PS-N_3	100[d]	-	8070	1,20
PLLGA	60[c]	2660	2870	1,23
PS-*g*-PLLGA	75[e]	10730	8370	1,68
PLLA	65[c]	1570	3520	1,30
PS-*g*-PLLA	69[e]	9640	15120	1,51

[a] Determined by ^{1}H NMR analysis.
[b] Determined by gel permation chromatography (GPC) using polystyrene standards.
[c] Conversion of monomers as determined gravimetrically.
[d] Conversion of the chlorine groups to azide groups.
[e] Yield of the Click reaction determined by ^{1}H NMR analysis.

The graft copolymer formation was also followed by FT-IR spectroscopy. The FT-IR spectrum associated with graft copolymers showed the presence of both components (PS and PLLA or PLLGA) in the structure (Figures 4.24 and 4.25). Peaks resonating at around 1800 and 1200 cm^{-1} were due to carbonyl groups and C-O bonds of the PLLA and PLLGA side chains whereas transitions from 1595, 1490, and 700 cm^{-1} and overtones between 1945 and 1805 cm^{-1} were specific to the PS main chain. As anticipated, the azide stretching band of the precursor polymers at 2100 cm^{-1} decreased significantly. However, it did not disappear completely probably due to the steric hindrance [133].

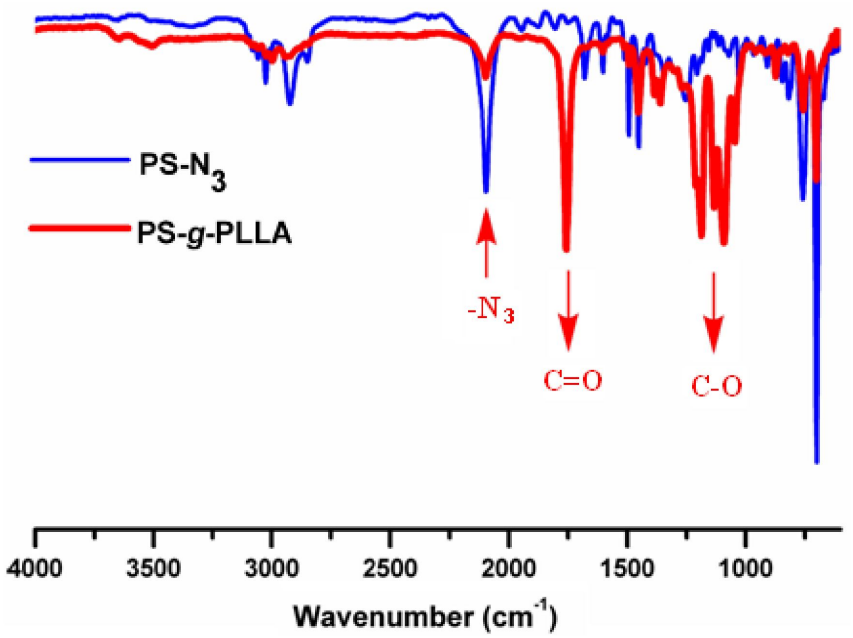


Figure 4. 24: FT-IR spectra of PS-N_3 and PS-*g*-PLLA.

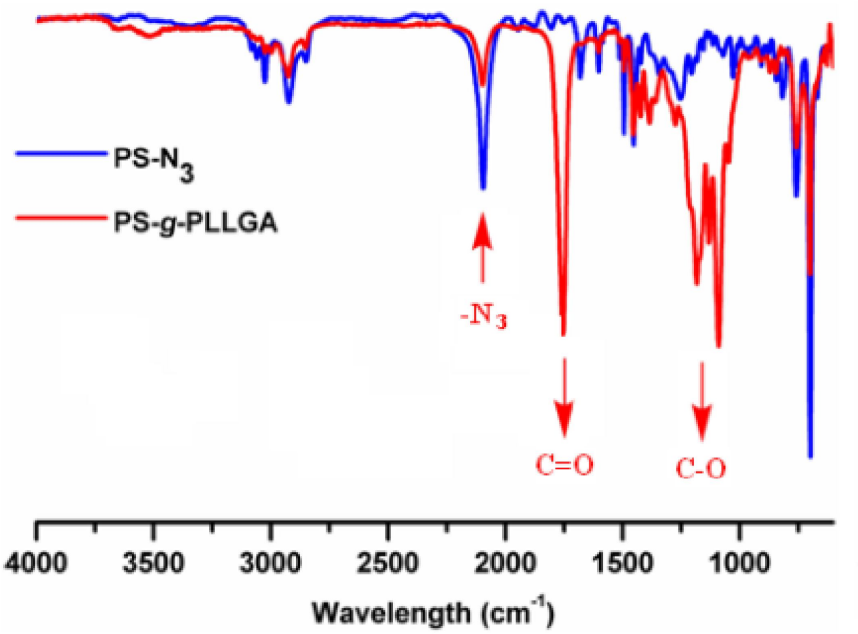


Figure 4. 25 : FT-IR spectra of PS-N_3 and PS-*g*-PLLGA.

Sucessful grafting was also confirmed by GPC measurements. In Figures 4.26 and 4.27, the normalized GPC curves of the individual precursor polymers as well as of the resulting graft copolymers were shown. In the elution curves of graft copolymers, although slight shoulders of the chromatographic peak corresponding to precursor polymers are noted, clear shifts toward lower retention times were observed. These

observations confirm that the described click approach is useful for the desired graft copolymer formation.

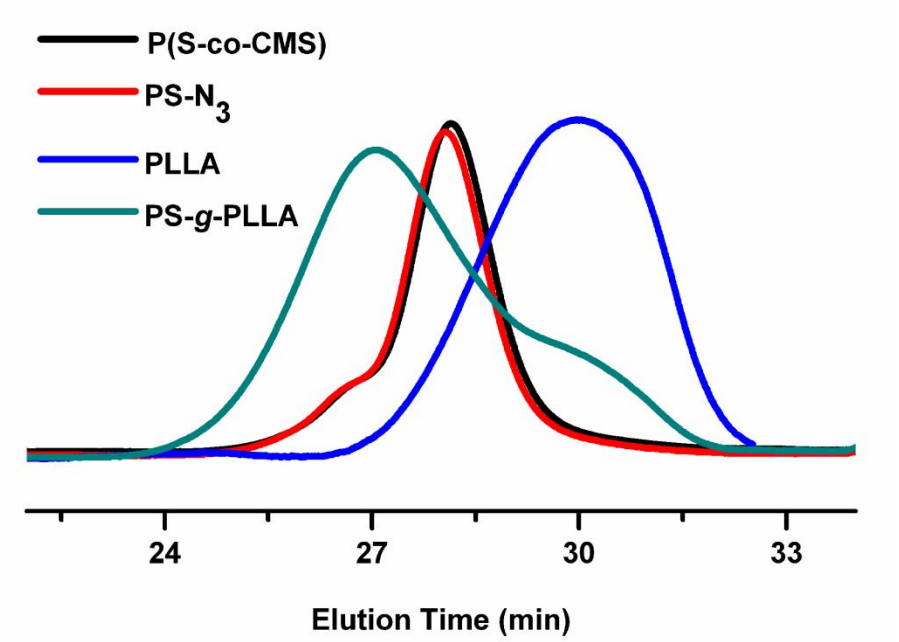


Figure 4. 26 : GPC traces of P(S-co-CMS), PS-N_3, PLLA and PS-*g*-PLLA.

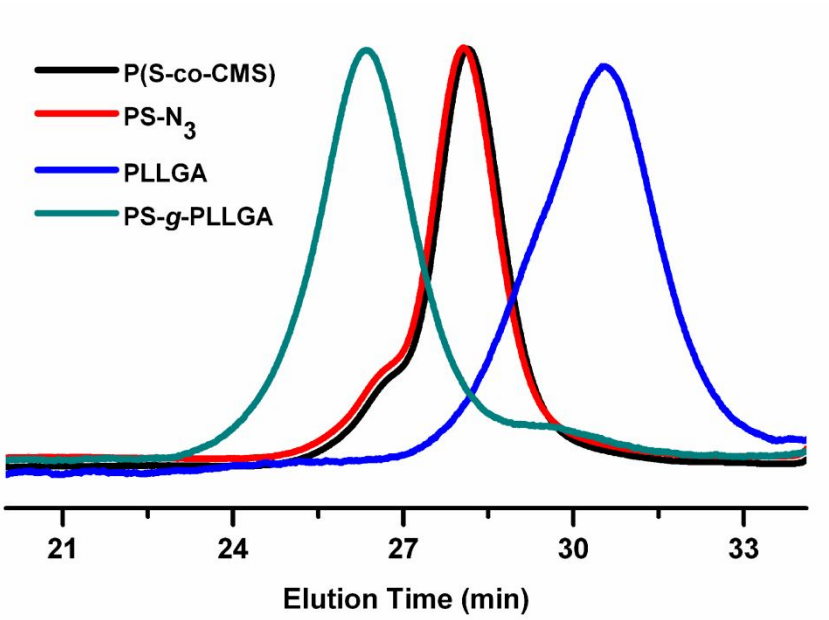


Figure 4. 27 : GPC traces of P(S-co-CMS), PS-N_3, PLLGA and PS-*g*-PLLGA.

The yields of polymerization, modification and click reactions and the molecular weight characteristics of the resulting polymers are collected in Table 4.4. As can be seen, the measured molecular weights generally fit with those determined by ^{1}H NMR analyses. Some discrepeancies observed may be due to the different

hydrodynamic volumes of the graft copolymers and strong interactions of the polar segments with the stationary phase of the GPC column. It is also noted that no side reactions such as coupling or chain scission occured during the azidation process and the molecular weight of the precursor polymer was preserved.

4.2.2 Hydrolytic degradation of graft copolymers

Preliminary studies were conducted to study the hydrolytic degradation behavior of the graft copolymers. The hydrolytic degradation behavior of the graft copolymers were carried out in phosphate buffer saline at pH 7.4 and 37˚C as described in the Experimental Section. Typical GPC curves of PS-*g*-PLLGA before and during hydrolytic degradation are shown in Figure 4.28.

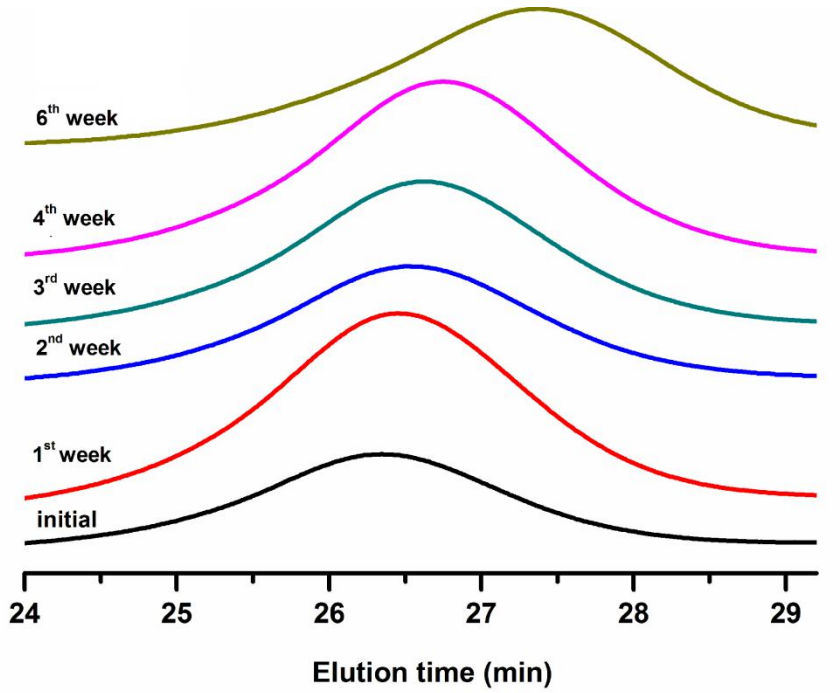


Figure 4. 28 : GPC traces of PS-*g*-PLLGA during hydrolitic degradation stage.

As can be seen, during hydrolysis the molecular weight peak of the graft copolymer is gradually shifted to a low-molecular weight region, thereby showing the hydrolytic degradation capability of the new graft copolymers synthesized. The degradation behavior of the graft copolymers was also followed by mass losses of the polymers. Figures 4.29 and 4.30 compare the molecular weight and mass losses of PS-*g*-PLLA and PS-*g*-PLLGA, respectively during the hydrolytic degradation.

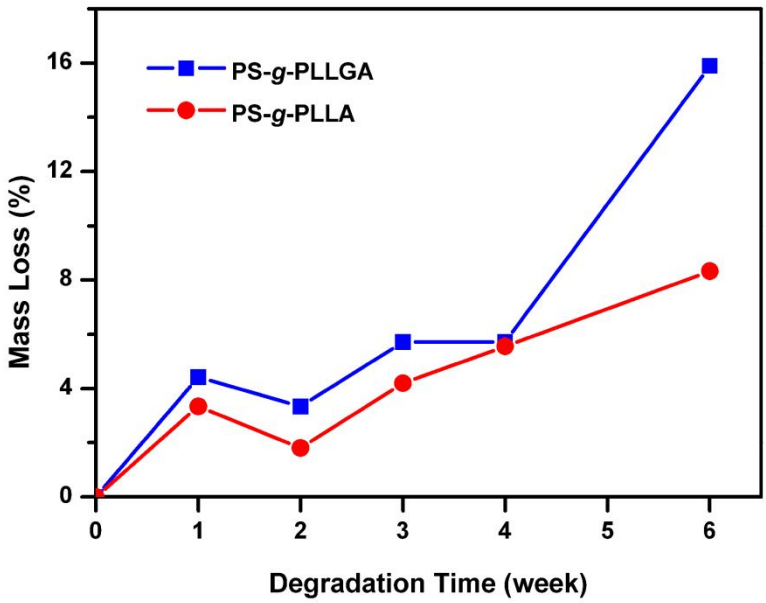


Figure 4. 29 : Mass losses for PS-*g*-PLLA and PS-*g*-PLLGA.

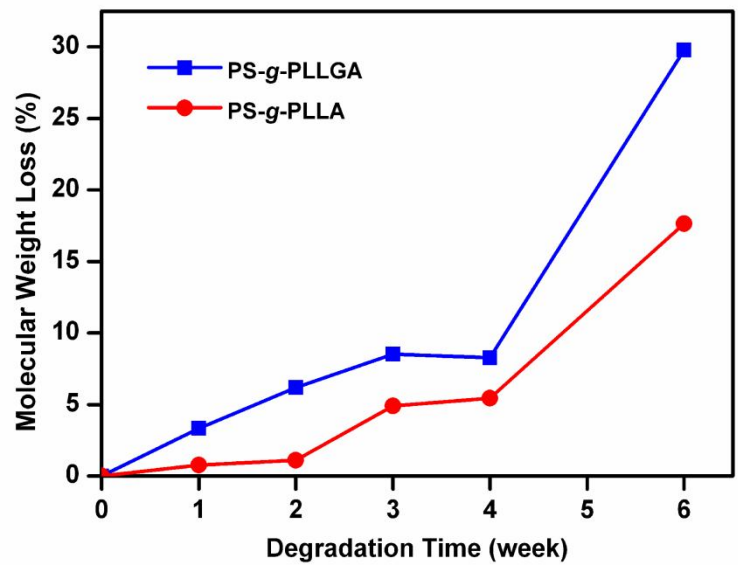


Figure 4. 30 : Molecular weight losses for PS-*g*-PLLA and PS-*g*-PLLGA.

In both cases, similar two-stage degradation patterns were observed. The initial slow degradation is leveled off at four weeks. The relatively faster rate observed after this point may be due to the autocatalytic degradation favored by the acid released.

Relatively faster rate of the degradation observed with the PS-*g*-PLLGA polymer is due to the presence of hydrophilic glycolide groups present in the structure. The degradations occured through ester groups (lactic acid and glycolic acid repeating units). This was confirmed by the ^{1}H-NMR spectroscopy. Although the ^{1}H-NMR

spectra of the chloroform extracted degradation products were complex for a definite estimation, they clearly indicated that the remaining product was mainly polystyrene.

4.2.3 *In vitro* release studies of PS-*g*-PLLA and PS-*g*-PLLGA

In this study, we describe the fabrication, characterization and *in vitro* release studies of microspheres of PS-*g*-PLLGA, PS-g-PLLA and PLLGA. PS-g-PLLA, PS-g-PLLGA and PLLGA were synthesized as described in section 3.3.2.

The *in vitro* release studies were performed at 37˚C in PBS at pH 7.4. Encapsulation efficiencies, drug loadings and initial burst releases of the microspheres are listed in Table 4.5. The optimized method of previous study involving the effect of conditions on particle size, surface morphology and controlled release behavior was used in this work.

Microspheres of these biocompatible copolymers fabricated through modified double emulsion/solvent evaporation method as described in section 3.3.1. Imatinib mesylate was used as a hydrophilic drug for encapsulation. Entrapment efficiencies and *in vitro* release studies of the imatinib mesylate microspheres were monitored by a high performance liquid chromatography (HPLC) assay. Stages of degradation and surface morphology of the loaded microspheres were determined by scanning electron microscopy (SEM).

Table 4.5: Encapsulation efficiencies (EE) and burst releases of drug loaded microspheres.

Polymer	EE (%)	Burst release (%)
PS-*g*-PLLA	77 ± 0,5	30,66 ± 0,28
PS-*g*-PLLGA	89 ± 1,44	8,34 ± 0,22
PLLGA	58 ± 3,32	4,65 ± 3,59

Molecular weights of the aliphatic polyester chains of PS graft copolymers were intended to keep closer to the molecular weight of the PLLGA copolymer for a proper comparison on controlled release profiles, size distribution and surface characteristics of the microspheres.

The degradation of polymeric microspheres were studied in PBS at pH:7.4 and 37 ºC. As shown in Figure 4.31, microspheres were in good spherical shape. The PS backbone of the graft copolymer provides a smooth surface without signs of major degradation during 35 days of incubation. PS-*g*-PLLGA graft copolymer showed nearly similar degradation behaviour with the PS-*g*-PLLA (Figure 4.32). The effect of glycolic acid units on the degradation was noted after 35 days. SEM images of microspheres of PLLGA copolymer indicate that the surfaces of the microspheres were smooth for the first 4 days (Figure 4.33). In a prolonged incubation, the microspheres started to lose smoothness and an increase in porosity of surface was observed by the initiation of the degradation. By day 25, the microspheres degraded to an extent that all spherical morphology was lost. On the other hand, microspheres of PS-*g*-PLLA remain smooth on 35th day as the first day.

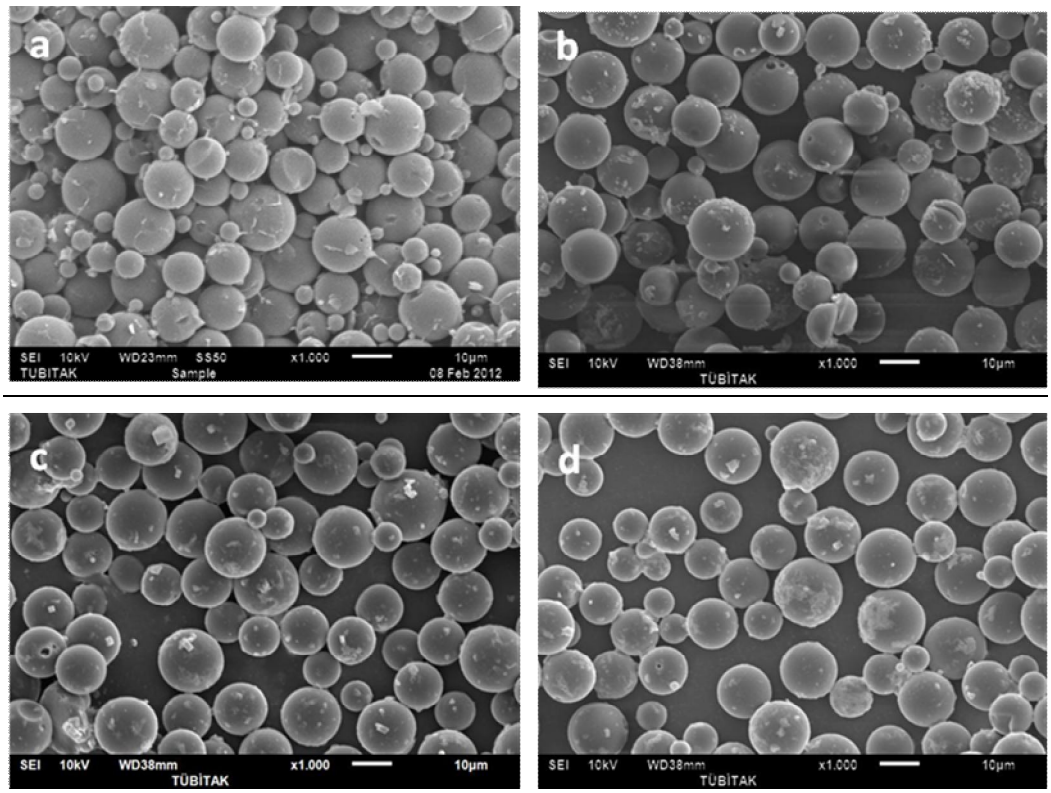


Figure 4. 31: SEM micrographs of PS-*g*-PLLA microspheres following *in vitro* degradation over a period of 35 days: a) 0 day; (b)4 days; (c) 19 days; (d)35 days.

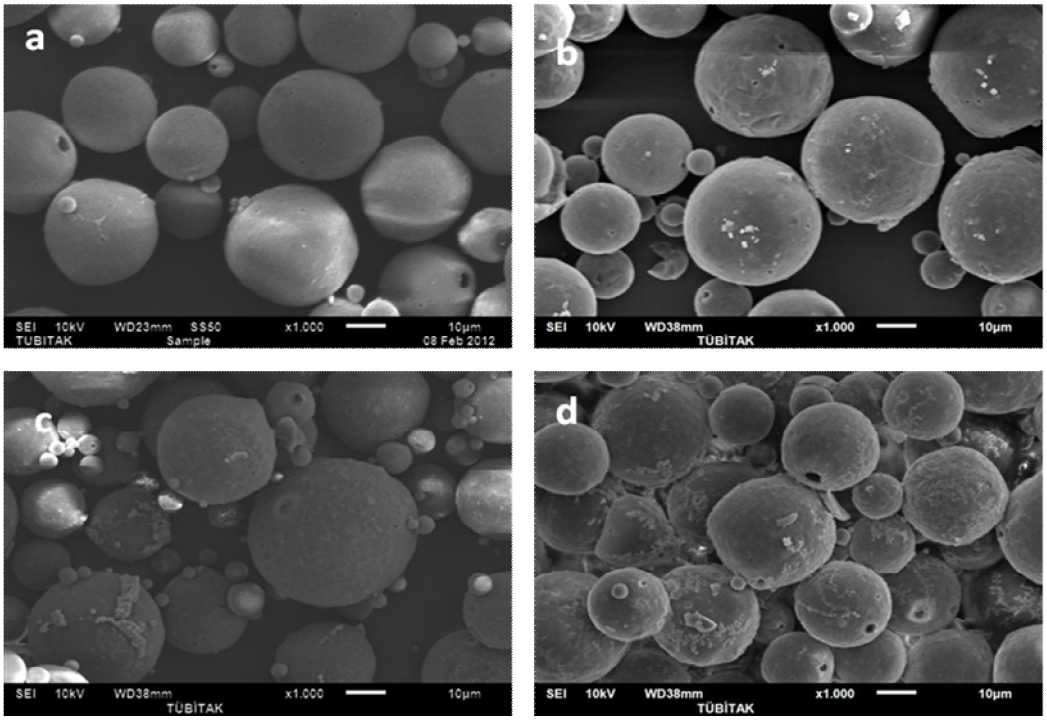


Figure 4. 32: SEM micrographs of PS-*g*-PLLGA microspheres following *in vitro* degradation over a period of 35 days: a) 0 day; (b)4 days; (c) 19 days; (d)35 days.

SEM photo of PLLGA microspheres points out the increasing porosity and loosing smoothness by the initiation of degradation due to the influence of glycolic acid units presence in the structure. On the other hand, microspheres of PS-*g*-PLLA remains as smooth as the first day. It is clear that PS backbone of graft copolymers contributes to retard the effect of glycolic units on degradation. This statement is independent of the molecular weight effect since M_w of aliphatic side chains of the graft copolymers close to the PLLGA copolymer. According to the morphological studies, it can be concluded that the composition of the polymers plays a dominant role in the degradation behaviour. The *in vitro* release studies of imatinib mesylate from PS-*g*-PLLGA, PS-*g*-PLLA and PLLGA microspheres were performed at 37˚C in PBS at pH:7,4 for 25-35 days. The influence of the structure of graft copolymers on daily and cumulative release of imatinib mesylate-loaded microspheres is shown in Figure 4.34 and 4.35, respectively. In the case of PS-*g*-PLLA, 153 µg/mL of the encapsulated IMM was released on the first day of incubation (Figure 4.34). For PS-*g*-PLLGA and PLLGA copolymer, the burst releases were 91 µg/mL and 15 µg/mL, respectively. Burst releases of the microspheres are quite high for PS-*g*-PLLA

suggesting the presence of the drug either stored close to the surface or remained on the surface during the preparation.

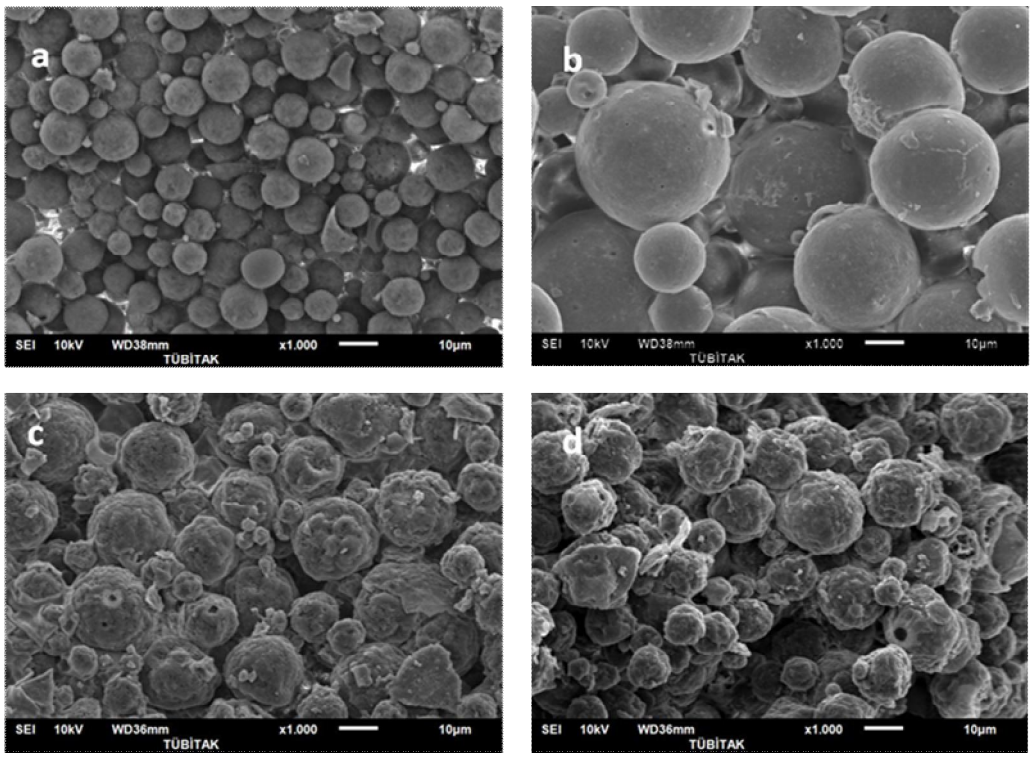


Figure 4. 33: SEM micrographs of PLLGA microspheres following *in vitro* degradation over a period of 25 days: a) 0 day; (b)4 days; (c) 10 days; (d)25 days.

Although such behaviour is not normally desired, in a previous study we showed that the burst release of imatinib mesylate is an important factor on inhibition tumor-induced neovascularization .

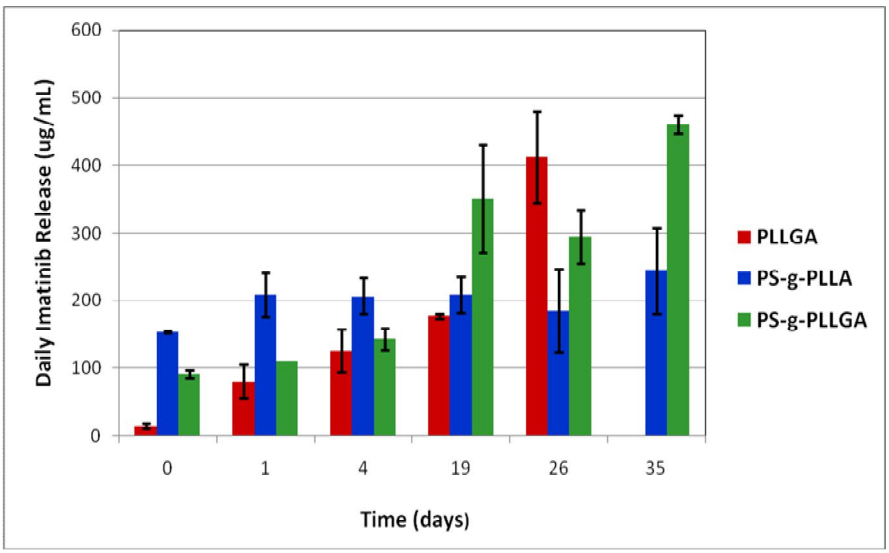


Figure 4. 34: Daily imatinib mesylate release profiles of PLLGA, PS-*g*-PLLA and PS-*g*-PLLGA microspheres.

Imatinib mesylate release increased up to 25 and 35 days for PLLGA and PS-g-PLLGA copolymers, respectively. Although there was no appearant degradation of PS-*g*-PLLA microspheres on the 35th day of incubation (vide ante, see Fig. 4.32), drug release was still carried on (Figure 4.35). It should also be pointed out that it showed an initial burst release followed by steady-state release until 35 days.

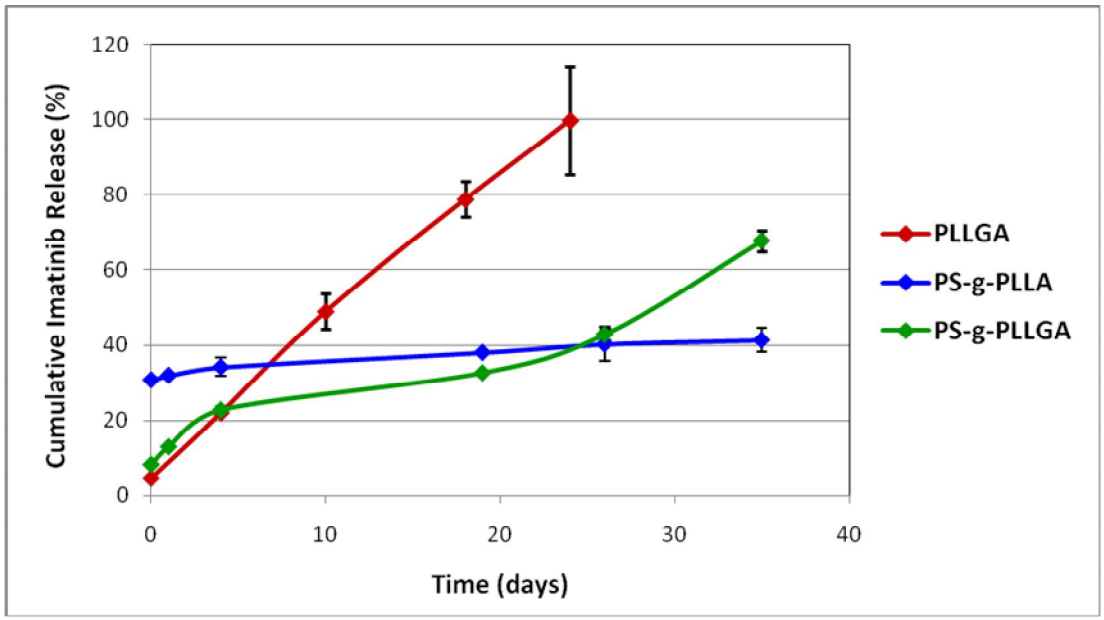


Figure 4. 35: Cumulative imatinib mesylate release profiles of PLLGA, PS-*g*-PLLA and PS-*g*-PLLGA microspheres.

Comparison of the graft copolymers of PS with PLLGA copolymer revealed that PS segments introduces durability and control the release of the drug that SEM images of the microspheres which is in agreement with the above statement. During the first 20 days, release profiles were similar for PS-*g*-PLLA and PS-*g*-PLLGA when burst effects were neglected. But, after 20 days, significant increase in release rates were observed for PS-*g*-PLLGA microspheres which can be explained in terms of higher degradation rate caused by increasing hydrophilicity and porosity of the surface.

In spite of low degradation profiles, PS-*g*-PLLA and PS-*g*-PLLGA released approximately 45% and 70% of total encapsulated drug during 35 days, respectively. On the other hand, PLLGA released the entire drug at the end of 25th day because of the glycolic acid units in the structure (Figure 4.35). Prolonged release in the former case indicates the influence of the diffusion rather than degradation provided by the polymer matrix.

4.3 Synthesis, Characterization and Pyrolysis of ABC Type Miktoarm Star PS-PLA-PEG Copolymer

Introduction of the click chemistry concept to the synthetic polymer chemistry enabled construction of various macromolecular blocks [137]. Click reactions are modular, high yielded rapid reactions with negligible by-product formation. The very well known click reactions include CuAAC [138-140] and Diels-Alder (DA) [137-142] reactions which are realized by the coupling of a conjugated diene and a dienophille. In the present study, we prepared a novel miktoarm star polymers possessing structurally different arms, namely PS, PEG and an aliphatic polyester, PLA. Such polymers are of interest particularly in biological application since respective hydrophobic, hydrophilic and biocompatible segments are linked together through a central core. It seemed appropriate to investigate their thermal degradation behavior since independent segment may influence their overall degradation behavior.

4.3.1. Synthesis and characterization of ABC type miktoarm star PS-PLA-PEG copolymer

The synthetic strategy is based on the design of a core molecule possessing appropriate functionalities for the initiation of the ring opening polymerization and two successive click reactions, namely thiol-ene and azide-alkyne cycloaddition reactions. The core was synthesized in a simple one-step reaction strategy. Thus, allyl glycidyl ether was reacted with sodium azide in the presence of ammonium choride using ethanol/water (Figure 4.36) with nearly quantitative yield (96%). The structure of the core was confirmed by ^{1}H-NMR and FT-IR analyses as shown in the experimental section.

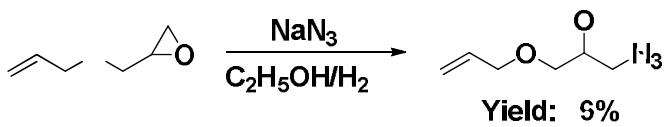


Figure 4. 36 : Synthesis of the core, 1-(allyloxy)-3-azidopropan-2-ol

Considering the possible involvement of the allylic group in the further reactions, in the first stage thiol-ene reaction was deliberately selected for the incorporation of the first arm to the core. Thus, ω-thiol functional polystyrene (PS-SH) was synthesized in a two-step protocol as described previously [146]. For this purpose, ω-bromo functional polystyrene obtained via Atom Transfer Radical Polymerization (ATRP)

is reacted with potassium xanthate where the ω-terminus is converted into xanthate functionality to yield PS-Xanth. Afterwards, the xanthate group is replaced with thiol functionality simply treating PS-Xanth with 1,2-ethandithiol in the presence of metallic zinc to give PS-SH. The overall process is given in Figure 4.37.

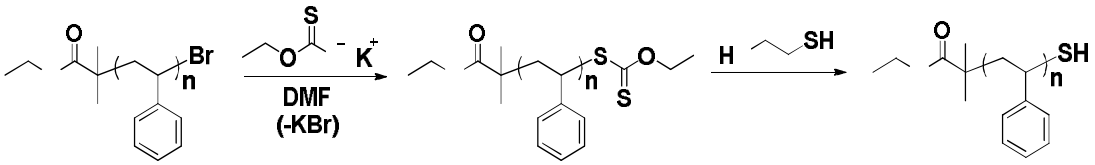


Figure 4. 37 : Synthesis of ω-thiol functional polystyrene, PS-SH.

In the later stage, PS-SH was clicked to the core by thiol-ene chemistry. Thus, PS-SH was reacted with the core in the presence of a photolabile compound, BAPO upon visible light irradiation. According to the ^{1}H-NMR data, the yield of the reaction is found to be realized with 95%. The thiol-ene process is shown in Figure 4.38.

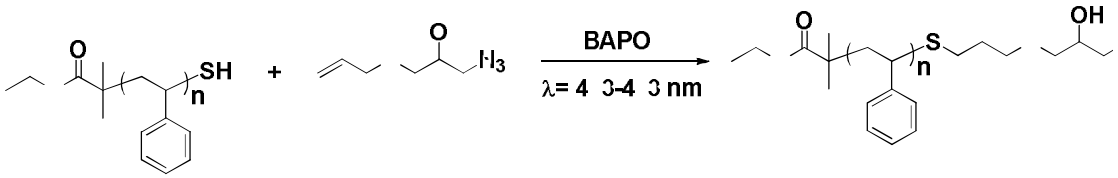


Figure 4. 38 : Thiol-ene click of PS-SH with the core, PS-core.

Next, L-lactide was polymerized from the hydroxyl functionality to afford the second arm of the star copolymer in the presence of $Sn(Oct)_2$ as catalyst. Parallel to this, acetylene functional PEG was synthesized via a simple esterification reaction between ω-hydroxy functional PEG (Me-PEG) with 4-pentynoic acid for the polymeric CuAAC click component (PEG-acetylene). Finally, PEG-acetylene was clicked to the azide functionality simply by CuAAC using CuBr as catalyst (Yield: 94%, according to the ^{1}H-NMR data). The overall process is demonstrated in Figure 4.39. The ^{1}H-NMR of the miktoarm star copolymer, PS-PLA-PEG, is shown in Figure 4.40. All characteristic peaks of each segment can be clearly identified confirming realization of the final structure.

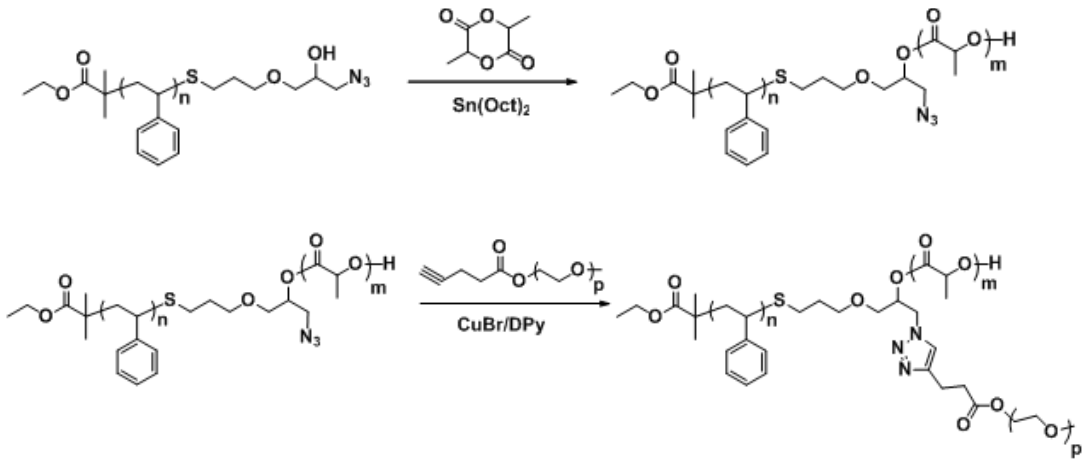


Figure 4. 39 : Construction of the second (PS-PLA-N_3) and third arm (PSA-PLA-PEG) of the star copolymer.

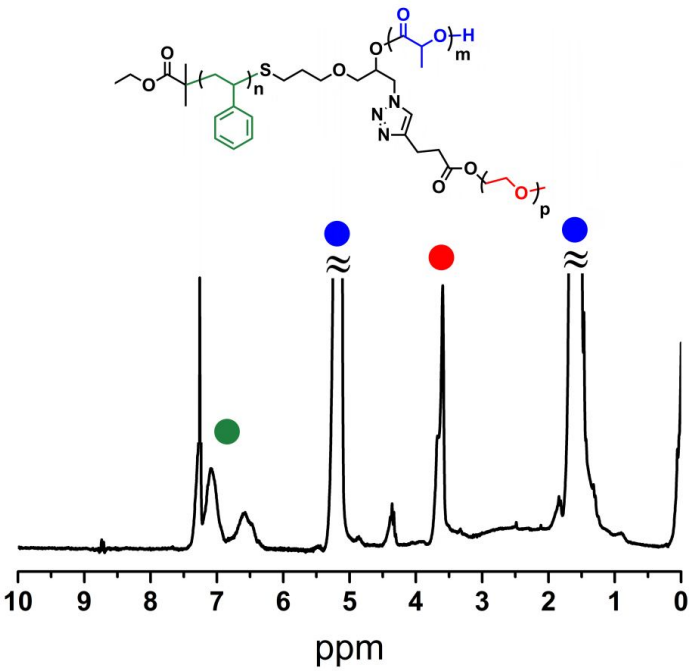


Figure 4. 40 : ^{1}H NMR of the miktoarm star copolymer.

The GPC chromatograms of the polymers in each step are depicted in Figure 4.41. All polymers display unimodal GPC traces confirming the absence of side reactions such as chain scission or any unwanted coupling reactions like disulfide formation.

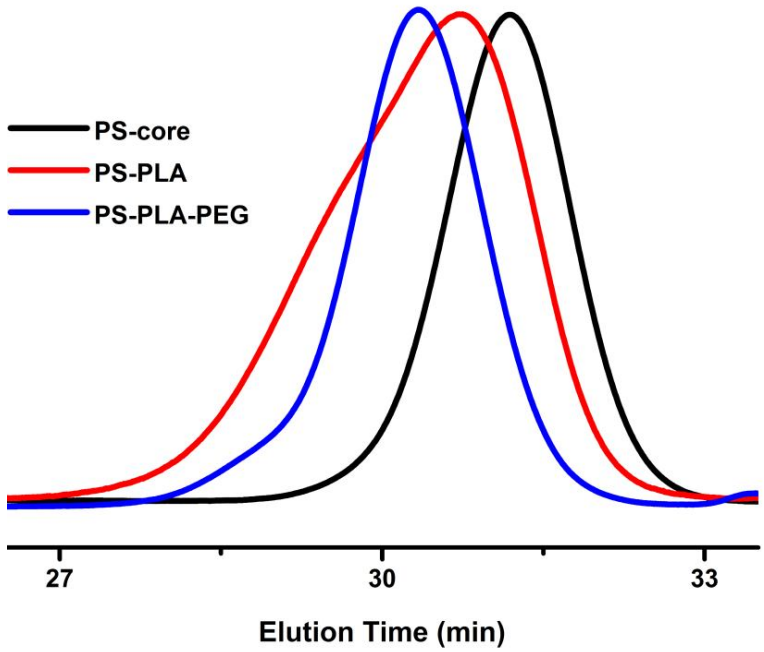


Figure 4. 41 : GPC traces of polymers obtained in each step.

The molecular weight characteristics of polymers obtained in each step are collected in Table 4.6.

Table 4. 6 : Molecular weight characteristics of polymers at various stages.

Polymer	$M_{n,NMR}$[a] (g·mol^{-1})	$M_{n,GPC}$[b] (g·mol^{-1})	M_w/M_n[b]
PS-Br	2030	1910	1.19
PS-Xant	-[c]	2200	1.20
PS-SH	1980	1950	1.18
PS-core	2270	2250	1.18
PS-PLA-N_3	3480	2860	1.32
PEG	1950	2100	1.15
PS-PLA-PEG	5940	3010	1.21

[a] Determined by ^{1}H NMR analysis.
[b] Determined by gel permeation chromatography (GPC) using polystyrene standards.
[c] Could not be determined due to partial insolubility.

4.3.2 Pyrolysis of of ABC type miktoarm star PS-PLA-PEG copolymer

The thermal degaradation behavior of the final miktoarm star copolymer PS-PLA-PEG was investigated by J. Hacaloglu et.al. Direct pyrolysis mass spectrometry was used to study the thermal degradation behavior of PS-PLA-PEG. As, pyrolysis mass spectra of polymers, especially the ones involving different chains, are usually very complex, for the elucidation of thermal degradation behavior of PS-PLA-PEG, DP-MS analysis of intermediate structures PS-SH, PS-core, PS-PLA-N_3 were also performed.

Thermal degradation of PS-PLA-PEG started just above 100 °C. The total ion current curve shows five overlapping peaks with maxima at 180, 250, 330, 370, and 450°C (Figure 4.42). Peaks diagnostic to PEG side chains appeared in the mass spectra at early stages of pyrolysis. Peaks due to decomposition of PLA chains were observed in the temperature range of 220 – 380 °C and those due to PS degradation were detected in the final stages of pyrolysis at around 441 °C. Single ion evolution profiles of diagnostic products of PEG, PLA and PS units are shown in Figure 4.39. In general a slight decrease in thermal stability compared to PS-PLA-N_3 was detected. Even the evolution of PS based products shifted slightly to low temperatures. It is known that during the thermal degradation of PEG, the fragments generated by cleavage of C-O and C-C bonds are stabilized by hydrogen transfer reactions, yielding low mass chains with methyl, ethyl, hydroxyl, aldehyde and unsaturated end groups [147-153]. It has been determined that at the onset of pyrolysis, products with ethyl and hydroxyl end groups are dominant [154]. The evolution profiles of diagnostic products of PEG showed two peaks, the first being more intense, with maxima at around 169 and 240°C. All products except the ones that can also be generated during the decomposition of PLA showed almost identical trends. The products involving unsaturated end groups were more pronounced even at low temperatures contrary to literature results. It may be thought that these end groups were mainly produced by elimination of H_2O from chains with alcohol end groups as shown in Figure 4.43. The low temperature peaks present in the evolution profiles of PLA based products were attributed to contribution of PEG based products with the same m/z value. Nevertheless, a slight decrease in the thermal stability of PLA chains compared to PS-PLA-N_3 was detected. The maxima of the two peaks present in the evolution profiles of PLA based products were shifted to

300 and 363°C from 308 and 377°C respectively. It may be thought that the possibility of decomposition of PLA chains by hydrolysis should increase significantly due to the evolution of H_2O during the degradation of PEG chains.

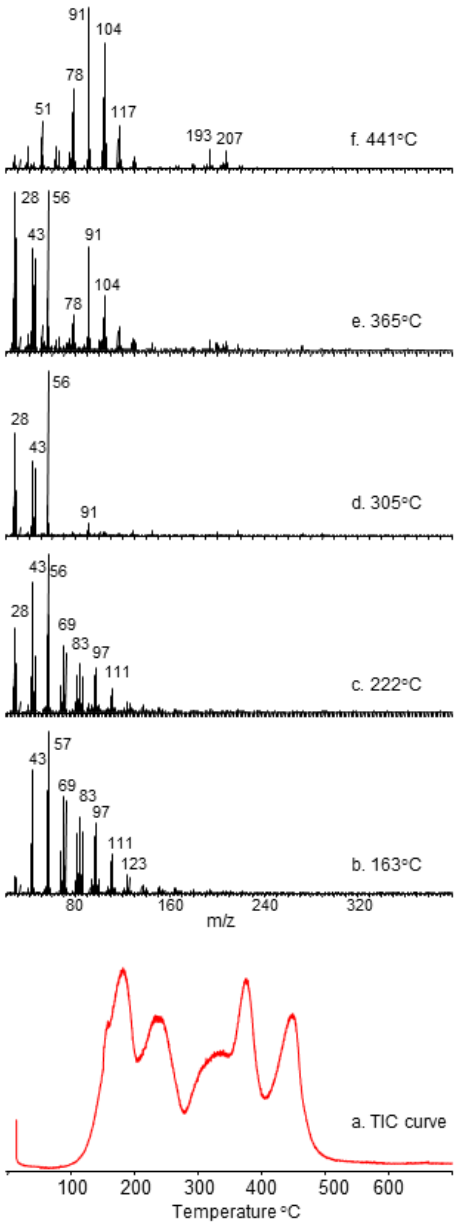


Figure 4. 42: a. TIC curve and pyrolysis mass spectra at b. 163, c. 222, d. 305, e. 365 and f. 441°C of PS-PLA-PEG.

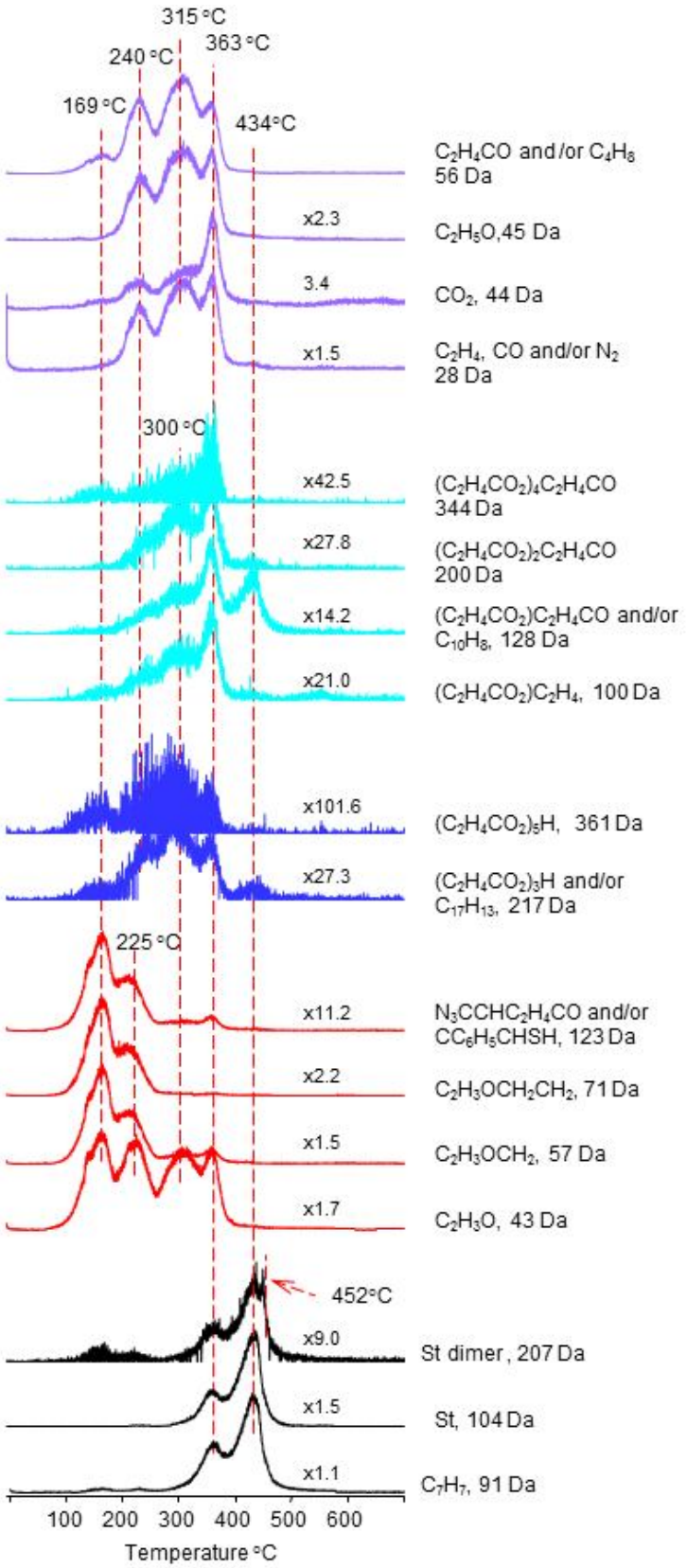


Figure 4. 43: Single ion evolution profiles of selected products recorded during the pyrolysis of PS-PLA-PEG.

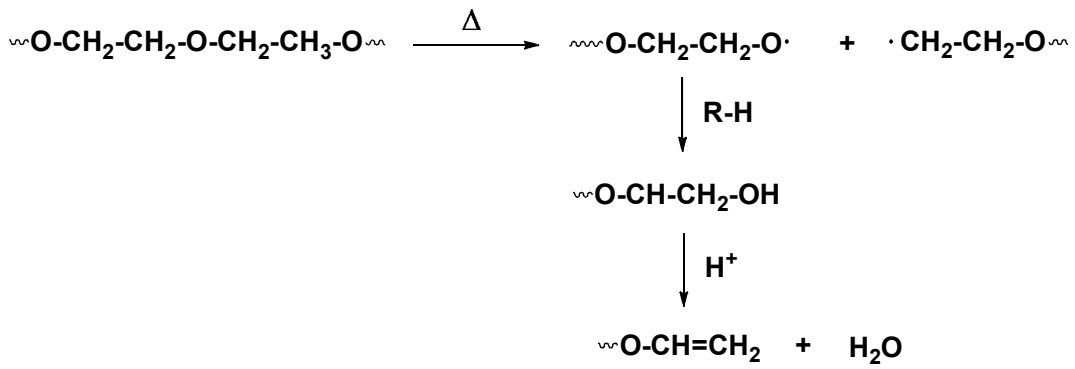


Figure 4. 44: Elimination of H_2O during the thermal degradation of PEG.

As a consequence, the chains bearing acid and alcohol end groups should be more dominant. This in turn, should enhance CO_2 and CO evolutions. Generation of $CH_3C_2H_2CH_3$, COOH, CO_2 and CO was detected at slightly higher temperatures again supporting the proposed degradation mechanism (Figure 4.44). A slight increase in the yields of CO_2 and CO was also noted. One point that should also be noticed was the significant increase in the intensities of the high temperature shoulder present in the evolution profiles of products with m/z=128 and 217 Da tentatively assigned to $C_{10}H_8$ and $C_{17}H_{13}$ fragments involving unsaturation and/or crosslinked structures. Again a high temperature peak in the evolution profile of 207 Da fragment at around 452 °C was detected.

These findings were in accordance to above proposals, and confirmed the increase in the extent of chains with acid end groups and anhydride units as a result of hydrolysis reactions due to the elimination of H_2O during the decomposition of PEG chains. Consequently, the increase in the extent of unsaturated and crosslinked units was supported.

5. CONCLUSIONS

New biopolymers are needed in biomedical applications because of the complexity of *in vivo* environments. Synthetic biopolymers have become an attractive alternatives caused by the difficulties on chemical modifications of biologically derived biopolymers and instead of having good biocompability but triggering an immune response in human body of biologically derived polymers. In this thesis, we describe novel synthesis of new biodegradable polymers to ensure the demand. These biodegradable polymers are expected to cover deficiencies of biopolymers and take place in practical applications.

In the first part, we aimed to prepare microspheres made of commercial biodegradable polymers at different conditions to optimize a method for further investigations. Polymers with a different molecular weights and compositions are used for comparison. Surface characteristics and controlled release profiles of synthesized microspheres are examined. As a result of this work optimized microspheres were chosen for *in vivo* experiments. The results indicate that a model drug loaded microspheres are a promising way for treatment.

In the second part, we have demonstrated that biodegradable graft copolymers of polystyrene and aliphatic polyesters of the following structure (Figure 5.1) could easily be synthesized by combination of controlled polymerization methods (NMRP and ROP) and click chemistry.

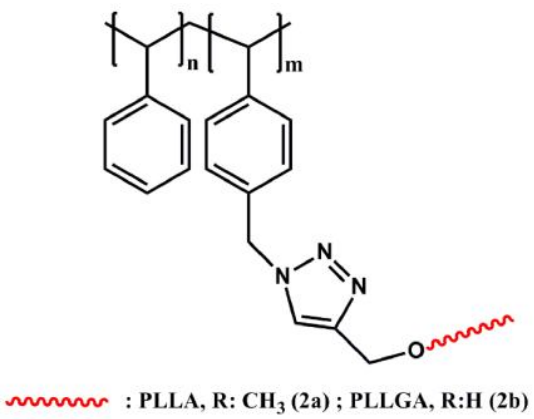


Figure 5. 1 : Chemical structure of PS-*g*-PLLA and PS-*g*-PLLGA graft copolymers.

The structural characterization of the graft copolymers were made by spectral and molecular weight analyses. The resulting graft copolymers showed slow hydrolytic degradation profile. The graft copolymers with PLLGA side chains degrade faster relatively due to the hydrophilic nature of the glycolic acid repeating units. Due to their slow degradation, these copolymers are ideally suitable for long-term drug delivery usage. *In vitro* release studies and surface morphologies of the miscropeheres formed from the graft copolymers confirm the statement.

In the third part, an ABC type miktoarm star copolymer possessing PS, PLA and PEG arms was synthesized by combining ATRP and ROP with two click chemistries, namely thiol–ene and copper catalyzed azide–alkyne cycloaddition (CuAAC) (Figure 5.2).

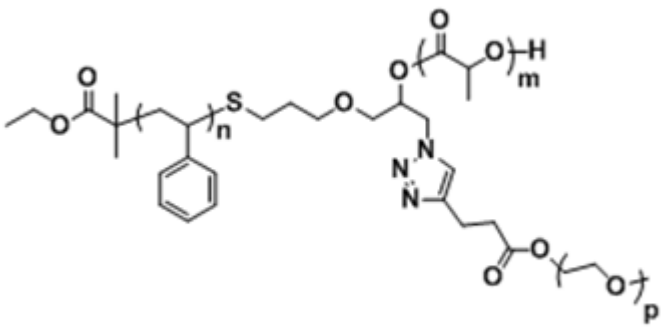


Figure 5. 2 : Chemical structure of ABC type miktoarm PS-PLA-PEG star copolymer.

All intermediates, related polymers at different stages and final PS-PLA-PEG miktoarm star copolymer were characterized by ^{1}H NMR, FT-IR, and GPC analyses. Thermal degradation studies are performed in order to obtain information for the future applications of the polymer in the biomedical field. DP-MS analyses indicated that the decomposition of PS and PEG chains occurred almost independently. On the other hand, as a consequence of hydrolysis of PLA chains by H_2O eliminated during the thermal degradation of PEG chains, thermal degradation of PLA was affected. As a result, increases in the yields of CO_2, CO and units involving unsaturation and/or crosslinked structure were detected.

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List of Publications and Patents:

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