Katrin Jammernegg, BSc

Modification and performance of biodegradable polyesters / copolyesters as drug encapsulation systems

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Ing. Adriana Gregorova, PhD.

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Kurzfassung

Diese Masterarbeit beschäftigt sich mit der Synthese und Modifikation von biologisch abbaubaren Copolyestern und deren Verwendung als Mikropartikel zur kontrollierten Freisetzung eines eingekapselten Wirkstoffes. Dazu wurden zwei unterschiedliche Copolyester durch Veresterungs – bzw. Polykondensationsreaktionen synthetisiert: einerseits Poly (butylene – adipate – co – butylene terephthalate), P-(BA-co-BT), welches außerdem auf zwei verschiedene Arten, durch Polyethylenglykol und Triallyl Isocyanurat, modifiziert wurde und andererseits Poly (glycerol sebacate – co – glycerol terephthalate), P-(GS-co-GT). Die erhaltenen Produkte wurden mit Hilfe von Gelpermeationschromatographie, Infrarot Spektroskopie, Kernspinresonanzspektroskopie, dynamischer Differentialkalorimetrie, Thermogravimetrie sowie durch Zugprüftests charakterisiert. Des Weiteren wurden Mikropartikel mit P-(BA-co-BT), P-(GS-co-GT) und verschiedenen Modifikationen von Polymilchsäure hergestellt und deren Eigenschaften miteinander verglichen. Coumarin und Triclosan wurden als Modellsubstanzen eingekapselt. Die erhaltenen Mikropartikel wurden mit UV – VIS Spektroskopie untersucht um die Einkapselungseffizienz und die Kinetik zur Wirkstofffreisetzung zu bestimmen und mit statischer und dynamischer Lichtstreuung sowie mit optischer Lichtmikroskopie und Rasterelektronenmikroskopie um die Größenverteilung und Struktur angeben zu können.

Anhand der erhaltenen Ergebnisse kann die erfolgreiche Synthese und Modifikation von P-(BA-co-BT) Copolyestern bestätigt werden, die einerseits zu einem höheren Vernetzungsgrad durch die Zugabe von Triallyl Isocyanurat und andererseits zu einer Verbesserung der Hydrolysefähigkeit durch den Einbau von Polyethylenglykol führte. Darüber hinaus gelang es, thermoplastische und zugleich lösliche P-(GS-co-GT) Copolyester zu synthetisieren. Durch die Herstellung von Mikropartikeln konnte gezeigt werden, dass die Größenverteilung und die Kinetik der Wirkstofffreisetzung stark vom verwendeten Polymer abhängig sind und dass es potenzielle Alternativen zu Polymilchsäure als Polymermaterialien für die Herstellung von Mikropartikeln gibt.

Abstract

The present master thesis deals with the synthesis and modification of biodegradable copolyesters in order to create drug encapsulation systems for a controlled drug release. Two types of copolyesters were synthesised via transesterfication and polycondensation reactions: 1) poly (butylene adipate – co- butylene terephthalate), P-(BA-co-BT) which was modified by triallyl isocyanurate or poly (ethylene glycol) and 2) poly (glycerol sebacate – co – glycerol terephthalate), P-(GS-co-GT). These copolyesters were characterised by gel permeation chromatography, infrared spectroscopy, nuclear magnetic resonance, differential scanning calorimetry, thermogravimetry and tensile testing. Moreover, the recent work compares drug encapsulation systems based on various biodegradable polymers: poly (lactic acid), P-(BA-co-BT) and P-(GS-co-GT). As model substances for the encapsulation coumarin and triclosan were investigated. The size and structure of prepared microparticles were characterised by static and dynamic light scattering, optical light microscopy and scanning electron microscopy. Encapsulation efficiency and drug release were determined by ultraviolet-visible spectroscopy.

Obtained results confirm successful synthesis and modification of P-(BA-co-BT) copolyesters, which are revealed by a high crosslinking degree by means of triallyl isocyanurate and an improvement of the degree of hydrolysis due to the implementation of poly (ethylene glycol). Furthermore synthesis of thermoplastic and soluble P-(GS-co-GT) copolyesters could be succeeded. The preparation of drug encapsulation systems showed that especially drug release and particle size distribution are strongly dependent on functional groups present in polymer matrix, polymer molecular weight and the degree of crystallinity.

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1. Theoretical background

1.1 Biodegradable polymers

Biodegradable polymers represent one of the most promising types of polymers for the future. Due to the fact of climate change, waste problems and very huge "plastic islands" destructing the sea and our ecological system, there has to be a rethinking in plastics policy. In recent years it became quite fashionable to use biodegradable plastics. Some big supermarket chains already replaced their plastic bags mostly made from LDPE through ones made out of starch blends. But there is still the problem concerning mechanical stability of these polymers and the most important factor, the price. Production of plastics like polyethylene, polypropylene, PET etc. is very cheap in comparison with biodegradable materials, because there are well established systems.

In general, the term biodegradable polymers refers to a group of plastics which can be converted into biomass, water and CO_2 (or CH_4) by microorganisms and / or hydrolysis within a certain period of time. [1] [2] The origin and composition are therefore immaterial; biodegradable polymers can either belong to the group of natural polymers like e.g. starch, chitin, proteins, cellulose or to the class of synthetic polyesters. Latter can again be divided into polymers derived from renewable resources like poly (lactic acid) or poly (hydroxyl alkanoates) and polyesters based on fossil resources, for example poly (butylene succinate), poly (butylene succinate adipate) and poly (butylene adipate-co-butylene terephthalate). [3] [4]

The crucial fact to gain a biodegradable polymer is the introduction of heteroatoms into its backbone to establish hydrolysable linkages. Dependent on the type of implemented heteroatom, various classes of polymers have been synthesised. To these belong polyesters, poly (ester-anhydrides), poly (ester-amides), poly (ester-urethanes), poly (ester-ureas), poly (amide-enamine) and poly (ester-ether). [3] [5]

Polyester

Polyesters are probably the most common group of synthetic biodegradable polymers. Usually they are synthesised either through ring opening polymerisation of cyclic monomers (lactide, lactone) or via a polycondensation reaction of hydroxyacids respectively diols and dicarboxylic acids which allow a great variety of different end products. The introduction of aromatic moieties leads to aromatic-aliphatic polyesters and can enhance mechanical and thermal properties. If monomers with more than two functional groups are implemented into the

process, networks with a high degree of crosslinking are established which show thermosetting properties. [3] [5] [6]

Poly (ester-amide)

Poly (ester-amides) are prepared to combine the best properties of polyamides and polyesters in one polymer. Whereas polyamides offer good thermal stability and mechanical properties including a high tensile strength which results from their ability to establish strong hydrogen linkages of their amide bonds with various chains, polyester provide better biodegradability. Poly (ester-amide) are synthesised by a polycondensation reaction of diamines with included ester bonds and dicarboxylic acids. To enhance the biodegradability of the polyamide part, α-amino acids are very often applied in these polymers. Because of this combination of desired properties, poly (ester-amides) are a promising class of polymers for biomedical applications in the future. [5] [6]

Poly (ester-urethane)

Poly (ester-urethanes) can be synthesised via a polycondensation reaction of hydroxyl end capped aliphatic polyesters and diisocyanates. Supplementary the prepared poly (ester-urethanes) can be chain extended by a further addition of diisocyanate and diamines. Through this reaction a polymer consisting of hard and soft segments can be established, whereas the poly (urethane – amide) parts are responsible for the hard segments and the poly (ester-urethane) for the soft segments. In general, poly (ester – urethanes) are applied in various fields like coatings, adhesives or as thermoplastics. [5] [6] [7]

Poly (ester-ether)

Poly (ester-ethers) are characterised by the implementation of ester and ether bonds in a polymer's backbone. Especially the introduction of polyethylene glycol (PEG) segments is well-known to accelerate the biodegradation due to their high hydrophilicity and is therefore combined with PET which offers excellent mechanical properties. The synthesis of poly (ester-ether) with PEG is particularly interesting for medical applications such as screws or sutures which should be able to degrade within a few months. [5] [6] [8]

However, biodegradable polymers in general offer many advantages. Some of them are listed below.

- Biodegradation: Due to their ability of biological degradation they are environmentally friendly and can help to gain control of waste problems caused by plastics.
- Biocompatibility: Most of the mentioned biodegradable polymers show good biocompatibility with the human body and can therefore be applied as medical devices such as screws or tissue scaffolds or drug encapsulation systems.
- Renewability: Some polymers like PLA and PHA are derived from renewable resources.
- Mechanical properties are often comparable to those of conventional polymers like PE, PP, PET, PS etc.

Of course biodegradable polymers also feature some disadvantages, which are discussed below: [9]

- High costs
- Production processes have to be optimised
- Not all polymers offer ideal material properties
- Thermal stability is sometimes insufficient (e.g. PCL has its melting temperature at 60 °C)

Following important synthetic biodegradable polyesters are covered in the next sub chapters:

- Poly (lactic acid) – PLA
- Poly (hydroxyalkanoates) – PHA
- Poly (butylene adipate – co- butylene therephthalate) – PBAT
- Poly (butylene succinate) – PBS
- Poly (caprolactone) – PCL

1.1.1 Poly (lactic acid)

Poly (lactic acid), abbreviated PLA, is a synthetic, aliphatic biodegradable polyester derived from renewable resources. It belongs to the family of polyhydroxyalkanoates. PLA can be synthesised either from lactic acid by a polycondensation reaction or from the cyclic lactide via ring opening polymerisation. Due to its good biocompatibility in the human body, it is often used for medical applications such as tissue scaffolds, implant devices, internal sutures and drug delivery systems. Furthermore, PLA is also applied as packing material for food, in the production of plastic cups, spoons and forks as well as in agriculture for mulch films. [10] [11] [12] [13]

Lactic acid (2-hydroxy propionic acid) occurs in the nature in two different enantiomeric forms, D- and L- lactic acid, whereas L- lactic acid is the predominant enantiomer. Both optically active forms are shown in Figure 1. Lactic acid is usually derived by a fermentation process of carbohydrates or (less common) via chemical synthesis. [3] [10] [11] [12] [14]

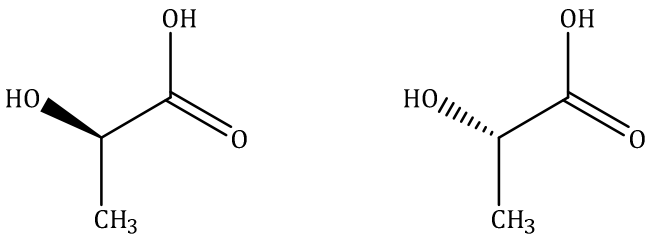

Figure 1: Enantiomers of lactic acid

The second precursor is lactide (3,6-dimethyl-1,4-dioxane-2,5dione)[12], a cyclic dimer of lactic acid. It occurs in three different stereoforms, D-lactide, L-lactide and meso-lactide (see Figure 2). Whereas D-lactide consists of two D- lactic acid monomers and L-lactide of two L-lactic acid monomers, meso-lactide is a combination of one D-lactic acid and one L-lactic acid monomer. Lactide can be derived as product from depolymerisation reactions of low molecular weight PLA. The obtained mixture of above mentioned isomers can be separated by distillation. [11]

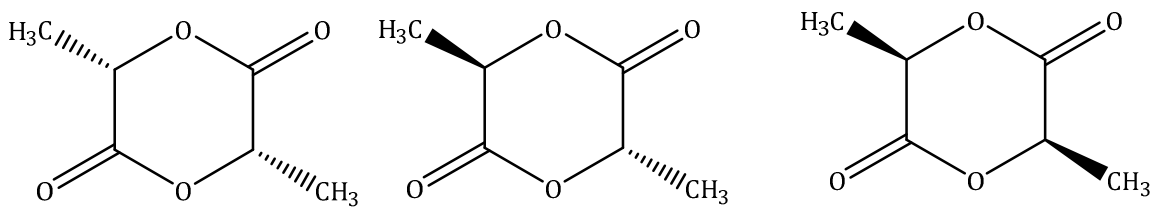

Figure 2: Diastereomers of lactide

In Figure 3 are three common synthesis routes of PLA shown.

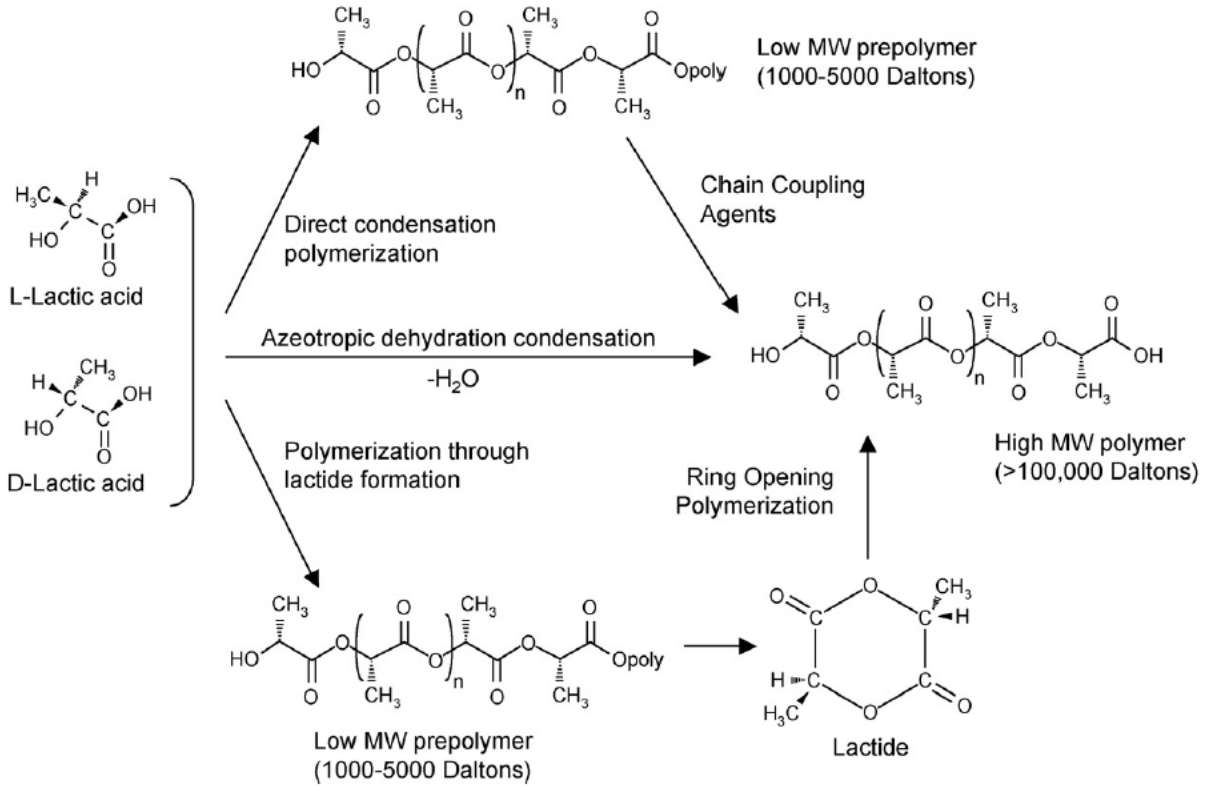

Figure 3: Syntheses routes of PLA (figure adopted from [10])

Direct condensation polymerisation

In direct condensation polymerisations lactic acid is applied as starting material. Usually this method is used very rarely because it only yields low molecular weight pre polyesters which have to be converted into high molecular weight polyester by help of chain coupling agents. These chain extenders, e.g. isocyanates, epoxides, anhydrides, react with the hydroxyl groups or the carbonyl end groups of the low molecular weight PLA. As a consequence, the resulting polymer may not be a homopolymer anymore, depending on the type of chain linker used. Moreover, they can diminish for example biodegradability and aside from that, unreacted oligomers, catalyst residues and chain coupling agents may require a further purification step which implies higher costs. [10][11[15]

Azeotropic polycondensation

Via azeotropic polycondensation reactions high molecular weight PLA can be achieved. Therefore lactic acid has to be dehydrated in a first step. This is done by means of a refluxing solvent with a high boiling point [11] which acts as entrainer for water, and an appropriate

catalyst. Due to continuous removal of the water the equilibrium is shifted to the side of the product and enables the synthesis of high molecular weight polyester. Because of the high amounts of catalyst required and the organic solvent which both have to be removed by a purification procedure, this type of reaction is very expensive. [11] [12]

Ring opening polymerisation

Ring opening polymerisation (ROP) of the cyclic lactide has been invented to reduce production costs of PLA. Nowadays it is by far the most common synthesis route for the production of high molecular weight PLA in industrial scale. Depending on the type of catalyst used, ring opening polymerisation can be performed via ionic (anionic or cationic) or coordinative mechanism. The only compound known to catalyse ROP by a cationic mechanism is trifluoromethane sulphonic acid and its methyl ester. [11] Examples for catalysts used in anionic polymerisation are alkoxides, for instance potassium methoxide. However, it was explored that the addition of less reactive transition metal compounds such as stannous octoate show high catalytic activity as well and lead to high molecular weight PLA. [11] [12]

Another advantage of ring opening polymerisation is the fact that it is possible to accomplish the synthesis with several methods such as melting, suspension, bulk and solution polymerisation. [11] [12] Nevertheless this type of polymerisation requires a purification step as well which is responsible for higher costs in comparison with conventional polymers. [12]

Copolymers of PLA

To enhance various properties of PLA, copolymerisations with other compounds have been intensively investigated. The most common copolymer based on PLA probably is poly (lactide-co- glycolide), abbreviated PLGA. Therefore glycolic acid is used as second type of monomer. Desired properties such as morphology and biodegradability can for instance be controlled by the lactide / glycolic acid ratio. [3] Other possibilities for comonomers are ε-caprolactone, γ-valerolactone or trimethylene carbonate. [14]

Moreover, PLA is already commercially available by different companies under their own trade names, e.g. Nature Works® from Cargill Dow (USA), Lacea® from Mitsui Chemicals, Lacty® from Shimadzu (both Japan) or Purac® from Purac Biochemicals (Netherlands). [3] [12] [14]

1.1.2 Polyhydroxyalkanoate

Polyhydroxyalkanoates (PHA) are biodegradable biopolymers derived directly from bacterial metabolism. Polymerisation takes place in specific microorganisms when there is a lack of nutrients such as nitrogen [3] and an excess of carbon. These living organisms produce these polymers as energy and carbon storages and accumulate them in their cells. Up to now about 250 [13] different types of microorganisms have been reported which are able to synthesise PHAs. The average molecular weights of produced polyesters ($2*10^5$ – $3*10^6$ Dalton) are dependent on the size and type of the bacteria. In Figure 4 is the general structure of PHA shown. The side chain residue marked "R" can for instance be hydrogen, methyl-, ethyl-, propyl-groups etc. Beside the various side chains PHAs can also be altered through different lengths of the main chain. These two parameters are responsible for the resulting thermal and mechanical properties. [3] [13] [14] [16]

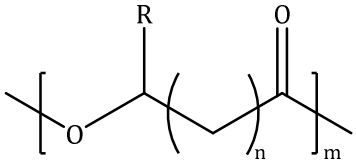

Figure 4: General structure of PHAs

Poly (hydroxybutyrate)

The most common type of PHAs is probably poly (3-hydroxybutyrate), abbreviated PHB. It was discovered in the 1920s of the last century by a French scientist called Lemoigne and is produced since then biotechnologically. The structure of PHB is illustrated in Figure 5. PHB is a thermoplastic, semi – crystalline polyester with a quite high crystallinity above 50 % and a melting temperature of about 180 °C. [3] [16] The main disadvantages of PHB are on the one hand its brittleness and on the other hand that its thermal degradation starts at the same temperature as it has its melting point. For these reasons PHB is rarely applied compared with other biodegradable polymers. [3] [14] [16] [17]

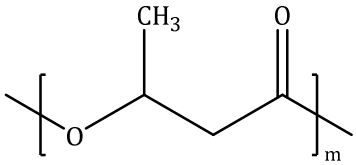

Figure 5: Structure of PHB

To overcome this problems, blending of PHB with different other materials like PCL and hydroxyvalerate has been intensively investigated. [3]

Poly (hydroxybutyrate – co – hydroxyvalerate)

Poly (hydroxybutyrate – co – hydroxyvalerate), abbreviated PHBV, is the most important copolymer of PHB. In comparison to pure PHB PHBV is less brittle and stiff. [3] [14]

PHB and PHBV are also produced in industrial scale by several companies with their own trade names, e.g. Biopol® from Monsanto (USA), Nodax® from Procter & Gamble (USA) or Biomer P® from Biomer (Germany). [3]

The production in industrial scale usually occurs in three steps in a bacterial fermentation process. It starts with the fermentation, proceeds with the isolation and purification and concludes with blending and palletising of obtained products. For fermentation an appropriate reactor is charged with mineral medium. Inoculation takes place by the addition of seed ferment, where the required bacteria are within. Beet sugar or corn steep liquor is in most cases used as carbon source and added until the production of PHA is completed. The whole fermentation process needs about 38 – 48 hours. [14] [16] [17]

In Figure 6 is as an example the synthesis of PHB with the bacterium *Ralstonia eutropha* illustrated. The whole process proceeds in three reaction steps including three different enzymes and starts with the condensation of two molecules of acetyl – CoA. [14] [16] [17]

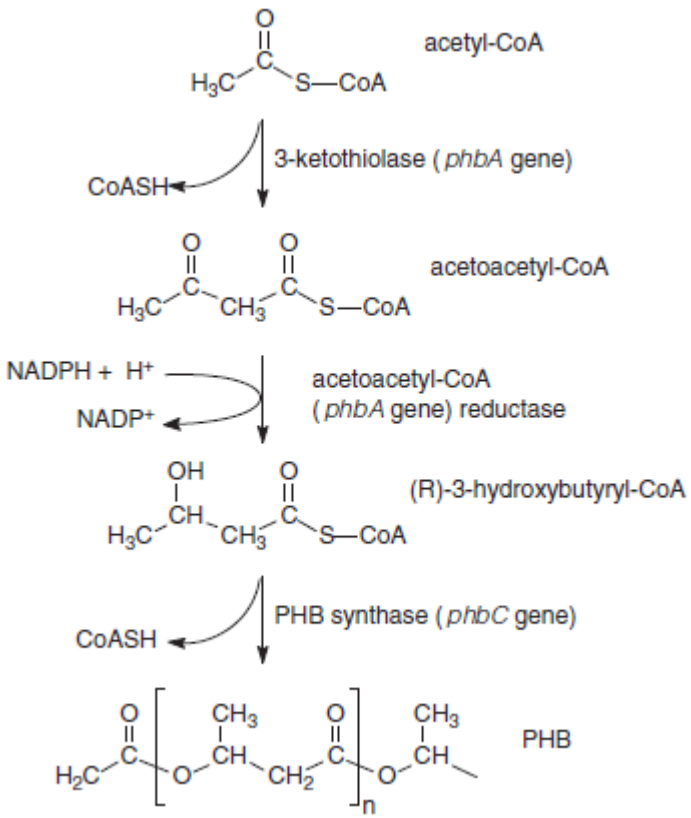

Figure 6: Reaction scheme of PHB in *Ralstonia eutropha* (figure adopted from [14])

1.1.3 Poly (caprolactone)

Poly (caprolactone), shortened PCL, belongs to the group of aliphatic, biodegradable polyester derived from petroleum based resources. It is synthesised via ring opening polymerisation of ε-caprolactone. These reactions are often catalysed by stannous octoate ($SnOct_2$) [3] [6] [14] and molecular weight can be controlled by the addition of short-chain alcohols. [14] As already discussed in ring opening polymerisation of lactide, also poly (caprolactone) can be synthesised within a cationic, anionic, radical or coordinative mechanism. Moreover, also an enzymatic mechanism has been investigated recently. [14] The general reaction scheme of poly (caprolactone) and its structure is shown in Figure 7.

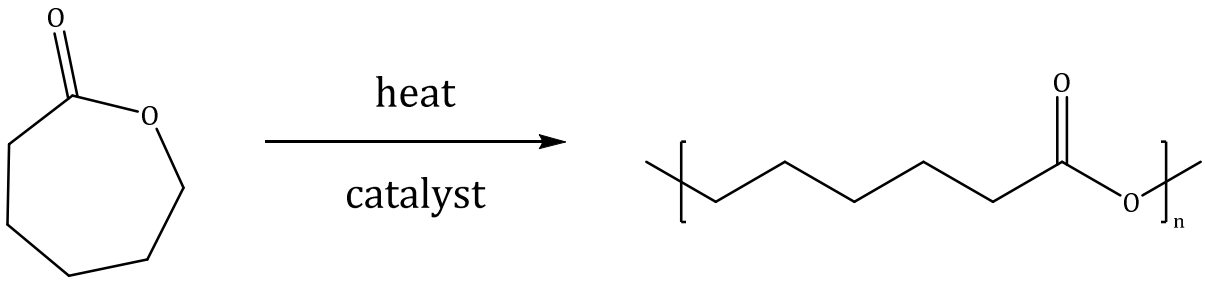

Figure 7: Reaction scheme and structure of PCL (figure adopted from [13])

Usually, synthesised PCL is a semi-crystalline polymer with a crystalline part of about 50%. [14] Thermal and mechanical properties are strongly dependent on its molecular weight. [13] Poly (caprolactone) has a very low glass transition temperature of about –60 °C whereas melting temperature is indicated in the literature with values of 58 – 65 °C. [3] [13] [14] Mechanical properties of high molecular PCL are comparable to those of low density and high density polyethylene. It shows a low tensile strength of 12 – 30 MPa and a high elongation at break of about 400 – 900 %. [13]

Due to its low melting and glass transition temperature, poly (caprolactone) is often used as compatibilizer as well as in polymer blends. PCL can be copolymerised with other lactones such as lactide, γ-valerolactone, glycolide, poly (ethylene glycol) or ε-decalactone. [14] Other possibilities of comonomers are polycarbonate, starch and polypropylene. [13]

Moreover, PCL is applied as packaging material for foods, in medicine as tissue scaffold and is an interesting polymer concerning drug delivery systems as well.[3] [13] [14] Nowadays it is produced in industrial scale by companies like Solvay (Belgium) under the trade name CAPA®, Tone® from Union Carbide (USA) and Celgreen® from Daicel (Japan). [3] [13]

1.1.4 Poly (butylene succinate)

Poly (butylene succinate), abbreviated PBS, belongs to the family of aliphatic, biodegradable polyesters especially to the group of poly (alkenedicarboxylates). The monomers are derived from fossil based resources. Usually PBS is synthesised via a polycondensation reaction of a diol and a dicarboxylic acid, for instance 1,4 – butanediol or ethylene glycol and succinic acid or adipic acid. Since the beginning of the 1990s poly (butylene succinate) and its relatives and copolymers poly (ethylene succinate) and poly (butylene succinate adipate) are commercially produced in large scale under the trade name Bionolle® by the company Showa High Polymers (Japan). [3] [5] [13] [14] [18]

Figure 8 shows the structural formula of poly (butylene succinate).

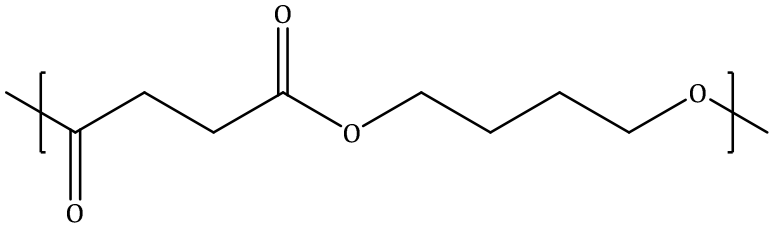

Figure 8: Structure of poly (butylene succinate)

The main problem of these polyesters was that only low molecular weights could be obtained for a long time. This has now successfully been overcome by the addition of chain extenders, e.g. diisocyanates like hexamethylene diisocyanate. [3] [5]

In general, poly (butylene succinate) is a white, thermoplastic, semi-crystalline polyester. Its melting temperature is between 90 – 120 °C and its glass transition temperature is around -45 °C up to -10 °C. [3] [18] Concerning mechanical properties, PBS indicates an elongation at break of about 330 % and a tensile strength of 330 kg/cm^2. [3] These data are comparable with common polymers like low density polyethylene, high density polyethylene and polypropylene. [3] [18] A big advantage of PBS is its good processability compared to other biodegradable polyester like PLA or PGA. However, there is also a drawback which is its bad biocompatibility. In recent years it was tried to overcome this problem by treating the polymers surface with plasma. [3]

Through the implementation of adipic acid into PBS, a copolymer called poly (butylene succinate adipate), abbreviated PBSA, is established. Due to the integration of adipate the polyester gets more elastic which is indicated by a higher elongation at break, but on the other side tensile strength gets worse. [3]

1.1.5 Poly (butylene adipate – co – butylene terephthalate)

In order to improve mechanical and thermal properties of aliphatic polyesters it was tried to introduce aromatic compounds into the polymers backbone. Poly (butylene adipate – co – butylene terephthalate), in the literature often abbreviated as PBAT, is such an aliphatic – aromatic biodegradable copolyester derived from petroleum based resources. It is synthesised in a process consisting of two steps. The first building block butylene – terephthalate is prepared via a transesterfication reaction of terephthalic acid and 1,4 – butanediol followed by a condensation of 1,4 – butanediol and adipic acid. The structural formula of the resulting PBAT copolyester is given in Figure 9. [1] [5] [6] [13] [14]

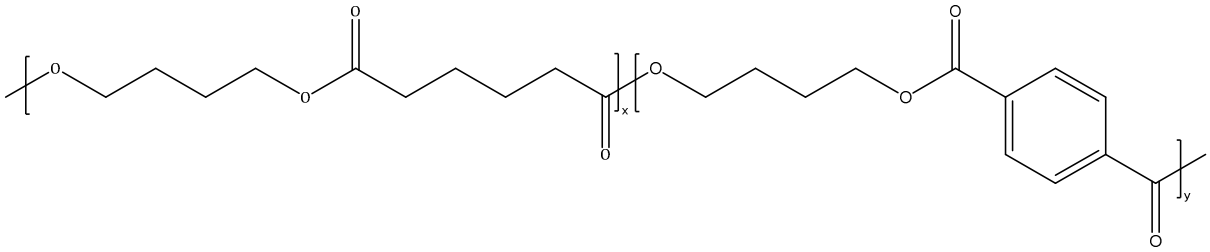

Figure 9: Structural formula of PBAT

Since aromatic moieties are known on the one side to decrease the ability of biodegradation of those copolyesters but on the other side to enhance mechanical properties such as tensile strength and elongation at break, there has be a compromise between these two parameters. It was found that the ideal amount of terephthalic acid is between 35 – 55 mol%, because above 55 mol% of terephthalic acid biodegradation decreases dramatically. [3] [9]

In general, PBAT is a semi – crystalline, thermoplastic copolyester. Ecoflex® for example has a melting temperature of about 110 – 115 °C. Regarding mechanical properties it shows a tensile strength of 32 – 36 MPa and elongation at break of 580 – 800 %. [13]

PBAT is commercially produced under the trade name Ecoflex® by BASF (Germany), Easter Bio® by Eastman Chemical (USA) and Origo - Bi® by Novamont (Italy). [1] [3] [13] It is mainly applied in agriculture as film sheets, in food industry as packaging material and one-way cups, forks, spoons etc.

1.2 Biodegradation

This sub chapter deals with the different guidelines and tests regarding biodegradation of polymers. Furthermore the mechanism of biodegradation of PLA will be discussed in detail.

As already mentioned in the introduction of this chapter, the main reason for biodegradation of polyesters is the implementation of heteroatoms into their backbones. Due to these introduced ester, amide or urethane linkages, hydrolysis is possible in contrast to plastics like PET or PE whose main chains consist only of carbon atoms. [19] [20]

Usually biodegradation involves four steps. [2] The initial step of the process is the breakdown of secondary structures, like crystals in a semi – crystalline polymer, to obtain transiently flexible chains. The reaction goes on with the second step, the cleavage of the ester (amide, urethane etc.) linkages to obtain smaller polymer units. These reactions are catalysed by enzymes. A further requirement for these cleavages is the ability of the polymer chain to dock on the correct site of the enzyme to enable catalysis. Usually concerning reaction is a hydrolysis and resulting products of these processes are oligomers and monomers of the regarding polymer. In the third step, obtained intermediate products have to be small enough to fit through the cell walls and membranes of the bacteria. Furthermore they have to be soluble in water. The last step consists of the conversion of the monomers and oligomers into biomass, water, carbon dioxide and / or methane and energy by the microorganisms. [2] [11] [12] [20] [21]

Important parameters influencing the biodegradation process are humidity and the level of oxygen. Biodegradation can occur under aerobic as well as under anaerobic conditions. [2] [11] [19] [21]

Beside the above described enzymatic catalysed reactions, degradation of the polymer can occur additionally through the impact of heat, UV – light and chemical hydrolysis and therefore attack the bulk of the polymer. These additional reactions are also necessary because enzymes are not small enough to get into the bulk of the plastics and so degradation is accelerated. So biodegradation can be described as a process that proceeds on the surface of a polymers material and therefore is a kind of erosion process. The most important parameter regarding the time of the biodegradation process is hence the thickness of the polymer sample. [2] [12] [19] [21]

In Figure 10 is a simplified reaction scheme of the degradation of PLA driven by hydrolysis as an example given. The main chain is fragmented step by step into smaller units (oligomers) due to

the cleavage of the ester bonds by means of water. In an ideal case, the resulting products at the end of the process are the used monomers, here lactic acid. [5] [12]

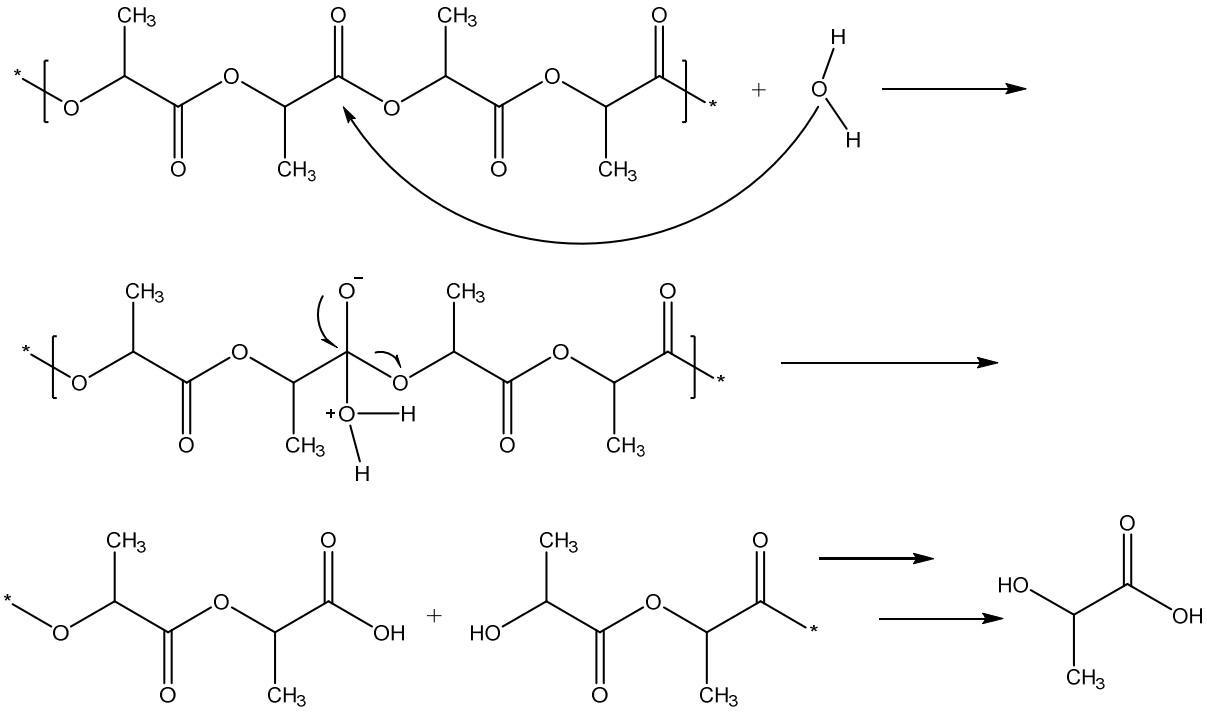

Figure 10: Reaction scheme of the degradation by hydrolytic scission of the main chain of PLA (figure adopted from [5])

1.2.1 Assessment of biodegradation

To evaluate above described progress and products of a biodegradation process, several standards have been established. They are scheduled by the European norm EN 13 432, by the American norm ASTM D 6400-04 and by the Japanese norm Green Pla. Furthermore there has been an intermediate standard (ISO 17088) introduced in 2008 which refers to the European and American norm. [2]

According to these standards several tests have been developed to evaluate the eco-toxicological impact of resulting degradation intermediates and products. The water soluble intermediates are investigated and assessed with the Daphnia test (DIN 38412 Part 30). Therefore 10 Daphnia are given into the test solution, which is incubated at 20 °C for 24 hours. If 9 out of 10 Daphnia are still able to swim in the test solution the next day, the test is passed. [2] [22] The plant growth test (EN 13432, Annex E) evaluates the influence of degradation products and intermediates on plant growth of different seeds in soil from compost tests of regarding polymer.[2] A third possibility to determine eco toxicity is the Earthworm Acute Toxicity Test (OECD guideline 207). Therefore a specific number of earthworms is abandoned in soil were plastics were composted. Beside the mortality rate of the earthworms, toxicity can be determined. [2] [23]

Moreover, also the product safety during the time of application is declared by these standards. For this purpose the irritation of eyes and skin (OECD guideline 404 + 405) as well as sensitisation (OECD guideline 406) are evaluated. Furthermore the acute oral toxicity (OECD guideline 423) and the possibility of mutagenicity (OECD guideline 471) are assessed. [2]

1.3 Drug encapsulation systems

Micro – and nanoparticles play a key role in the controlled release of active substances either in the human body as drug encapsulation systems or in other fields like agriculture for the release of herbicides. Biodegradable polyester and copolyester like PLA and its copolymers or poly (butylene adipate – co – butylene terephthalate) are suitable as matrix materials for these particles because they are non toxic, well hydrolysable and therefore predestined for biomedical applications. [24]

In general, it has to be distinguished between micro – and nanoparticles as well as between micro – and nanocapsules. While in former ones the drug is dispersed throughout the whole particle, in latter ones the drug is encapsulated in the centre of the particle and surrounded by a polymer shell. Furthermore there has to be differed between microparticles which are declared by a mean diameter > 1 µm and nanoparticles with a mean diameter < 1 µm. [24]

Hereafter various preparation procedures of micro – and nanoparticles will be discussed. The most common and most important methods are: [24] [25] [26] [27]

- Emulsion methods
- Nanoprecipitation
- Salting – out
- Spray drying method

The chosen preparation method is a main influencing parameter concerning characteristic properties like size distribution, encapsulation efficiency, morphology, drug release etc. Moreover, also temperature, stirring speed, type and concentration of the solvent (in most cases chloroform is chosen, other possibilities are for instance ethyl acetate, dichloromethane and acetonitrile), pH and type as well as concentration of the emulsifier are important parameters to ensure optimal interactions between drug and polymer. [24]

However, the stabilising agent is often seen as key part of the whole process. Due to the fact that its purpose is to build a very thin protection layer around the polymer and its drug, it has a great impact on the morphology and the velocity of drug release from the particles. In most cases poly vinyl alcohol (PVA) is used as surfactant with a concentration of 0.5 – 8 % (w/v). [24]

However, the most common preparation techniques are the emulsion method and the nanoprecipitation. Hence, these two methods will be discussed in detail in the following sub-chapters. [24] [28]

1.3.1 Emulsion methods

These techniques are based on the formation of an emulsion between an aqueous and an oil phase respectively between two oil phases. Therefore the polymer is dissolved in an organic solvent (o-phase) whereas the emulsifier (e.g. PVA, poly ethylene glycol, gelatine, alginate, polyvinylpyrollidone, methyl cellulose etc.) forms the aqueous phase. Through the removal of the solvent the prepared micro – and nanoparticles are able to harden. This can be either achieved through vaporisation or extraction of the solvent. In the vaporisation process, the solvent is removed under reduced pressure while extraction is performed by stirring the emulsion a few hours (e.g. overnight) with the magnetic stirrer. [24] [29] [30]

According to Hnaien et al. [29], vaporisation of the solvent results in smaller particles (9.1 ± 0.1 µm) than extraction (14.4 ± 0.3 µm). This fact is explained due to the faster removal of the solvent under reduced pressure. [29]

Simple Emulsion (o/w) technique

The simple emulsion technique, also known as oil-in-water (o/w) method, is usually used for the encapsulation of hydrophobic drugs. Most of them are very well soluble in organic solvents and can therefore, together with the polymer, be dissolved for instance in chloroform to form the oil phase. This oil phase is subsequently poured into the water phase which contains the emulsifier. The emulsion can be established either through stirring with a magnetic stirrer or by means of a high speed homogenizer. [25] [28] [31] [32] [33] The suspension can further be ultrasonicated for a few minutes to gain smaller nanoparticles. [25] The solvent is removed either by extraction or vaporisation as described above. Examples for drugs which are encapsulated with the oil-in-water technique are paclitaxel, different local anaesthetics, chlorpromazine, neuroleptics thioridazine etc. [28]

Double Emulsion ($w_1/o/w_2$) technique

The double emulsion technique is applied when a hydrophilic drug shall be encapsulated. For this purpose the drug is dissolved in water and forms the inner aqueous phase (w_1), whereas the polymer is dissolved in an organic solvent and represents the oil phase (o). By mixing those both phases, a first emulsion (w_1/o) is established. The final ($w_1/o/w_2$) emulsion is afterwards prepared by pouring the first emulsion carefully into the second aqueous phase (w_2), which contains the stabilising agent. Afterwards the solvent has to be removed again either by vaporisation or extraction to harden the micro- and nanoparticles. [24] [28] [29] [30] [34] [35]

The double emulsion method is usually applied for the encapsulation of peptides and proteins and shows quite high encapsulation efficiencies. Sometimes particles including hydrophobic drugs are prepared with this technique too. [28]

Emulsion (o/o) technique

The oil-in-oil (o/o) emulsion technique represents an altered form of the simple emulsion method. It is used for drugs which are in general declared as hydrophobic, but are nevertheless slightly soluble in water, e.g. hydrocortisone (solubility: 280 µg/mL in water) [28]. Since particle formation with the oil-in-water technique would lead to very low encapsulation efficiencies in this case, it is necessary to overcome this problem by the introduction of a second oil phase. A general procedure starts with the dissolution of the drug and the polymer in an appropriate organic solvent to create the first oil phase (o_1). This first oil phase is than slowly poured into the second oil phase (o_2), which consists of an emulsifying agent dissolved in an oil like mineral oil or seed oil. It is important to note that neither the polymer nor the drug should be able to dissolve in this second oil phase. The purification step is a little bit more complicated here and includes among others a washing step with a non-polar solvent like heptane. [24] [28]

Emulsion (s/o/w) technique

In general, the solid-oil-water (s/o/w) method is used when the target active substance cannot be dissolved in an organic solvent or in water. This technique is normally used very rarely. For a successful application of this method, the drug has to have very low particle sizes (1 – 10 µm), otherwise a complete encapsulation is not possible. The solid-oil-water method is used for active substances like levonorgestrel, β-estradiol [28] or proteins. [24]

1.3.2 Nanoprecipitation

Nanoprecipitation is primarily used for the formation of particles enclosing hydrophobic drugs. A typical procedure starts with the dissolution of the drug and the polymer in an appropriate organic solvent. This established organic phase is further slowly dropped into the aqueous phase. The organic solvent is evaporated during this step through gentle stirring of the suspension. By adding the organic solution drop wise into the aqueous phase, nanoparticles precipitate. The advantages of the nanoprecipitation technique are on the one hand the formation of small particles (nanometre scale) including a narrow size distribution and on the other hand the avoidance of large amounts of organic solvents. [24] [28] [36] [37]

Figure 11 shows a schematic comparison of nanoprecipitation and emulsion – based techniques. Although it seems at a first appearance that they are roughly the same, closer examination reveals quite big distinctions. The main differences concerning the experimental setup are on the one hand that in nanoprecipitation no emulsifying agent is involved and on the other hand that the organic solution is added drop wise while the aqueous phase is stirred in contrast to emulsion methods where the organic phase is poured into the aqueous one and vigorously stirred afterwards. [24]

However, there are many examples shown in the literature comparing particles formed with nanoprecipitation and emulsion – based methods. All of them show the same results, especially regarding particle size: particle sizes obtained from nanoprecipitation are smaller than the one from emulsion methods, but therefore the encapsulation efficiency of drug encapsulation systems prepared with emulsion method is significantly higher than with nanoprecipitation. [24] [38] [39] But it was also mentioned that efficiency is strongly depending on the type of encapsulated drug. The particle size is usually dependent on the stirring speed, the presence of a stabilising agent and the molecular weight of the polymer. In general it can be concluded that particles enclosing hydrophobic drugs should be prepared with simple emulsion method or nanoprecipitation and hydrophilic drugs including proteins should be encapsulated with the double emulsion method. [24]

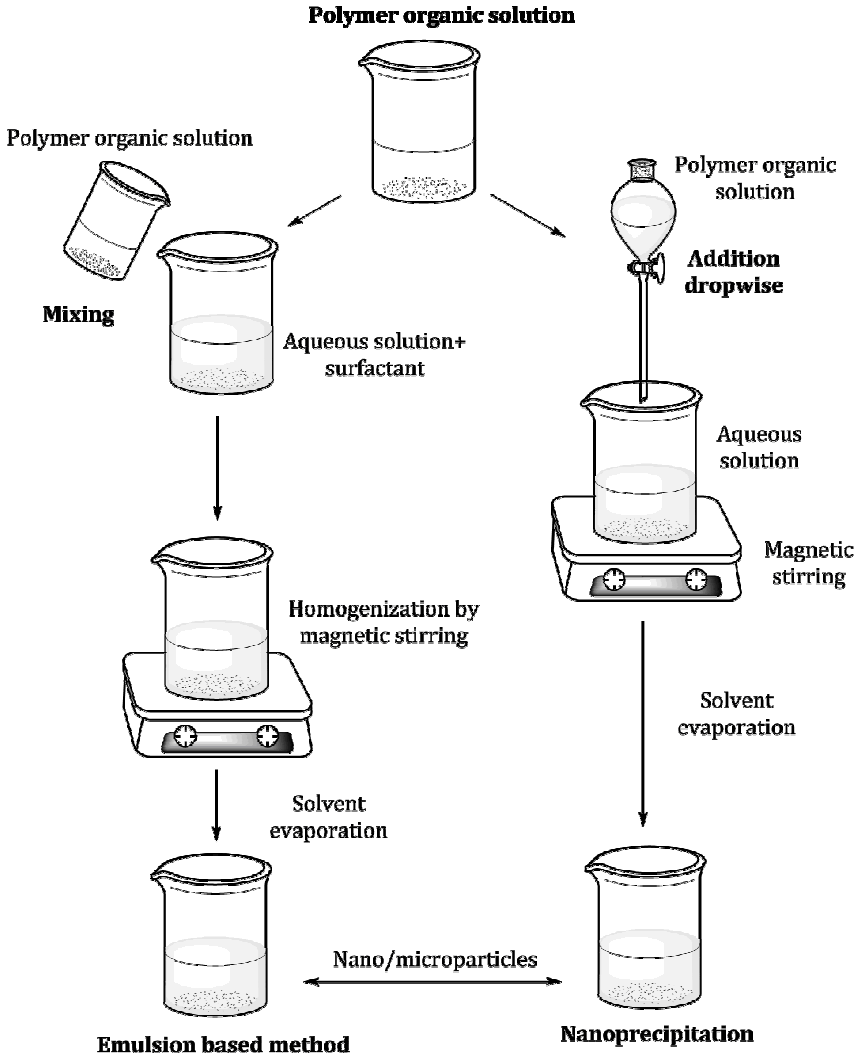

Figure 11: Schematic comparison of emulsion methods and nanoprecipitation (figure adopted from [24])

2. Research objectives

1) Chemical modification of poly (butylene adipate – co – butylene terephthalate) copolyesters to reach variability during hydrolysis in phosphate buffered saline solvent (PBS).

    a) Synthesis of poly (butylene adipate – co – butylene terephthalate) copolyesters with poly (ethylene glycol) and triallyl isocyanurate.

    b) Synthesis of new copolyesters based on glycerol, sebacic acid and dimethyl terephthalate.

2) Determination of molecular weight properties, chemical constitution and thermal properties of the prepared copolyesters.

3) Selecting of polyesters as suitable drug encapsulation systems and encapsulation of the model substances (coumarin and triclosan). Comparison of encapsulation efficiency, particle size distribution and drug release of the selected copolyesters with common or modified poly (lactic acid).

3. Experimental

3.1 Materials

In Table 1 is a list with all used chemicals, which are commercially available, shown.

Table 1: List of chemicals

Substance	CAS-number	Manufacturer	Purity	M_w [g.mol^{-1}]
Dimethyl terephthalate	120-61-6	Fluka	>99%	194.19
Dimethyl terephthalate	120-61-6	Acros Organics	99%	194.19
1,4 – Butanediol	110-63-4	Roth	⩾99.5%	90.12
Glycerol	56-81-5	Sigma Aldrich	⩾99.5%	92.09
Adipic acid	124-04-9	Merck	Pure	146.14
Sebacic acid	111-20-6	Merck	98%	202.25
Polyvinyl alcohol 4-88	9002-89-5	Fluka	-	~31000
Chloroform	67-66-3	VWR-Chemicals	-	119.38
Dichloromethane	75-09-2	Fisher Chemicals	-	84.93
Tetrahydrofuran	109-99-9	Roth	⩾99.5%	72.11
Methanol	67-56-1	-	-	32.04
Pentane	109-66-0	-	-	72.15
Tetrabutyl orthotitanate	5593-70-4	Sigma Aldrich	97%	340.3
Coumarin	91-64-5	Sigma Aldrich	⩾99%	146.14
Triclosan	3380-34-5	Fluka	⩾97.0%	289.54
Polyethylene glycol 1000	25322-68-3	Roth	-	950-1050
Triallyl isocyanurate	1025-15-6	Sigma Aldrich	98%	249.27
Sodium chloride	7647-14-5	VWR-Chemicals	-	58.44
Potassium chloride	7447-40-7	Fluka	>99.0%	74.56
Potassium dihydrogen phosphate	7778-77-0	Sigma Aldrich	⩾99%	136.09
$CDCl_3$+0.03% TMS	865-49-6	Eurisotop	99.8%	-
DMSO D6	2206-27-1	Eurisotop	99.80%	-

All chemicals were used without any further purification except 1,4-butanediol which was cleaned via distillation to remove eventual impurities. Polyethylene glycol (PEG) has a hydroxyl value of 107 – 118 mg KOH/g and can therefore be classified as OH-terminated.

3.2 Syntheses

For the processing of microparticles were used following biodegradable polyesters and copolyesters: 1) poly (lactic acid) with different molecular weights and functional end groups, 2) poly (butylene adipate – co – butylene terephthalate) copolyesters and 3) poly (glycerol sebacate – co – glycerol terephthalate) copolyesters.

3.2.1 Polylactides

PLA, PLA/SA and PLA/SA/CY which were used for the preparation of drug delivery systems (see Figure 12) were synthesised by Berger and Gregorova [40].

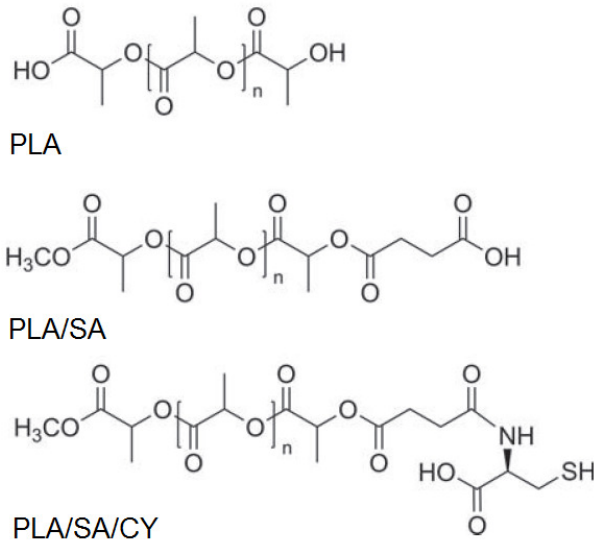

Figure 12: Poly(lactic acid) synthesised by azeotropic condensation from L-lactic acid (a) PLA, (b) sample modified by succinic anhydride - PLA/SA and (c) sample modified in two steps by succinic anhydride and cysteine - PLA/SA/CY (Figure adopted from [40])

PLA-0 and PLA-5400 are based on industrial polylactide PLA 3015D (Nature Works, USA) with Mw = 35 600 g.mol^{-1} but with different crystallinity degree. Based on X-ray PLA-0 and PLA-5400 have crystallinity of 0% and 53.2%, respectively (Kaltenböck Ilena, Bachelor work: Modification of PLA´s crystallinity, Graz University of Technology, August 2012). All types of PLA samples are summarised in Table 2.

Table 2: Overview and description of used types of PLA

Sample	Description	Additional information
PLA	PLA synthesised via azeotropic polycondensation; 95 % L-lactic acid	M_w=35600 g.mol^{-1}; M_n=21400 g.mol^{-1}; PDI=1.7
PLA/SA	PLA with end capped carbonyl groups; 95 % L-lactic acid	M_w=8500 g.mol^{-1}; M_n=4100 g.mol^{-1}; PDI=2.1
PLA/SA/CY	PLA with end capped thiol groups; 95 % L-lactic acid	M_w=10200 g.mol^{-1}; M_n=5400 g.mol^{-1}; PDI=1.9
PLA original pellets	PLA 3051D purchased from Nature Works without any treatment; 96 % L-lactide	Purity: > 98 %
PLA-0	PLA 3051D thermoformed for 0 sec.	Crystallinity: 0 %
PLA-5400	PLA 3051D thermoformed for 5400 sec.	Crystallinity: 53.19 %

3.2.2 Poly (butylene adipate-co-butylene terephthalate) copolyesters, P-(BA-co-BT)s

Copolyesters P-(BA-co-BT)s used throughout diploma work were synthesised according to the work of Milinkovic et al. 2014 [41]

Namely, 5.82 g (0.03 mol) dimethyl terephthalate (DMT) and 10.7 mL (0.12 mol) 1,4-butanediol are weighed into a 250 mL three-necked round bottom flask equipped with a Dean – Stark apparatus combined with a condenser and an inlet for the nitrogen. The educts are heated up to 160 °C under N_2 atmosphere and 22 µL of tetrabutyl orthotitanate (TBOT) as catalyst is added. The reaction is held for 2 hours under these conditions. Then 10.23 g (0.07 mol) of adipic acid and 22 µL of TBOT are added and the reaction mixture is heated up to 180 °C for 1 hour under N_2 to remove water. Afterwards the temperature is raised to 255 °C and first a vacuum of about 43 mbar is applied for 1 hour to get rid of residual 1,4-butanediol, followed by a vacuum of 4 mbar for another 1.5 hours. Subsequently the reaction is cooled to room temperature and dissolved in about 60 mL of chloroform overnight. Finally the product is precipitated in 250 mL cold methanol, filtrated and dried for at least 2 hours at the vacuum line followed by 2 days at 60 °C in the vacuum drying cabinet.

Synthesis of P-(BA-co-BT) copolyester with TAIC

10.7 mL (0.12 mol) 1,4 – butanediol and 5.82 g (0.03 mol) DMT are weighed into a three necked round bottom flask equipped with a Dean-Stark apparatus, a condenser and an inlet for the nitrogen. As soon as the reactants are heated to 160 °C under N_2 and a homogenous solution is established, 22 µL of TBOT as catalyst are dropped into the mixture. After 2 hours, 1 mol% of

TAIC is added and the reaction is stirred for another hour. Then, 10.23 g (0.07 mol) of adipic acid are put into the reaction mixture, the temperature is raised to 180 °C and continued for 1 hour. Subsequently the flask is heated up to 260 °C and the pressure is reduced to 40 mbar for 1 hour and thereafter to 4 mbar for 1.5 hours. After the product is cooled down to room temperature, it gets dissolved in 60 mL of chloroform, precipitated in 250 mL of cold methanol, filtrated and dried at first for 2 hours at the vacuum line and in the end overnight in the vacuum drying cabinet at 60 °C.

Synthesis of P-(BA-co-BT) copolyester with PEG

5.82 g (0.03 mol) of DMT and 10.7 mL (0.12 mol) of 1,4 butanediol are put into a three-necked round bottom flask equipped with a Dean-Stark apparatus, a condenser and an inlet for the nitrogen. First the reaction mixture is heated up to 160 °C under N_2, then 22 µL of TBOT are added and stirred for 2 hours. Subsequently 2.02 g (0.002 mol; 7.5 wt% related to the overall mass) of PEG are added and the temperature is raised to 180 °C. After 1 hour 10.23 g (0.07 mol) of adipic acid are given to the reaction mixture and it is stirred for another hour under these conditions. Then the reaction is heated to 260 °C and firstly a vacuum of 40 mbar is applied for 1 hour and finally the pressure is reduced to 4 mbar for another 1.5 hours. Following that the product gets dissolved in 60 mL of chloroform, precipitated in 250 mL cold methanol and filtrated. The resulting product is first dried for 2 h on the vacuum line and finally put into the vacuum drying cabinet at 60 °C overnight.

3.2.3 Poly (glycerol-sebacate –co – glycerol terephthalate) copolyesters, P-(GS-co-GT)

Basically, the whole process is divided into two steps, whereas the first step is kept the same in all experiments, while the second step is varied.

Step 1: Synthesis of glycerol – terephthalate pre-polyester

The required amounts of glycerol and DMT (see Table 3) are weighed into a three-necked round bottom flask equipped with a Dean-Stark apparatus, a condenser and an inlet for nitrogen. The reactants are heated to 160 °C under N_2 atmosphere. When DMT is completely molten, 1 wt% of TBOT is added and the mixture is stirred for 2 hours.

Step 2: Synthesis of P-(GS-co-GT) copolyester

Subsequently sebacic acid is put into the flask, the temperature is raised to 180 °C and again 1 wt% of TBOT is dropped into the solution. The reaction is continued under various time, temperature and pressure conditions A, B, and C (see Figure 13).

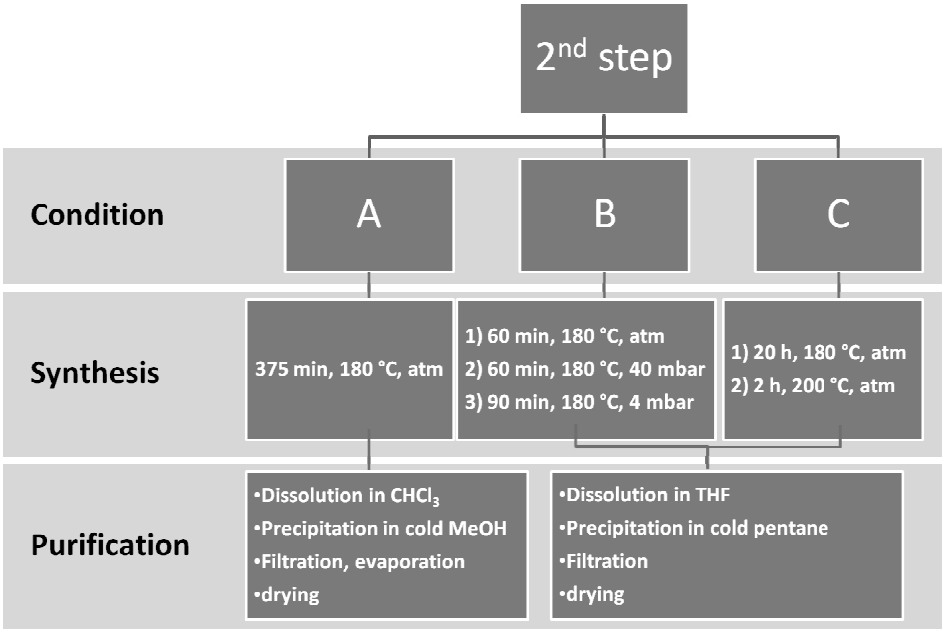

Figure 13: Flow chart of the 2nd reaction step

Condition A: Standard reaction time and atmospheric pressure

The reaction is continued for 6.25 hours at 180 °C under N_2 atmosphere. Then the product is dissolved in 30 mL of chloroform, precipitated in about 150 mL of cold methanol, filtrated and residual washing solution is evaporated at the rotavapor. The obtained white product is dried in the desiccator for at least two days.

Condition B: Shorter reaction time and vacuum

For samples where vacuum is applied, the reaction is in the beginning held for 1 hour under above described conditions and thereafter the pressure is first reduced to 40 mbar for 1 hour and then further to 4 mbar for 1.5 hours. Temperature is kept all the time at 180 °C. For purification, the resulting product is dissolved in THF, precipitated in cold pentane and finally filtrated. In the end it is dried in the desiccator for 3 days.

Condition C: Prolonged reaction time and atmospheric pressure

The reaction mixture is stirred firstly for 20 hours at 180 °C under N_2 atmosphere and finally the temperature is raised to 200 °C for another 2 hours. When resulting product is cooled to room temperature, it gets dissolved in 30 mL of THF, precipitated in 150 mL of cold pentane and filtrated. To bring the process to an end, the product is dried for 3 days in the desiccator.

Table 3: Overview of the compositions of prepared P-(GS-co-GT) copolyester

Sample	Protocol Nr.	Glycerol [mol]	Fraction [%][a]	DMT [mol]	Fraction [%][b]	Sebacic acid [mol]	Fraction [%][b]
Option A: 2h+6.25h							
P-(GS-co-40-GT)	Cop-57	0.0047	17	0.0112	40	0.0168	60
P-(GS-co-30-GT)	Cop-59	0.0047	17	0.0084	30	0.0196	70
P-(GS-co-50-GT)	Cop-60	0.005	17	0.015	50	0.015	50
P-(GS-co-44-GT)	Cop-55	0.0047	17	0.0124	44	0.0157	56
P-(GS-co-44-GT)+¼ glycerol	Cop-62	0.007	25	0.0124	44	0.0157	56
P-(GS-co-44-GT)+⅓ glycerol	Cop-51	0.00935	33	0.0124	44	0.0157	56
Option B: 2h+1h+2.5h vacuum							
P-(GS-co-30-GT)+vacuum	Cop-68	0.0094	17	0.0168	30	0.0393	70
P-(GS-co-44-GT)+vacuum	Cop-69	0.007	25	0.0124	44	0.0157	56
Option C: 2h+22h							
P-(GS-co-20-GT)-24h	Cop-76	0.0125	25	0.01	20	0.04	80
P-(GS-co-30-GT)-24h	Cop-77	0.0125	25	0.015	30	0.035	70
P-(GS-co-40-GT)-24h	Cop-78	0.0125	25	0.02	40	0.03	60

[a]: given fraction is related to the overall amount of sebacic acid and DMT. [b:] given fractions represent the ratio between sebacic acid and DMT.

3.3 Preparation of microparticles

The main aim of this work was to prepare, characterise and compare microparticles based on various (above mentioned) biodegradable polyesters and copolyesters. Microparticles were prepared with two different emulsion based methods: the simple emulsion method and the double emulsion method. The chosen preparation technique is dependent on the used polymer.

3.3.1 Double emulsion ($w_1/o/w_2$)

100 mg of the particular polymer are dissolved in 5 mL of dichloromethane together with either 10 mg of coumarin or triclosan. 500 µL of an aqueous solution containing 1% (w/v) of PVA are added and thereupon mixed with the high speed homogenizer Ultraturrax at 14200 rpm for 3 minutes to form the inner w_1/o phase. After that the dispersion is dropped via a syringe into a flask containing 30 mL of a 0.5 % (w/v) PVA-solution and mixed again at 11000 rpm for 3 minutes to create a $w_1/o/w_2$ phase. Subsequently the obtained suspension is transferred into a

flask containing 150 mL of 0.25 % (w/v) PVA solution and stirred for at least 2 hours at 500 rpm to evaporate the organic solvent. Finally the suspension is centrifuged for 5 min at 3500 rpm and washed twice with methanol/water 20:80 to remove residual PVA and coumarin respectively triclosan. For a better distribution of the particles, 0.1 wt% of D-mannitol is added to the particles, homogenised with the vortex and dried overnight at the lyophilisator.

3.3.2 Simple emulsion (o/w)

1 g of polymer is dissolved in 10 mL of chloroform. 10 mg of coumarin are added after the complete dissolution of the polymer. Subsequently the organic phase is dropped into 40 mL of 0.5 % (w/v) PVA solution and an emulsion is formed by mixing with the high speed homogenizer Ultraturrax for 10 min at 14000 rpm. During this process the flask is cooled with an ice bath. To remove the solvent, the emulsion is stirred overnight at 500 rpm. To bring the process to a close, prepared microparticles were obtained by centrifugation at 3500 rpm for 5 minutes, washed twice with methanol / water 20:80 and put into the lyophilisator overnight for drying.

3.4 Processing and characterisation methods

Film processing

Dried copolyesters were thermoformed by using a thermohydraulic press (Collin P 200PV) under vacuum in three steps: 1) temperature in the range of melting temperature + 10 °C, a pressure of 5 MPa and time of 3 min, 2) temperature in the range of melting temperature + 10 °C, a pressure of 10 MPa and time of 5 min, and 3) temperature of 30 °C, a pressure of 10 MPa and time of 7 min. From obtained films, specimens in shoulder bar form were punched out and further analysed by tensile testing.

Gel permeation chromatography (GPC)

To obtain average molecular weights M_w and M_n as well as polydispersity indicies (PDI) gel permeation chromatography was accomplished. The setup consists of a L6000A Merck - Hitachi pump, separation columns of Polymer Standards Service (5µm grade size) and a refractive index detector from Wyatt Technology. The columns were calibrated with polystyrene standards from Polymer Standards Service. Samples were dissolved in THF and measured at room temperature. THF was used as eluent with a flow rate of 1.0 mL/min and an injection volume of 112.0 µL.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy was done with a Bruker Alpha-P FTIR-Spectrometer in ATR mode. Measurements were performed between 4000 – 400 cm^{-1} with a resolution of 4 cm^{-1}. For background measurements 24 scans were run, for sample measurements 64 scans.

Nuclear magnetic resonance (NMR)

^{1}H, ^{13}C, APT, COSY and HSQC spectra were recorded at room temperature with a Bruker Avance III spectrometer operating at 300 MHz and a 500 MHz spectrometer. All samples were dissolved in $CDCl_3$ or DMSO D6 with 0.03% TMS as internal standard. ^{1}H spectra for qualitative analysis were obtained with 1 s delay and 16 scans, while spectra for quantitative analysis were done with 5 s delay and 64 scans, respectively 10 s delay and 32 scans. ^{13}C data were recorded with 2 s delay and 1024 scans, APT with 2 s delay and 256 scans. For ^{1}H measurements ca. 10 mg sample were dissolved in 800 µL solvent, for all other experiments 30 mg were dissolved in 800 µL $CDCl_3$ or DMSO D6. The following abbreviations were taken for identification: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet), dd (double of doublets).

Simultaneous thermal analysis (STA)

Data from differential scanning calorimetry (DSC) and thermogravimetry (TG) were obtained with a Netzsch STA 449 C. About 5 mg of the sample were weighed into an aluminium pan and heated from 20 °C up to 550 °C with a heating rate of 10 °C/min. Measurements were performed under helium atmosphere with a flow rate of 50 mL/min.

From TG-curves the onset temperature of thermal decomposition (T_o) and from the 1st derivative the maximal degradation temperature (T_{max}) could be determined. DSC data provided information about the melting temperature (T_m) and melting enthalpy (ΔH_m).

Differential scanning calorimetry (DSC)

Further DSC measurements were accomplished with a DSC Pyris Diamond from PerkinElmer under nitrogen atmosphere with a flow rate of 20 mL/min. About 5 mg of the sample were heated up in an aluminium pan from 20 °C to 160 °C (1st heating cycle) with a heating rate of 10 °C/min. Cooling step occurred from 160 °C to -20 °C with a cooling rate of 10 °C/min. The 2nd heating cycle started from -20 °C up to 130 °C again with a heating rate of 10 °C/min. Beside melting temperature (T_m) and melting enthalpy (ΔH_m), glass transition temperature (T_g) could be determined as well from the second heating cycle.

Mechanical testing

Mechanical properties were determined with Autograph AGS-X 5 kN from Shimadzu. All samples were analysed in tensile test mode in plate form with a speed of 10 – 15 mm/min. Specimens were punched out from thermoformed films in shoulder bar form with a gauge length of 20 mm. All presented data are averages derived from at least 3 measurements.

Optical light microscopy

Optical light microscopy was carried out on an Olympus BX60 equipped with an Olympus E-520 camera. For analysing, samples were dispersed on a glass slide with a few drops of distilled water and coated with a cover glass.

Static / dynamic light scattering

Static light scattering experiments were done with the particle size analyser Cilas 1064 wet. The range of measurement was between 0.04 and 500.00 µm. Before analysis samples were dispersed in water together with K_2PO_4 by means of the homogeniser HD2200 from Bandelin electronics for 30 s at a speed of 6000 rpm.

Dynamic light scattering was carried out with a Zetasizer Nano ZS from Malvern Instruments.
All samples were measured at least with one of the above mentioned methods in triplicates.

UV-VIS

UV-VIS measurements were accomplished with a UV1800 from Shimadzu. Experiments were performed in the measuring mode absorbance and single scan mode with a light source wavelength of 340.8 nm as well as a slit width of 1.0 nm. Experiments were done in quartz cells.

For drug release experiments 10 mg of freeze – dried microparticles are dispersed in 20 mL of phosphate buffer saline solution (PBS) at a pH of 7.4. During the time of UV-VIS measurements all samples are stored in a water bath at 37 °C under gentle stirring.

PBS was prepared by dissolving 3.281 g NaCl, 0.0891 g KCl, 0.730 g $Na_2HPO_4 \cdot 2H_2O$ and 0.103 g KH_2PO_4 in 320 mL of distilled water. The pH of 7.4 was adjusted with a pH-meter by the addition of a few drops of either NaOH solution or HCl. Finally the remaining 80 mL of water were added. PBS was stored at 4 °C in the fridge.

Concentration of encapsulated drug is determined by measuring a sample taken from the whole supernatant obtained from centrifugation.

To determine the concentration, calibration curves of coumarin in methanol/water 20:80, coumarin in PBS and triclosan in methanol/water 80:20 were measured in a range between 1 µg/mL and 20 µg/mL for PBS, respectively between 5.76 µg/mL and 55 µg/mL for methanol/water solutions.

Scanning electron microscopy (SEM)

SEM – images were taken on a VEGA-II from Tescan. Before the analysis particles were coated with gold – platinum layer. Electrons were accelerated with a voltage of 10.00 kV.

Contact angle measurements

Contact angles were measured from pressed film samples with a Krüss DAS 100, Drop Shape Analyzer. Data were calculated in curve fitting mode. All samples were analysed 5 times.

4. Results and discussion

4.1 Properties of polymer matrices used for the processing of microparticles

4.1.1 P-(BA-co-BT) copolyesters

Poly-(butylene adipate-co-butylene therephthalate), abbreviated P-(BA-co-BT), copolyesters were synthesised via a polycondensation reaction as already described by Milinkovic et al. [41]. The reaction is divided into three steps. First, a pre polyester is prepared by the transesterfication of dimethyl terephthalate and 1,4 - butanediol where methanol is distilled out. In the second step, adipic acid is added and a low molecular weight random copolyester is generated which is turned into high molecular copolyester in the third step through the usage of reduced pressure and higher temperatures. The reaction scheme is shown in Figure 14.

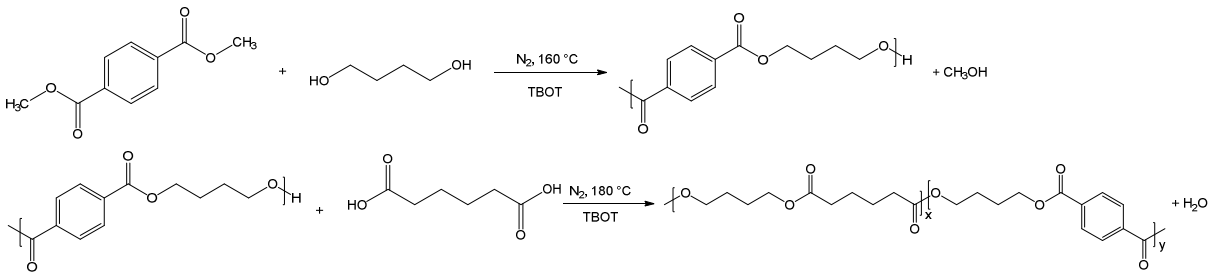

Figure 14: Reaction scheme of the synthesis of P-(BA-co-BT) copolyester

Modifications

Above described copolyester was altered in two different ways to implement new moieties and therefore enhance various properties. To achieve better mechanical properties and a higher molecular weight, copolyesters were modified with a triallyl isocyanurate called TAIC. TAIC is a crosslinking agent which consists of a functionalised triazine ring and is e.g. often used in rubbers. [42] Witt et al. [9] showed by means of a compost simulation test that the addition of isocyanates has no significant influence regarding the degradation behaviour of P-(BA-co-BT) copolyesters. [9]

Most of the time chemical crosslinking agents are added either at the end of the reaction [9] or an already prepared polymer is molten and by means of peroxide or irradiation, radicals are generated to enable the reaction. [43] In the present work triallyl isocyanurate was added after the first reaction step, because at this point more free functional groups are available than in the completed product and a second synthesis can be avoided. It is proposed that through the high temperatures (ca. 260 °C) radicals are generated and therefore the double bonds get activated

and are able to react (compare Figure 15). Furthermore it is feasible that the triazine ring of the triallyl isocyanurate is thermally cracked and hence the carbonyl bond is able to react with hydroxyl groups of the prepolymer, 1,4 – butanediol and adipic acid too. The resulting product can be classified as poly (ester – urethane).

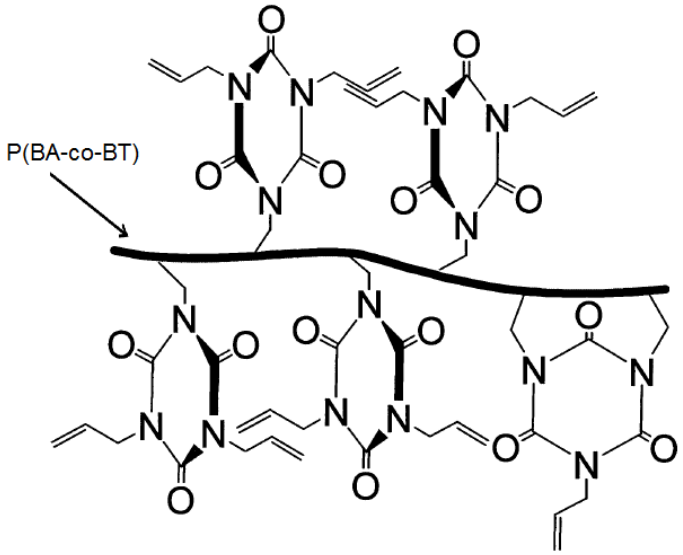

Figure 15: Crosslinked P-(BA-co-BT) copolyester with TAIC (Figure adopted from [60])

Furthermore, as a second possibility, polyethylene glycol (PEG) was used to generate a copolyester, which should be more hydrophilic than the unmodified one. PEG is also used sometimes in combination with PLA due to the fact, that a higher degree of hydrolysis accelerates the process of degradation. [15] A possible reaction scheme is illustrated in Figure 16.

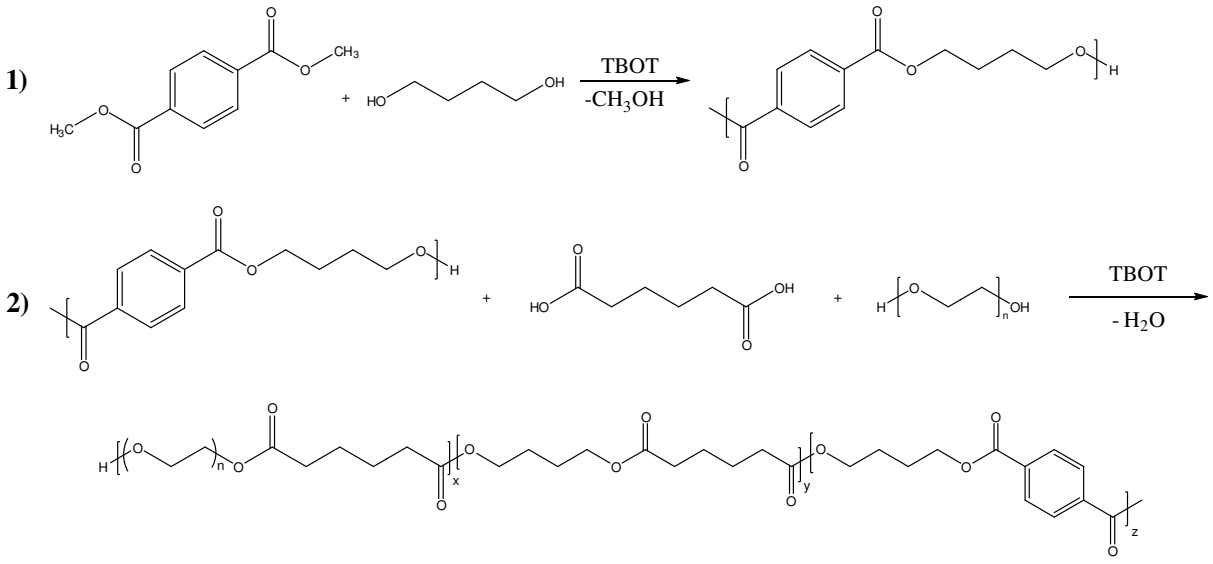

Figure 16: Reaction scheme of the synthesis of P-(BA-co-BT)+PEG

Influence on structural and molecular weight properties

Table 4 shows the average molecular weights and composition of synthesised copolyesters. The molecular composition was determined through comparison of peak integrals at 8.1 and 2.3 ppm in the ^1H spectrum and was theoretically kept the same in all syntheses with 30 mol% butylene terephthalate (BT) and 70 mol% butylene adipate (BA).

Table 4: Overview of molecular weight and composition of the P-(BA-co-BT) copolyester

Sample	BA/BT [mol%]	M_w [g.mol^{-1}]	M_n [g.mol^{-1}]	PDI
P-(BA-co-30-BT)	67.76/32.24	29870	17830	1.68
P-(BA-co-30-BT)+1% TAIC*	69.22/30.78	40970	22240	1.84
P-(BA-co-BT)+PEG	68.66/31.34	25080	14820	1.69

* GPC analysis was done during synthesis before cross-linking happened.

The results presented in Table 4 indicate that the unmodified copolyester P-(BA-co-30-BT) contains 32.24 mol% BT which is close to the theoretical value of 30 mol%. Average molecular weight M_w with 29870 g.mol^{-1} and polydispersity index of 1.68 is in accordance with data published by Milinkovic et al. [41]. They obtained about 34000 g.mol^{-1} and a PDI of 2 for P-(BA-co-30-BT). For all syntheses a quite narrow size distribution for polycondensations, declared by the PDI, could be reached. This phenomenon seems to be characteristic of this kind of copolyesters because Gan et al. [1] and Witt et al. [9] presented PDI < 2 as well. According to theory polydispersity indices of polycondensations are at least 2. A possible explanation for PDI values lower than 2 can be the applied purification process where low molecular weight fractions and unreacted monomer residues were mostly eliminated.

As can be also seen from Table 4, P-(BA-co-BT) copolyester modified with TAIC shows a molecular composition very close to the theoretical value (30.78 mol% BT) and furthermore one aim due to the addition of triallyl isocyanurate, the increase of molecular weight, was successfully accomplished from about 30000 g.mol^{-1} up to 40000 g.mol^{-1}.

Addition of PEG decreased molecular weight from 30000 g.mol^{-1} to 25000 g.mol^{-1}. Molecular composition determined from ^1H – NMR revealed that prepared copolyester consists of 68.66 mol% BA and of 31.34 mol% BT. Therefore amount of polyethylene oxide incorporated in copolyester structure calculated from ^1H-NMR is very low with an overall amount of about 2 mol%. Nevertheless it is high enough to influence various properties, among others hydrophilicity which will be discussed later.

In general, molecular weight in polycondensation reactions is strongly dependent on the stoichiometric ratio of the educts and the extent of conversion. In order to achieve high molecular weight copolyesters equilibrium of the reaction has to be shifted to the product side. [5] [44] This could be obtained by using a Dean-Stark apparatus for removal of the condensates methanol and water from the product as well as by the addition of an external catalyst, tetrabutyl orthotitanate. Because polycondensation reactions are also known to be very sensitive concerning water and humidity, the first and second reaction step took place under nitrogen atmosphere and all educts and glass devices were dried overnight at 50 °C.

For all reactions the extent of conversion for the first reaction step was evidenced by the amount of methanol distilled out. Calculations devoted conversions of DMT with 1,4 – butanediol between 98 – 99%.

In Figure 17 is the ^{1}H – NMR spectrum of P-(BA-co-30-BT) shown. By means of the peaks at 8.1 ppm responsible for the aromatic part and 2.3 ppm for the aliphatic one, above mentioned molecular compositions could be determined by using following equations 1 – 2 [59]:

$$f_{A=\frac{A_{2.3}}{(A_{2.3}+A_{8.1})}} \quad (1)$$

$$f_{T=\frac{A_{8.1}}{(A_{2.3}+A_{8.1})}} \quad (2)$$

Nevertheless the main purpose of the NMR was to determine the exact structure of the synthesised copolyesters. Therefore, ^{13}C, COSY, HSQC and HMBC spectra were recorded in addition to ^{1}H spectrum with a 500 MHz device. The correlation between peaks and proposed structure is given below in Figure 17. The protons of butanediol (magnified segment between 4.0 – 4.5 ppm) are split into four triplets. By means of the two-dimensional spectra the presented sequences (ABA, ABT and TBT; A- adipic acid, B- 1,4-butanediol, T-dimethyl terephthalate) could be determined. Furthermore, integration of those peaks revealed that the most common sequences in the synthesised copolyester are ABA blocks. In general, NMR investigations revealed the same peaks and chemical shifts as reported in previous papers. [1][59] Moreover, NMR investigations confirmed that all prepared copolyesters can be classified as random copolyesters. The presented NMR assignments are also valid for the other modified copolyesters.

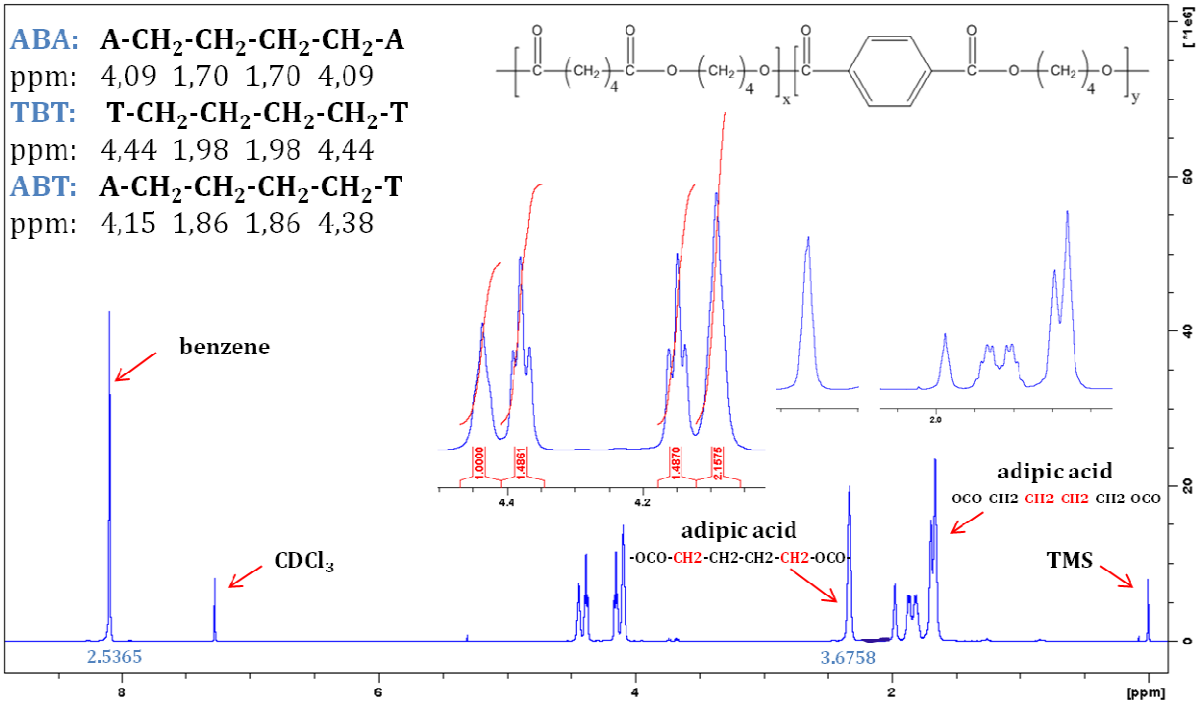

Figure 17: ^{1}H-NMR of P-(BA-co-30-BT)

In Figure 18 is the ^{13}C – NMR of P-(BA-co-BT) shown. Of particular interest are here again the carbon atoms of butanediol. Through magnification of the areas at 25 ppm and 64 ppm it is possible to identify and assign the separated peaks concerning the different sequences (ABA, TBT, ABT).

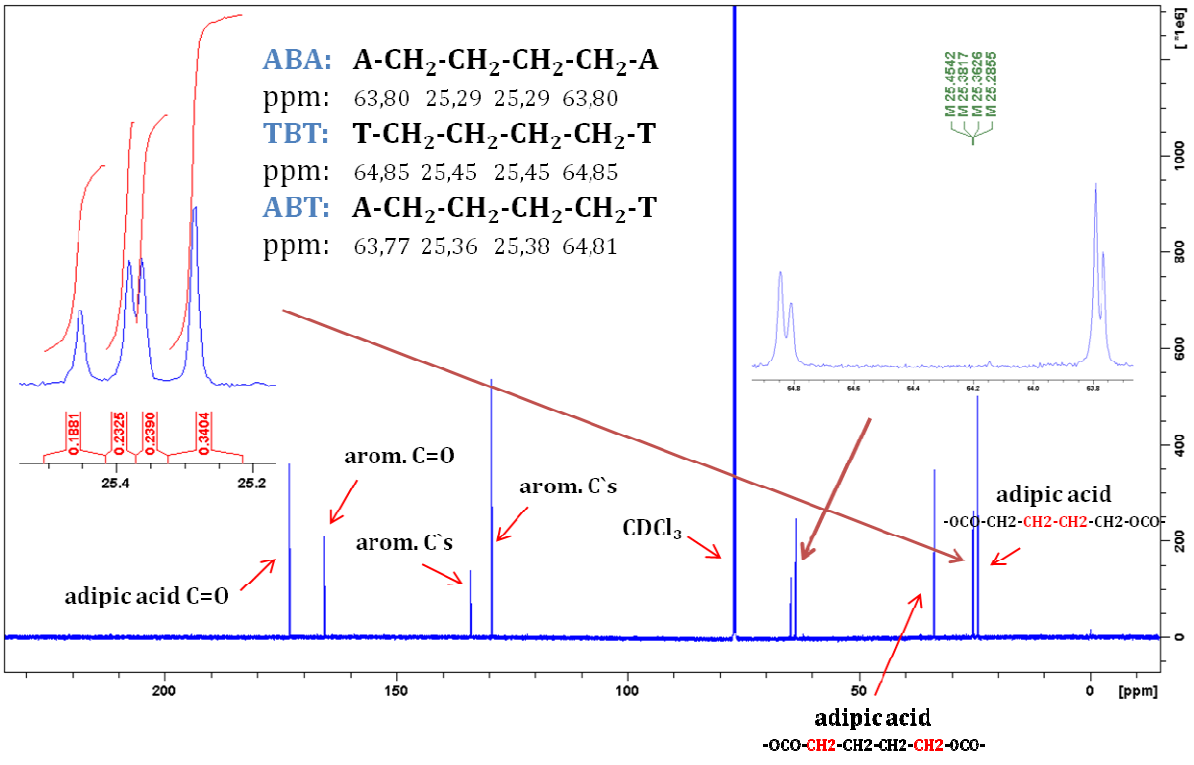

Figure 18: ^{13}C – NMR of P-(BA-co-30-BT)

In the following Figure 19 – 21 are the H, H-COSY, HSQC and HMBC spectra shown whereby it was possible to determine before presented structure of the copolyester.

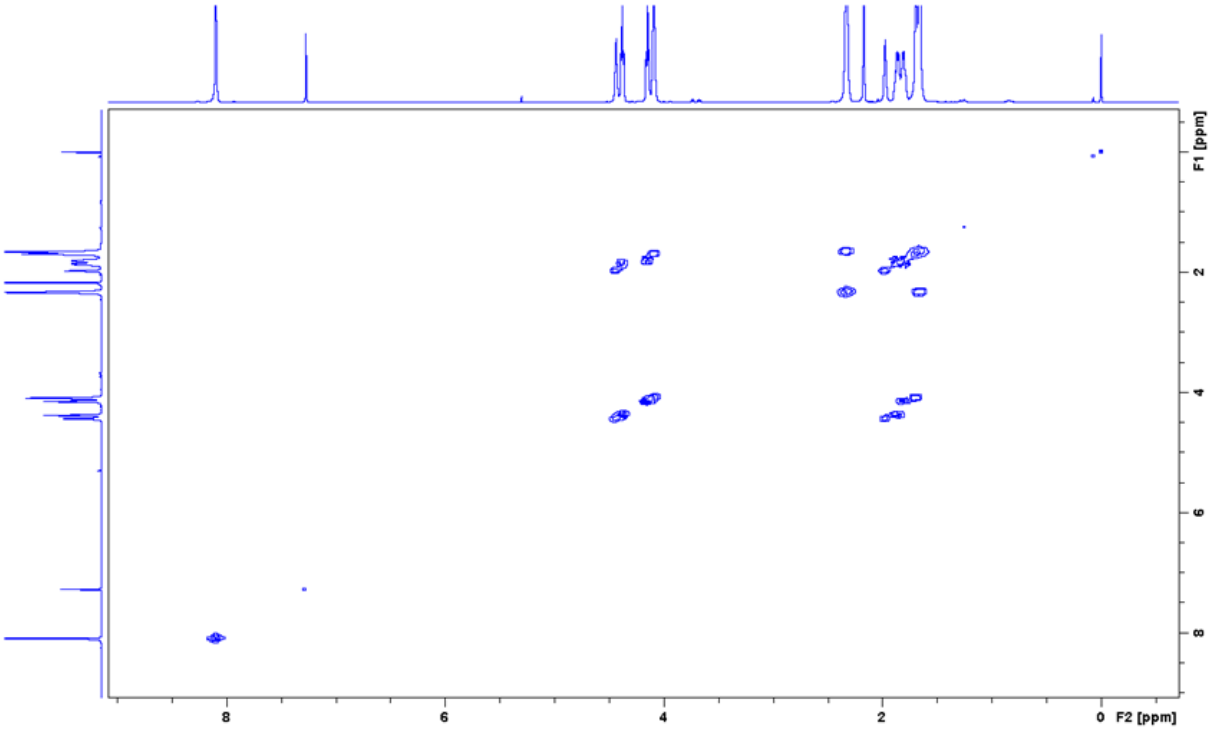

Figure 19: H,H-COSY of P-(BA-co-30-BT)

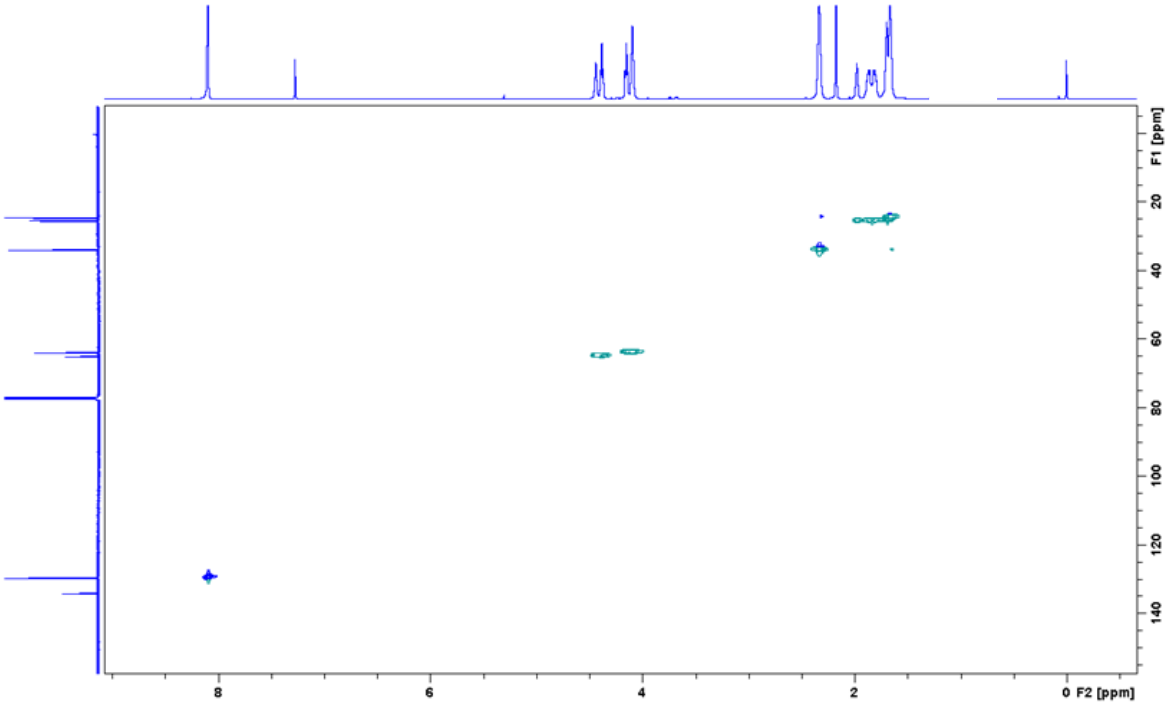

Figure 20: HSQC of P-(BA-co-30-BT)

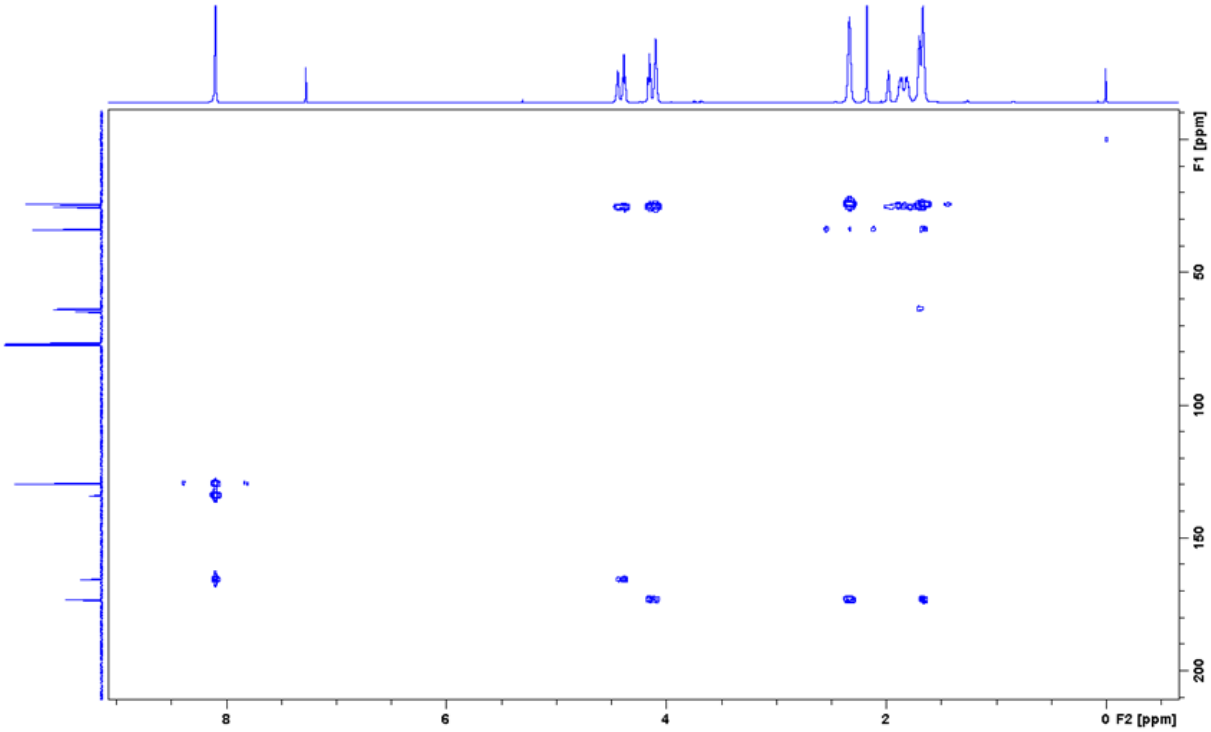

Figure 21: HMBC of P-(BA-co-30-BT)

To determine the structure of synthesised P-(BA-co-BT) + PEG copolyester supplementary to ^{1}H and APT spectra, H, H – COSY and HSQC were recorded with a 500 MHz NMR-device. All peaks in ^{1}H-NMR (see Figure 22) remain the same chemical shift as in previous discussed spectra presented, with exception of additional peaks (marked in Figure 22) occurring at 3.66 ppm attributable to inner CH_2 groups of poly (ethylene glycol) and at 3.7 and 4.15 ppm referring to the protons of the end groups of PEG. Due to the chemical shift of end groups of PEG, it can be determined that PEG reacted. Furthermore Pavelkova et al. [15] also presented peaks of PEG at 3.6 – 3.7 ppm and 4.2 – 4.4 ppm. The below presented structure of the copolyester is just a suggestion, because it was impossible to determine it exactly.

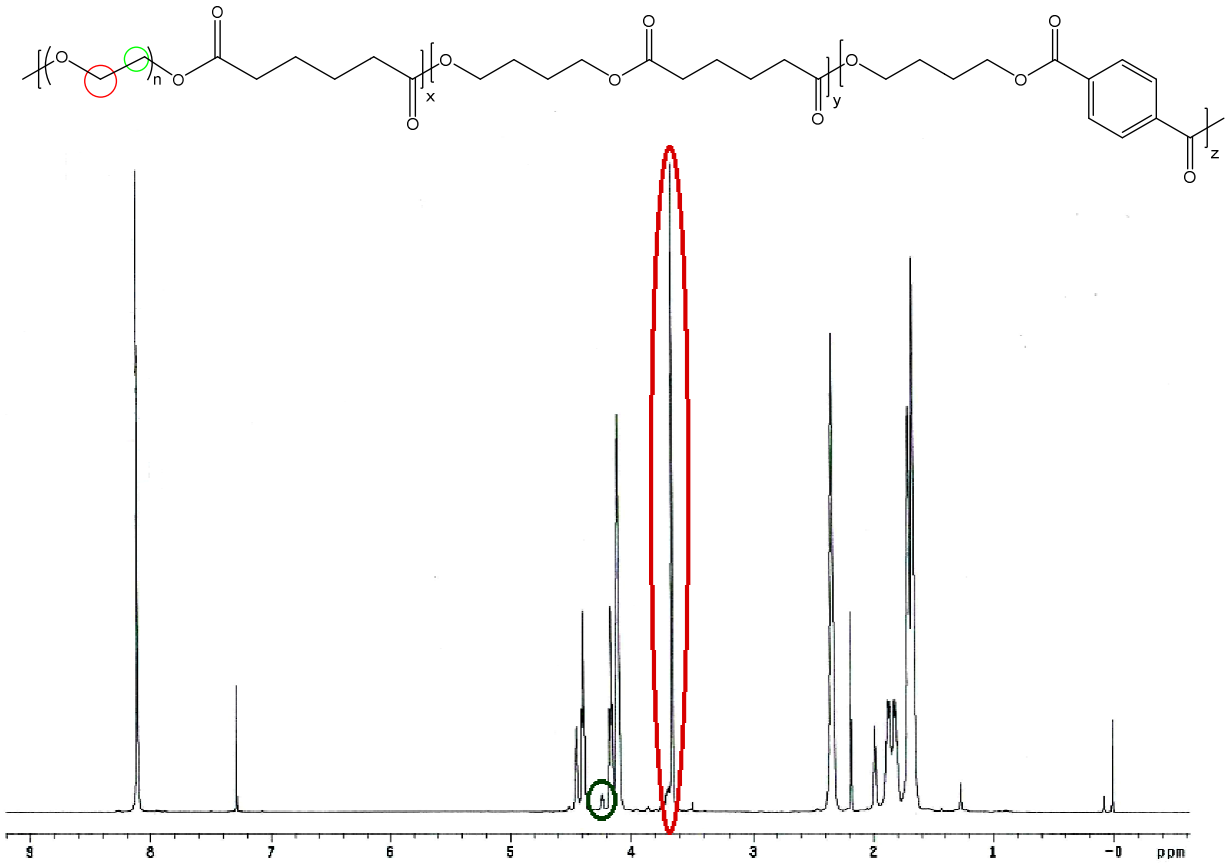

Figure 22: ^{1}H – NMR of P-(BA-co-BT)+PEG

To ensure that NMR investigations regarding the structure of prepared copolyesters are true, ATR – IR spectra of all mentioned samples were recorded supplementary. It can be seen from Table 5 that all characteristic vibration bands occur.

Table 5: Overview of characteristic IR-bands for P-(BA-co-BT)

	P-(BA-co-30-BT)	**P-(BA-co-BT)+TAIC**	**P-(BA-co-BT)+PEG**
Description	**Wave number [cm^{-1}]**		
CH_2, ν_{as}	2954.58	2953.40	2955.15
CH_2, ν_{as}			
CH_2, ν_s	2873.16	2874.21	2871.48
C=O, ν	1712.37	1713.03	1713.36
-C=C, ν, skeleton vibration	1504.34	1504.40	1504.31
-C=C, ν, skeleton vibration	1460.00	1461.72	1458.25
CH_2, δ, scissor vibration	1409.81	1409.64	1409.87
CH_2, δ_{as}	1392.68	1393.83	1391.03
CH_2, $\delta_{,s}$	1367.29	1367.88	1360.15
C-O-C, $\nu_{as,}$ benzene	1267.59	1268.11	1267.54
C=O, ν_s	1253.44	1254.24	1252.05
C-O-C, ν_{as} aliphatic	1164.91	1165.29	1165.89
C-O-C, ν_s aliphatic	1141.32	1141.67	1139.20
C-O-C, ν_s benzene	1119.31	1120.17	1118.54
C-O, δ, absorption	1102.63	1102.50	1102.43
CH, δ, out of plane	1017.61	1017.73	1017.57
CO, ν_s	938.97	951.46	939.72
CH, δ, out of plane	874.13	874.57	874.12
CH, δ, out of plane	727.99	728.52	728.38

ν: stretching vibration, ν_{as}: asymmetric stretching vibration, ν_s: symmetric stretching vibration, δ: bending vibration, δ_{as}: asymmetric bending vibration, δ_s: symmetric bending vibration

The most important peaks are also marked in Figure 23 – 25. Characteristic for ester bonds is of course the very sharp stretching vibration band of the carbonyl moiety at ~1710 cm^{-1}. Furthermore symmetric and asymmetric stretching vibrations of C – O – C bonds are located in the fingerprint region at 1100 – 1300 cm^{-1}, which allow a distinction between aliphatic and aromatic ester linkages. Peaks appearing at wavenumbers between 700 and 1000 cm^{-1} can be dedicated to out- of-plane bending vibrations of the benzene ring of terephthalate. The observed substitution pattern in this region confirms 1,4 – para – constitution of the aromatic compound.

Moreover peaks at 1504 and 1460 cm^{-1} refer to skeleton vibrations of the benzene ring. Aliphatic chains due to adipic acid are represented by asymmetric and symmetric stretching vibration bands of CH_2 groups at 2955 cm^{-1} respectively 2874 cm^{-1}. Presented wavenumbers and above described attributions were determined with the help of two books [45] [46] and are consistent with those published in the literature, e.g. by Y.-X. Weng et al. [47] and T. Kijchavengkul et al. [48]

While all characteristic peaks of P-(BA-co-BT) occur in all ATR – IR spectra, a distinction between modified and unmodified copolyesters is not possible with this kind of method. The reason for that is that on the one hand PEG possesses no additional functional groups which may result in further peaks and on the other hand concentration of TAIC is very low, so the attributable vibration bands at 1691 cm^{-1} for the carbonyl group and at 1627 cm^{-1} for C=C for TAIC [43] cannot be observed.

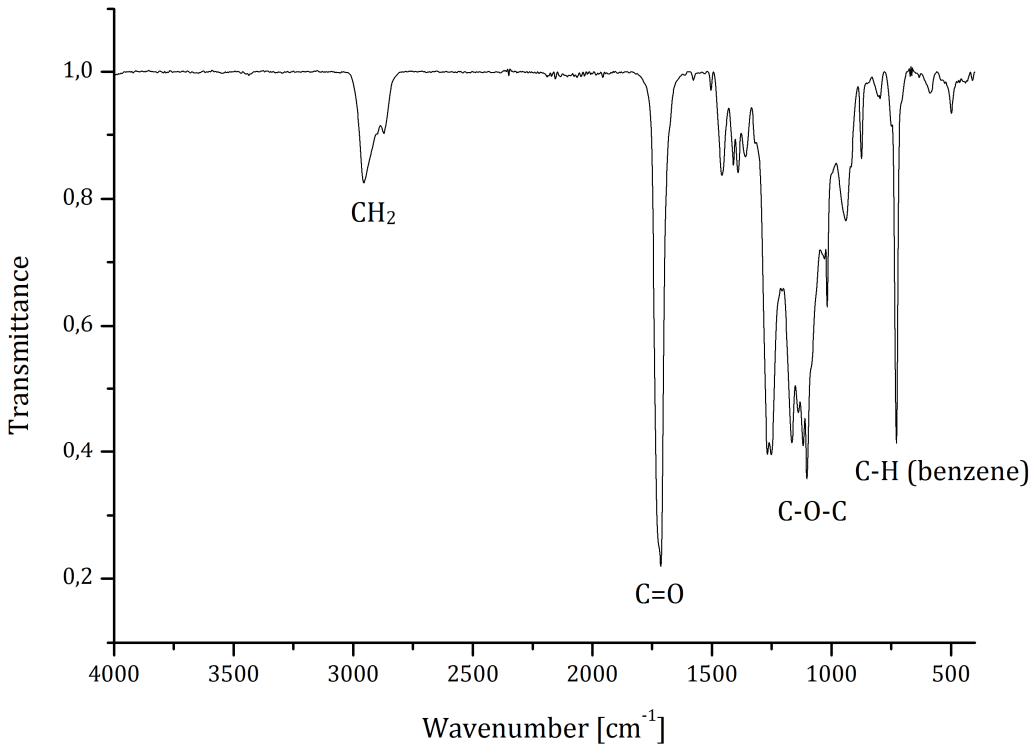

Figure 23: ATR – IR spectrum of P-(BA-co-BT) +PEG

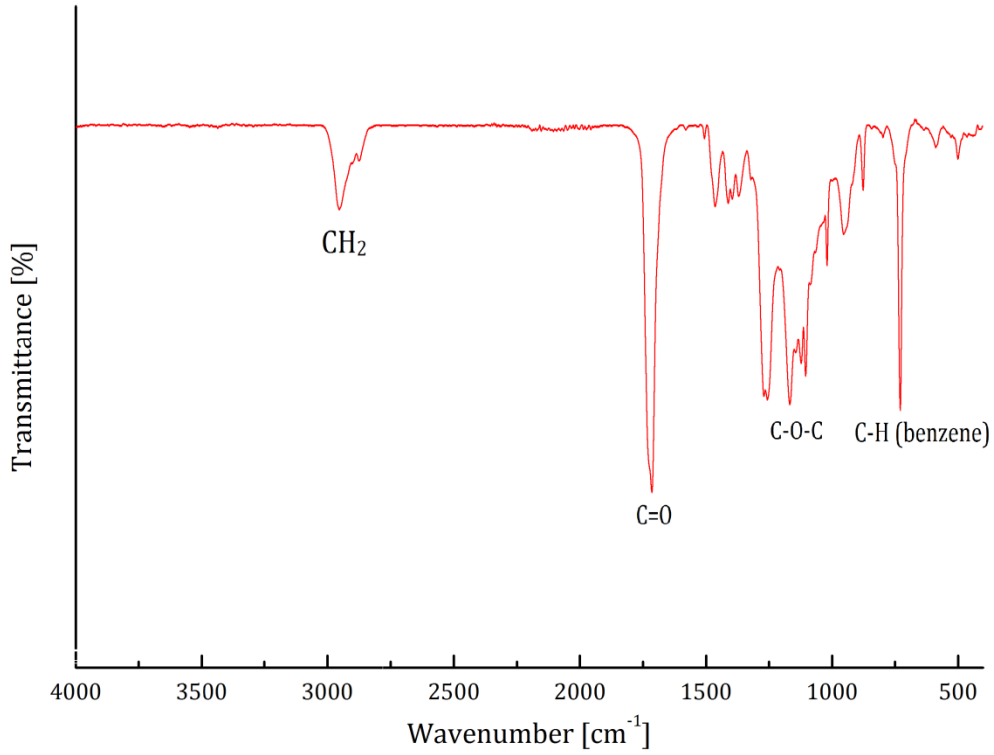

Figure 24: ATR – IR spectra of P-(BA-co-BT) +1% TAIC

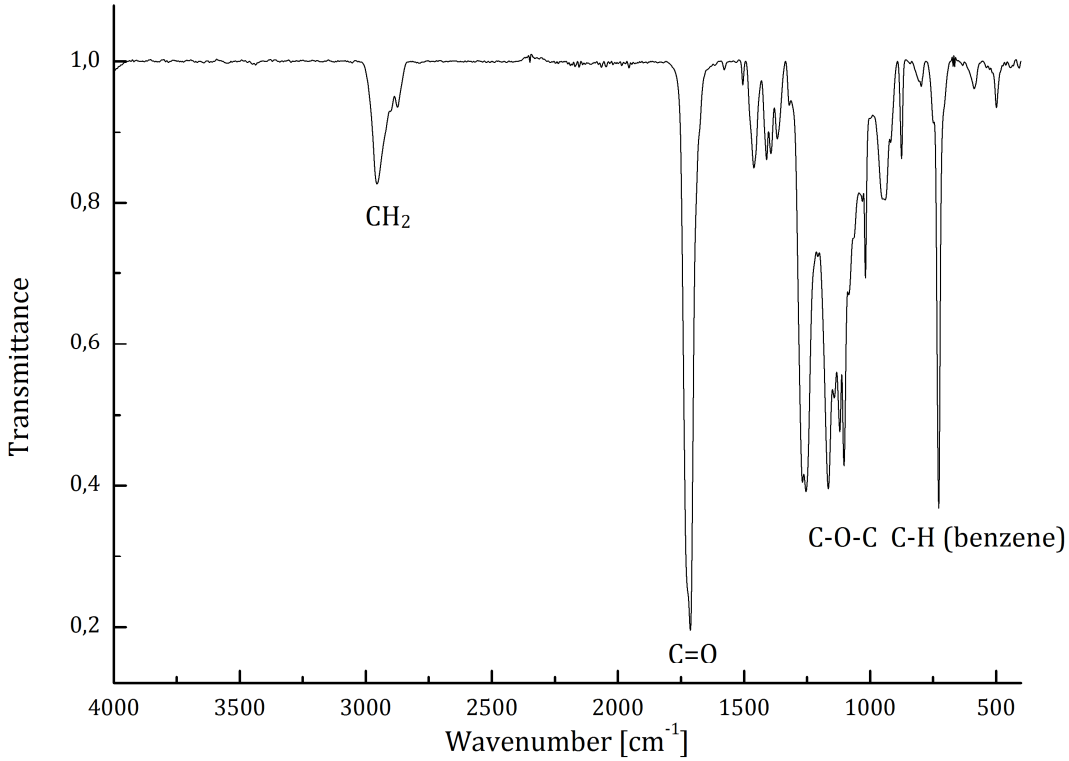

Figure 25: ATR – IR spectrum of P-(BA-co-30-BT)

Hydrophilicity of prepared films was determined by contact angle measurements with water. The results are presented in Table 6. P-(BA-co-BT)+PEG has a about 10° lower contact angle as unmodified P-(BA-co-30-BT) which confirms that modification with PEG leads to a more hydrophilic copolyester.

Table 6: Results obtained from contact angle measurements of P-(BA-co-BT) copolyesters

Sample	Contact angle Θ [°]
P-(BA-co-30-BT)	68.5 ± 6.8
P-(BA-co-30-BT)+1% TAIC	67.7 ± 5.2
P-(BA-co-BT)+PEG	59.3 ± 4.1

Influence on thermal properties

In Table 7 is a summary of data obtained from thermal analysis given. Melting enthalpy (ΔH_m) of P-(BA-co-30-BT) has a value of 38.7 J/g. Because melting enthalpy is a parameter referring among others to crystallinity (compare equation 3) it can be concluded that the sample consisting of 30 mol% butylene – terephthalate is a semi – crystalline copolyester and not an amorphous polymer. This is also in accordance with the general assumption that aromatic blocks are responsible for crystalline and aliphatic parts for amorphous behavior. [8] However, Z. Gan et al. [1] presents data with T_m = 73 °C and ΔH_m = 3.0 J/g for P-(BA-co-BT) copolyester with 30 mol% BT. These values are significantly lower than the ones obtained in our experiments. But similar results concerning melting temperature are presented in a paper published by Witt et al. [9] They indicate a melting temperature of 79 °C and a melting enthalpy of 7.7 J/g for a copolyester containing 31 mol% BT and 69 mol% BA.

$$X[\%] = \frac{\Delta H_m - \Delta H_c}{\Delta H^\circ_m} \qquad (3)$$

Modification of copolyester with TAIC lead to a slightly lower crystallinity whereas melting temperatures stays more or less the same. Implementation of PEG does neither have an effect on melting enthalpy nor on melting temperature, which can be explained by the low amount (2 mol%) incorporated. Maximal temperatures and onset temperatures of thermal degradation stay more or less constant.

Table 7: Summary of data achieved by STA and DSC measurements for P-(BA-co-BT) copolyester

	TG		DSC				
Sample	**T_0 [°C]**	**T_{max} [°C]**	**T_{m1} [°C]**	**ΔH_{m1} [J/g]**	**T_{m2} [°C]**	**ΔH_{m2} [J/g]**	**$\Sigma \Delta H_m$ [J/g]**
P-(BA-co-30-BT)	381.8	409.8	82.2	38.7	-	-	38.7
P-(BA-co-30-BT)+1%TAIC	372.6	407.3	81.2	31.6	113.0	2.6	34.2
P-(BA-co-BT)+PEG	380.6	407.5	80.2	41.7	113.0	2.4	44.1

In Figure 26 is a segment of obtained DSC – curves of P-(BA-co-BT) copolyesters plotted. The higher melting enthalpies of the first melting point are clearly visible through the big peak at around 80 °C whereas the second melting points with the corresponding low melting enthalpies are hardly observable.

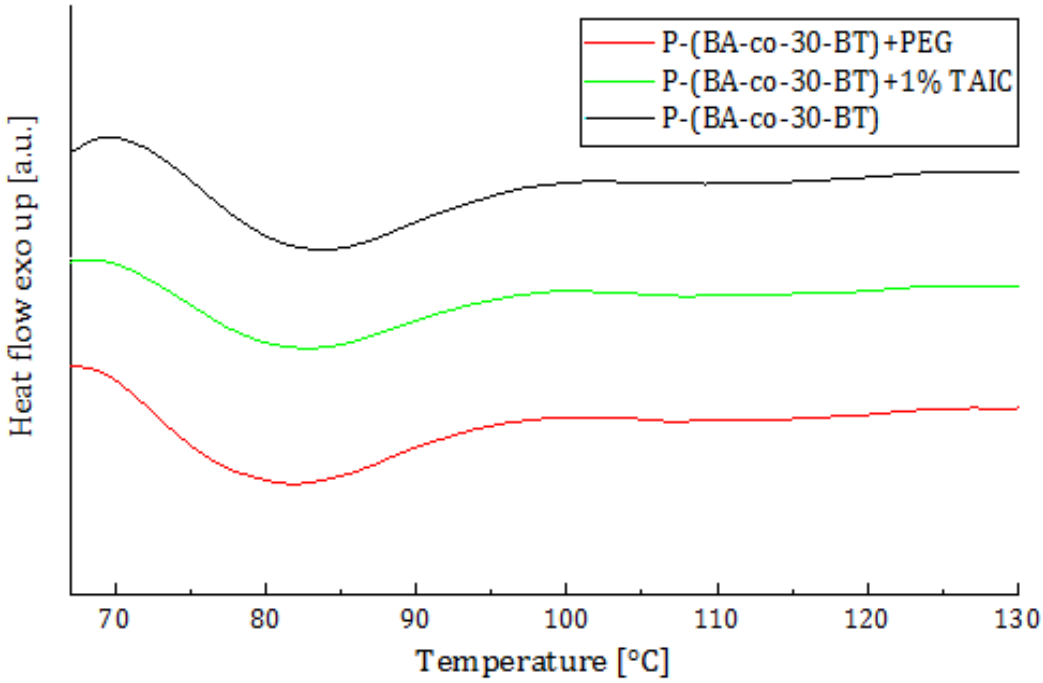

Figure 26: Comparison of DSC-curves obtained from STA of P-(BA-co-30-BT) copolyesters

The curves resulting from thermogravimetric analysis are shown in Figure 27. From this graph the beginning of degradation, described by the onset temperature, can be determined. It can be seen from the plot that the melting behaviour is the same for all three samples. Nevertheless, sample modified with TAIC degradades a little bit faster in the first phase than the other copolyesters. A possible explaination may be that the bonds between TAIC and the polyester break down easier at higher tempeatures than the copolyester itself. Moreover, maximal degradation temperatures (T_{max}) are calculated form the first derivative of these plots.

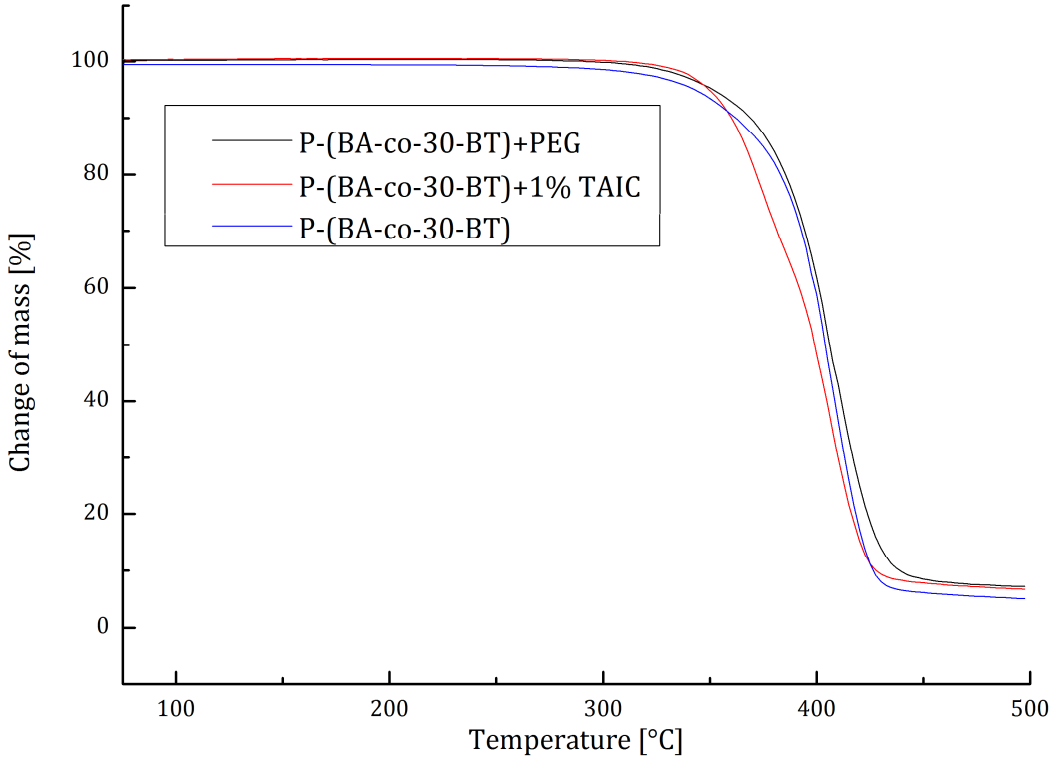

Figure 27: Comparison of TG-curves obtained from STA of P-(BA-co-30-BT) copolyesters

Influence on mechanical performance

Table 8 presents data achieved from mechanical testing by an uniaxial tensile test mode. For analyses 5 specimens were punched out in shoulder bar form of a pressed film of each of the three copolyesters. Results given in Table 8 are average values from 3 measuring runs and their corresponding standard deviations. In Figure 28 is the stress – strain curve for P-(BA-co-30-BT) as an example shown. It provides among others information about the elongation at break (ε_B), tensile strength at the maximum (σ_B), Young's modulus (E) and maximal applicable force (F_{max}). The mathematical correlations are given by following equations 4 – 6

$$E\ [MPa] = \frac{\sigma_2 - \sigma_1}{\varepsilon_2 - \varepsilon_1} \quad (4)$$

$$\varepsilon_B[\%] = \frac{L - L_0}{L_0} * 100 \quad (5)$$

$$\sigma_B[MPa] = \frac{F_{max}}{S_0} \quad (6)$$

with σ as tensile stress [MPa], ε as tensile strain (elongation), L as maximal obtained length of the specimen [mm], L_0 as length of specimen at the beginning [mm], S_0 as area of the specimen [mm^2]. [49]

In general, tensile strength for plastics is determined from the slope between 0.05 and 0.25% elongation from the stress – strain curve.

Table 8 indicates that the two different types of modifications lead to completely different results concerning mechanical properties. Unmodified P-(BA-co-30-BT) copolyester has a Young's Modulus of 13.2 MPa, an elongation at break of 502.4 % and a tensile strength at the maximum of 4.2 MPa. These values identify this polymer as a rather elastic, soft and ductile material. Milinkovic et al. [41] reached for example a Young's modulus of 10.8 MPa, whereas Witt et al. [9] presented results for tensile strength at break of about 7.9 MPa and for elongation at break of 650 %, for samples containing the same molar ratios as ours. However, mechanical properties can be controlled for instance by the amount of terephthalate added [9] or alternatively, as described by means of the present work, by modifying agents.

Crosslinking via triallyl isocyanurate did not only increase molecular weight but most of all elastic behaviour. P-(BA-co-30-BT)+1% TAIC reached a value of elongation at break of 1023.7 % which is twice as much as unmodified copolyester showed. Tensile strength and maximal force increased also marginally. The incorporation of PEG resulted in a very rigid polymer and is therefore not useable as material for stable films.

Table 8: Summary of the results obtained from mechanical testing

Sample	**Max. Force [N]**	**Young modulus [MPa]**	**Elongation at break [%]**	**Tensile strength at maximum [MPa]**
P-(BA-co-30-BT)	9.7 ± 1.0	13.2 ± 3.0	502.4 ± 101.6	4.2 ± 0.4
P-(BA-co-30-BT)+1% TAIC	11.3± 0.04	13.8 ± 5.3	1023.7 ± 52.1	6.7 ± 0.2
P-(BA-co-BT)+PEG	1.9 ± 0.4	10.1 ± 5.0	15.0 ± 3.7	0.9 ± 0.2

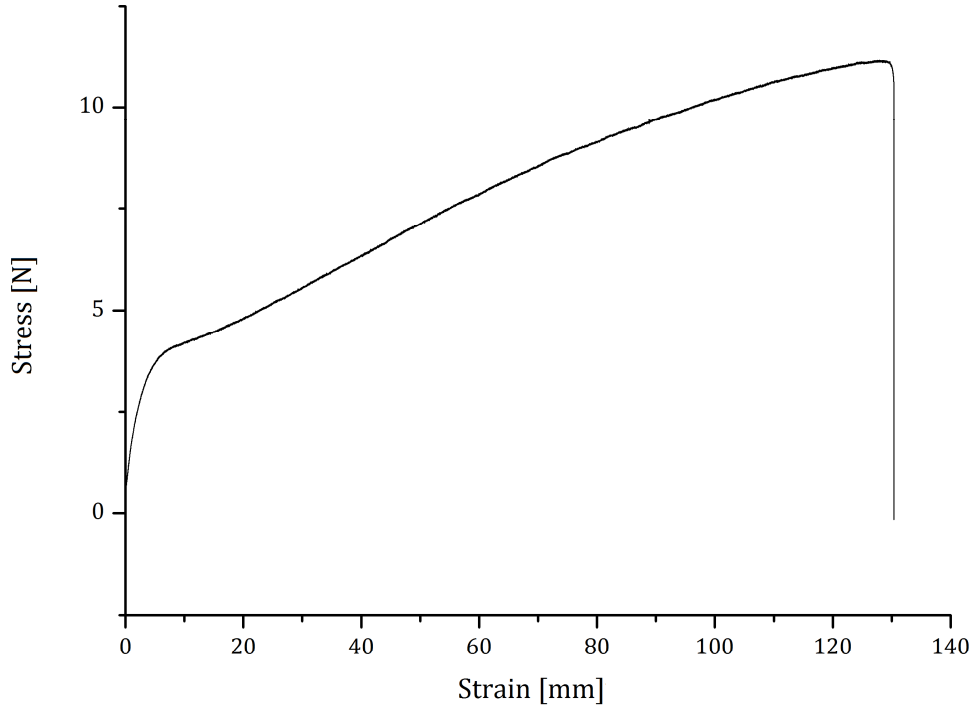

Figure 28: Stress – strain curve of P-(BA-co-30-BT)

Summary

P-(BA-co-BT) copolyesters with a fraction of 30 mol% BT were altered in two different ways. On the one hand crosslinking with 1 mol% triallyl isocyanurate resulted in an increase of molecular weight from 29870 $g.mol^{-1}$ up to 40000 $g.mol^{-1}$. Furthermore mechanical properties could be improved, especially elongation at break of P-(BA-co-30-BT) with the addition of 1% TAIC could be more than doubled. The second modifying agent was PEG whose implementation led to a third block. The aim of creating a more hydrophilic copolyester could successfully be reached and is confirmed by results obtained from contact angle measurements. The structures of all samples were identified by NMR and IR spectroscopy. Thermal analyses showed that modifications of P-(BA-co-30-BT) had no big influence on thermal properties.

4.1.2 P-(GS-co-GT) copolyesters

Referring to the work described in chapter 4.1.1 and a polyester called poly-glycerol-sebacate prepared among others by Y. Wang et al. [50] we combined these two concepts in order to create a copolyester based on glycerol, dimethyl terephthalate and sebacic acid. These types of copolyesters already exist, but mainly only as thermosetting materials and alkyd resins [51] [52] and not as polymer with thermoplastic properties. A further aim of our syntheses was also to prepare soluble copolyesters to use them as matrix materials for drug encapsulation systems. Because glycerol belongs to the group of triols we considered to reach on the one hand an even more crosslinked network than with 1,4 – butanediol in P-(BA-co-BT) copolyesters and on the other hand due to the introduction of aromatic units a more stabilised system than poly-glycerol-sebacate. A further advantage is that glycerol is received in large amounts as side product in the process of making biodiesel, whereas 1,4 – butanediol is obtained from fossil based resources. This implies another step towards "green chemistry".

It was tried to synthesise these copolyesters with different ratios of DMT, sebacic acid and glycerol under various reaction conditions. The proposed (theoretical) reaction scheme is given in Figure 29.

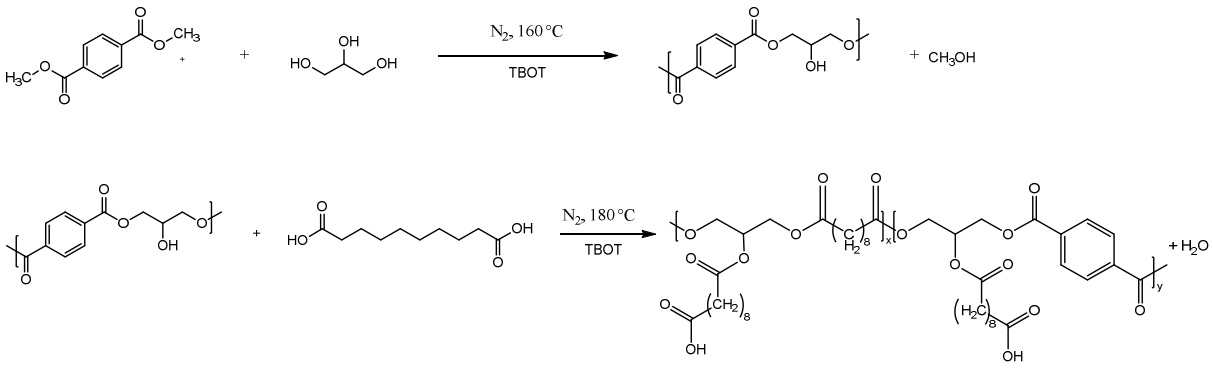

Figure 29: Reaction scheme of the synthesis of P-(GS-co-GT)

The obtained copolyesters were used as polymer material for drug encapsulation systems (see chapter 4.2) as well as for the preparation of films.

Comparison of copolyesters with various DMT/SA - ratios but same amount of glycerol

Table 9 shows average molecular weights of prepared copolyesters containing glycerol-terephthalate ratios between 30 and 50 mol%. The amount of glycerol is thereby the same in all reactions with ⅙ of overall amount of substance of dimethyl terephthalate and sebacic acid. As can be seen in Table 9, increasing amounts of glycerol-terephthalate tend to result in higher

average molecular weights, but also broadens the distribution which is indicated by the PDI. In general, obtained molecular weights are very low (1990 – 8370 g.mol^{-1}). This can be explained by the fact, that polycondensation reactions only lead to high molecular weight polymers when a high extent of reaction (>99 %) is achieved. [5] [44] Because performed syntheses were terminated after 6.25 hours, monomer conversion was for sure not completed and chromatograms obtained from GPC show that there are several additional low molecular weight oligomer fractions. But complete conversion was not desired in this case, because the aim was that the synthesised polymers are soluble and therefore the degree of crosslinking has to be quite low. These two requirements are necessary for their application as matrix material for drug encapsulation systems. Another reason for the low molecular weight is for certainty, that the amount of glycerol (⅙ related to the overall amount of substance) is too low to provide enough hydroxyl groups for all functional groups of dimethyl terephthalate and sebacic acid. Noticeable is also the fact that the copolyester containing 50 mol% of glycerol-terephthalate has a slightly lower M_w than P-(GS-co-44-GT), whereas there is a big gap between P-(GS-co-40-GT) and latter one.

Table 9: Summary of average molecular weights of P-(GS-co-GT) copolyester

Sample	M_w [g.mol^{-1}]	M_n [g.mol^{-1}]	PDI
P-(GS-co-30-GT)	1990	1319	1.51
P-(GS-co-40-GT)	3120	1569	1.99
P-(GS-co-44-GT)	8366	2443	3.42
P-(GS-co-50-GT)	7081	2293	3.09

Solubility tests featured that all above mentioned samples are very good soluble in THF and DMSO, slightly poorer in chloroform and insoluble in dichloromethane, methanol and ethyl acetate. But dissolution in chloroform and dichloromethane can be enhanced by adding a few drops of THF to the solution.

Figure 30 presents a comparison of ^{1}H-NMR spectra between those four copolyesters. All peaks remain the same chemical shift which indicates that all syntheses reveal the same product. An exact correlation between the individual peaks and the proposed structure is impossible because there are too many opportunities. The structure presented in Figure 29 only shows one possible arrangement of dimethyl terephthalate and sebacic acid units with glycerol. Aside from that, P-(GS-co-GT) copolyesters can be classified also as random copolyesters like P-(BA-co-BT).

However, the peak at 8.12 ppm indicates the aromatic moiety, whereas the small peaks at 4.16 and 4.29 ppm represent CH_2 groups of glycerol and peaks at 1.29 – 2.37 ppm are responsible for CH_2 groups of sebacic acid. Due to the comparison of these chemical shifts of the obtained

product with those of the educts, it can be concluded that polycondensations have been successful.

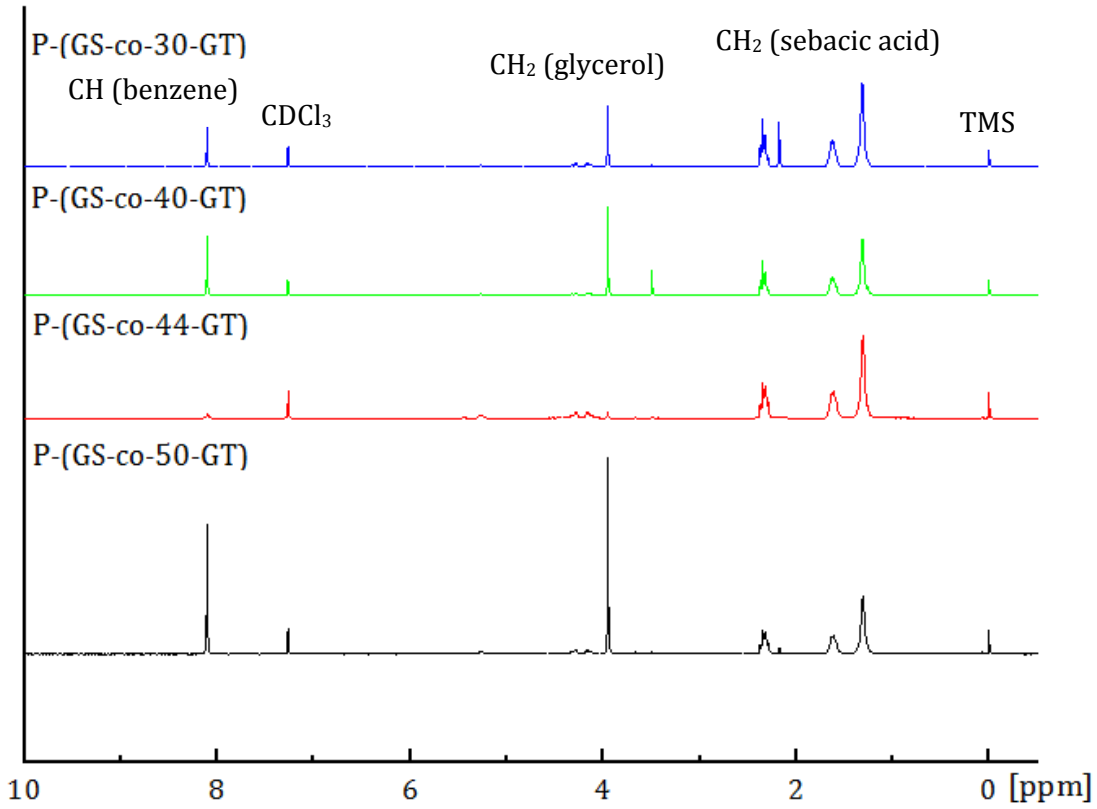

Figure 30: Comparison of ^{1}H-NMRs of samples with different DMT and sebacic acid ratios but same glycerol concentration

Figure 31 shows ATR-IR spectra of obtained copolyesters. The recorded spectra show all distinctive vibration bands typical for copolyesters. The sharp peak detected at about 730 cm^{-1} is characteristic for aromatic compounds. Increasing intensity of this peak is in accordance with theoretical growing amount of butylene-terephthalate. All carbonyl bands are broader compared with P-(BA-co-BT) samples. Detailed information of observable vibration bands is given in Table 5.

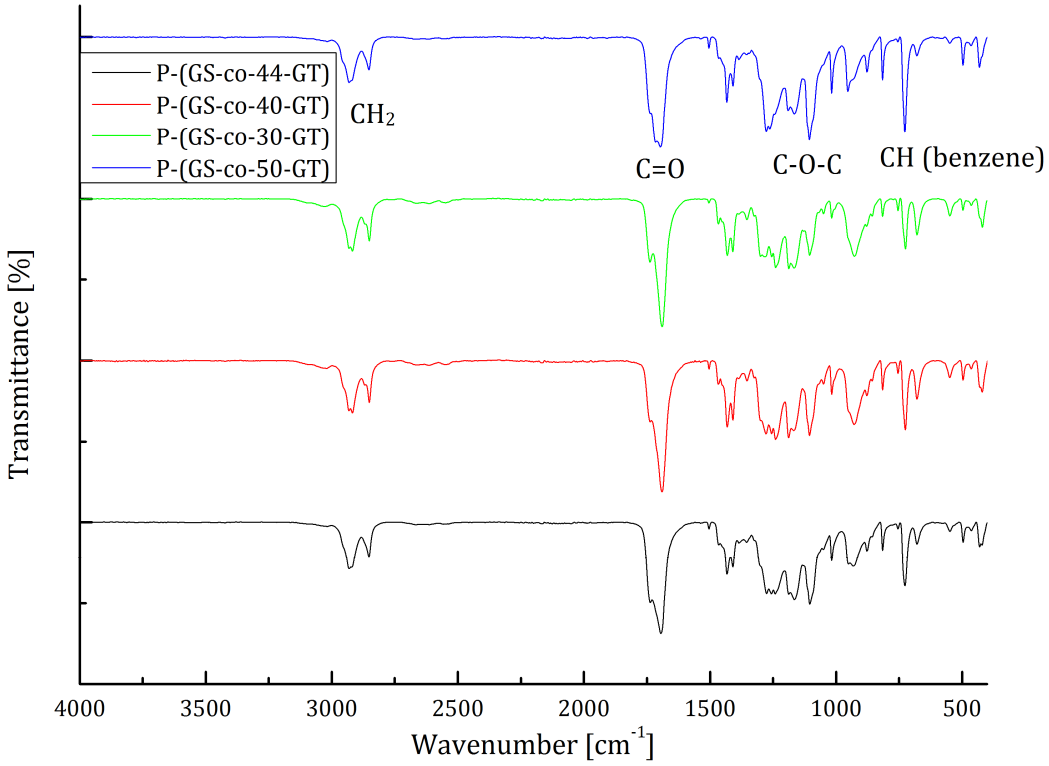

Figure 31: Comparison of ATR-IR spectra of P-(GS-co-GT) with various DMT/SA ratios

In Table 10 is an overview of results achieved from thermal analysis by DSC and STA given. All samples exhibit at least 2 melting temperatures, whereas the first one is at about 68 °C for copolyesters with 30 and 40 mol% GT respectively 100 °C for other both. Second melting temperature is for former ones at around 113 – 117 °C and for P-(GS-co-44-GT) at 123 °C respectively for P-(GS-co-50-GT) at 128 °C. Melting temperatures rise with increasing amount of terephthalate which is in line with general theory described in literature as well as with the assumption that polymers with a higher molecular weight also exhibit melting peaks at higher temperatures. [1] [41] Furthermore very high melting enthalpies of all samples with up to 63.4 J/g suggest semi – crystalline polymers with a very high crystalline ratio.

Data obtained from thermogravimetric analysis show onset temperatures between 156 – 199 °C and maximal degradation temperatures of 188 – 210 °C. Noticeable is the fact that P-(GS-co-30-GT) has the highest onset temperature as well as maximal degradation temperature. Nevertheless these data indicate quite thermally unstable copolyesters, which may be explained again by the short reaction time and hence it is obvious that the prepared samples are not really copolyesters but a kind of oligomers instead. Prolongation of the reaction time resulted in an improvement of thermal stability of about 200 °C (see chapter 4.2.3). Figure 33 also shows that thermal degradation occurs in two stages, which is as well a result of thermal instability.

Table 10: Overview of thermal properties of P-(GS-co-GT) copolyester

	TG		DSC				
Sample	**T_0 [°C]**	**T_{max} [°C]**	**T_{m1} [°C]**	**ΔH_{m1} [J/g]**	**T_{m2} [°C]**	**ΔH_{m2} [J/g]**	**$\Sigma \Delta H_m$ [J/g]**
P-(GS-co-30-GT)	198.7	210.4	68.1	7.1	113.0	42.8	49.9
P-(GS-co-40-GT)	169.5	204.0	65.2	2.2	117.6	61.2	63.4
P-(GS-co-44-GT)	155.9	187.7	100.0	34.5	122.7	23.6	58.1
P-(GS-co-50-GT)	169.6	201.7	101.3	4.8	128.2	7.3	12.1

Figure 32 and 33 illustrate DSC and TG curves obtained from STA measurements.

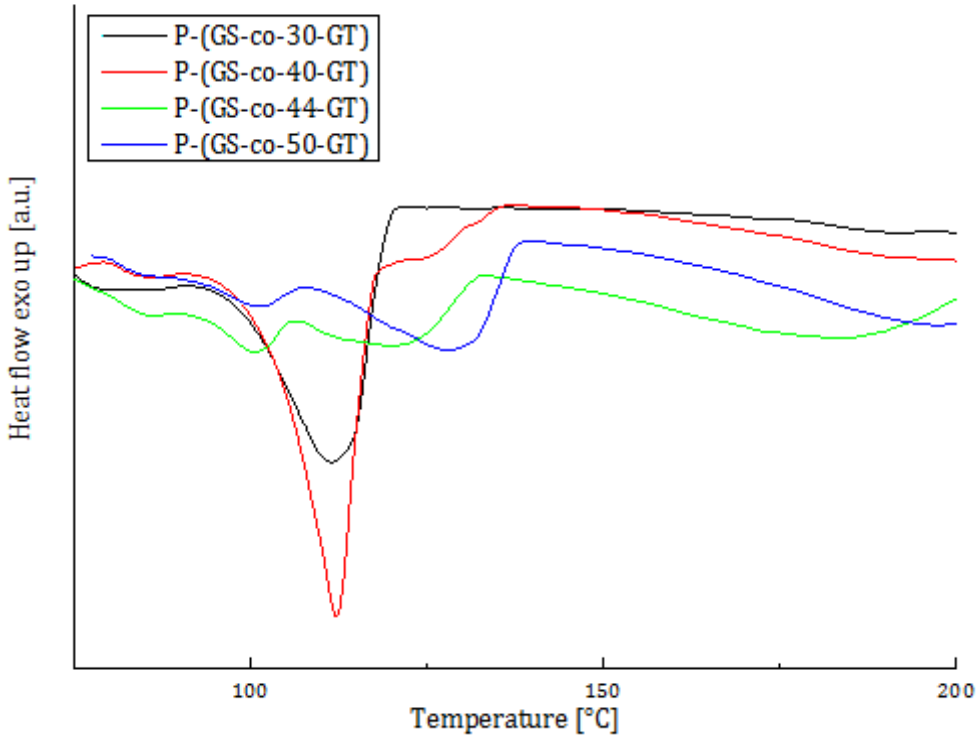

Figure 32: Comparison of DSC – curves of P-(GS-co-GT) copolyesters with various DMT/SA ratios

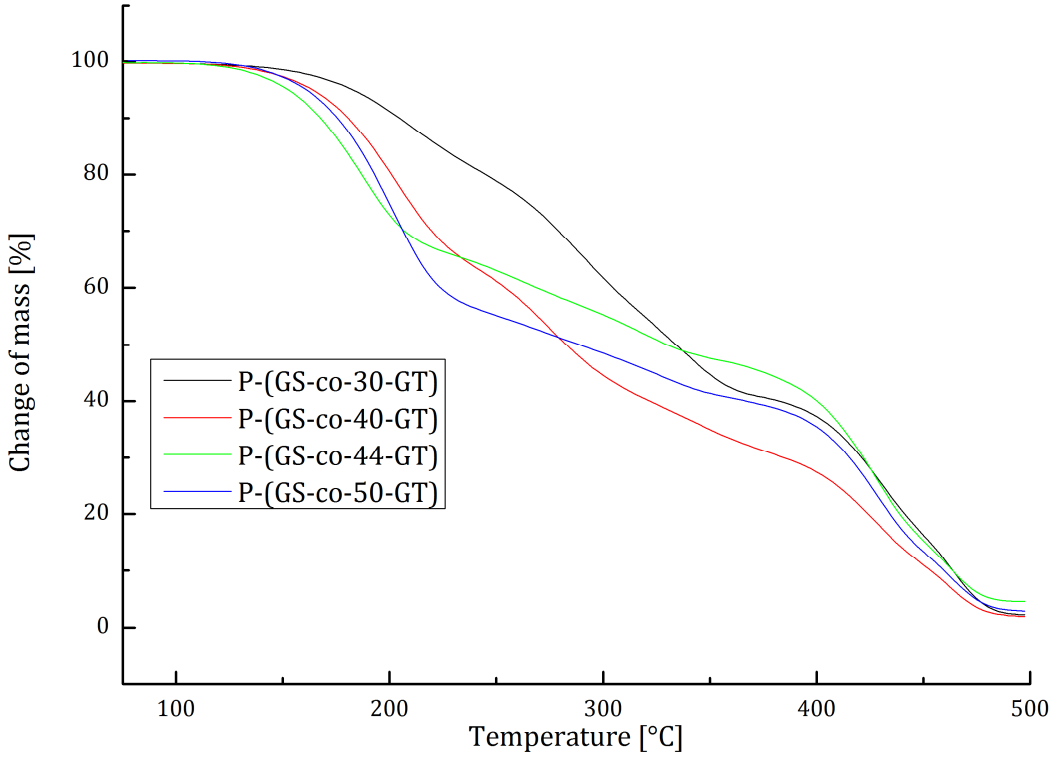

Figure 33: Comparison of TG – curves of P-(GS-co-GT) copolyesters with various DMT/SA ratios

It was also tried to press films with obtained copolyesters, it also worked but it turned out that they are very brittle, whereas the film consisting of P-(GS-co-50-GT) is among the others the most promising one. The reason for that is for sure the low molecular weight and furthermore the small amount of glycerol added. Another explanation could be that this polymer is quite amorphous compared to the other ones.

But as a practical application, three of them were successfully implemented as polymer matrix materials for drug encapsulation systems (see chapter 4.2).

Influence of the amount of glycerol

Besides varying DMT / sebacic acid ratios, the impact of different glycerol concentrations concerning thermal and molecular properties was also investigated. Therefore the sample with the highest molecular weight, P-(GS-co-44-GT) was chosen. Because glycerol possesses three hydroxyl groups in contrast to 1,4 – butanediol, which is only able to esterify with two moieties and hence added in excess, crosslinking with glycerol occurs quite rapidly when added abundantly. That is why it was in the beginning attempted to start with ⅓ of glycerol related to overall amount of substance. But this resulted in a too crosslinked, insoluble network, for what

reason it was not possible to measure molecular weight by GPC. The results of remaining samples are presented in Table 11.

Table 11: Overview of average molecular weights of P-(GS-co-GT) with various amounts of glycerol

Sample	M_w [g.mol^{-1}]	M_n [g.mol^{-1}]	PDI
P-(GS-co-44-GT)+⅙ glycerol	8366	2443	3.42
P-(GS-co-44-GT)+¼ glycerol	4833	1988	2.43
P-(GS-co-44-GT)+⅓ glycerol	nd*	nd*	nd*

**nd: not determined*

Data achieved by GPC measurements seem to be quite controversial, because molecular weight of P-(GS-co-44-GT) +⅙ glycerol is higher than that of P-(GS-co-44-GT) +¼ glycerol. P-(GS-co-44-GT) +⅓ glycerol has certainly the highest M_w. For all prepared copolyesters could be observed, that a higher molecular weight also broadens the molecular weight distribution. The higher PDI results from a great variety of arrangements concerning esterfication of dimethyl terephathalate, sebacic acid and glycerol.

In Figure 34 are the ^{1}H-NMRs of synthesised P-(GS-co-GT) copolyester shown.

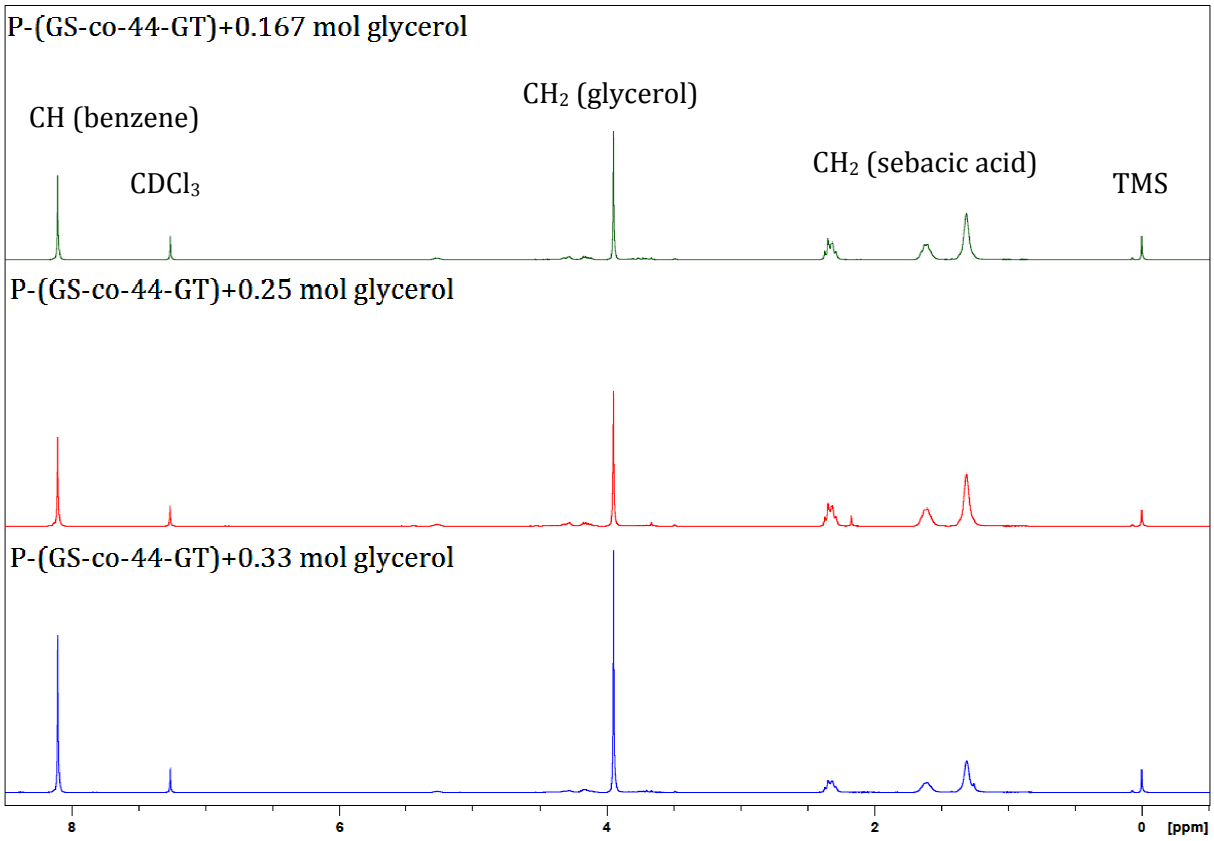

Figure 34: Comparison of ^{1}H-NMRs of samples with various amounts of glycerol but constant DMT/sebacic acid ratio

As a second method for (qualitative) investigations concerning structure of copolymers, ATR-IR spectra were recorded to ensure all requested functional groups are really implemented. The results are illustrated in Figure 35.

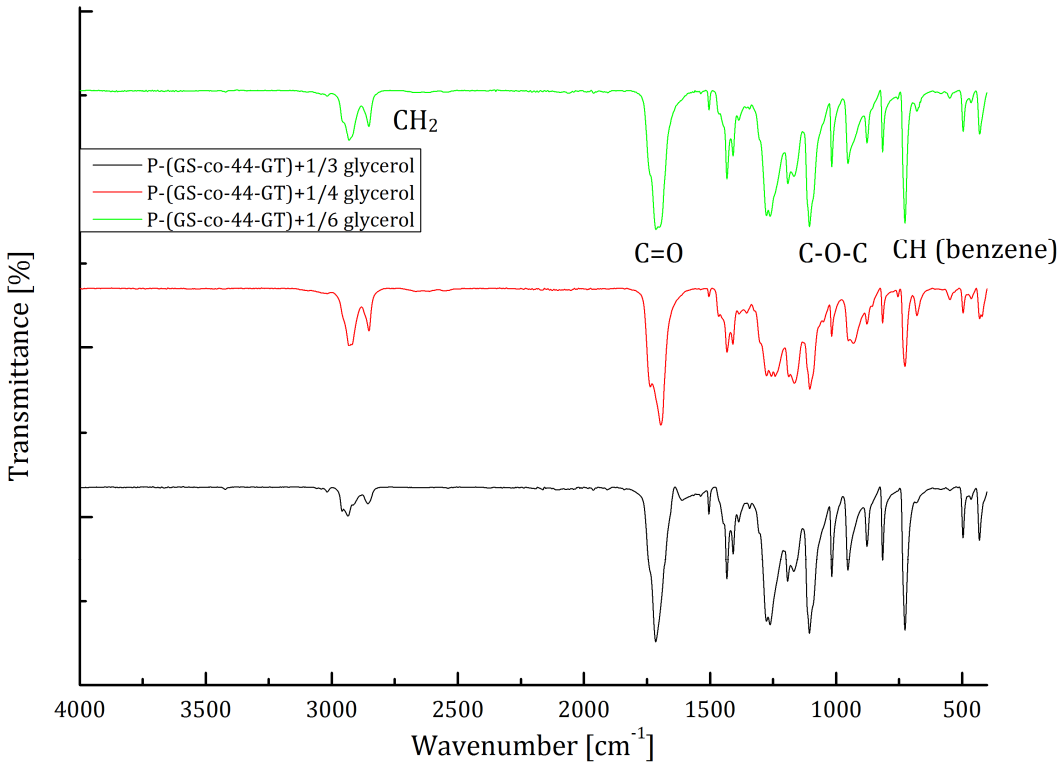

Figure 35: ATR-IR spectra of P-(GS-co-44-GT) with various amounts of glycerol

Qualitative investigations of these spectra lead on to an explanation in case of significant lower molecular weight of P-(GS-co-44-GT) +¼ glycerol. Intensity of the peak at 727 cm^{-1}, which is typical for aromatic compounds, is lower than those of the other two samples. One is forced to come to the conclusion that P-(GS-co-44-GT) +¼ glycerol has not reacted properly especially in the 1st reaction step. The exact correlation of vibration bands and functional groups is given in Table 5.

Table 12 shows outcomes of STA measurements. Regarding thermogravimetric analysis it is observable that onset temperatures T_0 of samples with ⅙ respectively ¼ amount of glycerol have the same value (155 °C) while the one containing ⅓ of glycerol starts to degrade about 20 °C higher at 172 °C. The same is valid for maximal degradation temperatures (T_{max}), which are in a range between 187.7 °C and 207.2 °C. Melting temperatures of all samples are nearly the same, but slightly increase with rising amount of glycerol. Melting enthalpies are quite high (50.3 – 76.5 J/g) and indicate semi-crystalline polymers with a very high crystalline ratio.

Table 12: Results from STA-measurements of P-(GS-co-GT) with various amount of glycerol

	TG		DSC				
Sample	T_0 [°C]	T_{max} [°C]	T_{m1} [°C]	ΔH_{m1} [J/g]	T_{m2} [°C]	ΔH_{m2} [J/g]	$\Sigma \Delta H_m$ [J/g]
P-(GS-co-44-GT)+⅙ glycerol	155.9	187.7	100.0	34.5	122.7	23.6	58.1
P-(GS-co-44-GT)+¼ glycerol	156.7	189.7	-	-	124.7	50.3	50.3
P-(GS-co-44-GT)+⅓ glycerol	172.2	207.2	77.9	7.8	128.2	68.7	76.5

It was also tried to press films with thermoforming under vacuum, but only copolyester with ⅓ of glycerol showed film formation. High crystallinity is visible through twinkle areas in the film. From mechanical properties of view, it seems to be very flexible and not very brittle. Mechanical testing was not possible, because the film is easily breaking down into smaller pieces which is attributable to its low molecular weight.

Influence of reaction conditions and time

Due to the fact that above described syntheses did not result in copolyesters with a moderate average molecular weight, it was tried to overcome this problem with two alternative reaction pathways. On the one hand it was attempted to synthesise requested polymers through the application of vacuum, based on the method used for P-(BA-co-BT) and on the other hand by prolongation of the reaction time from 8.25 hours to 24 hours in total. Moreover the purification process was changed. Because samples described in 4.2.1 and 4.2.2 were better soluble in THF than in chloroform which suggests the assumption that these copolyesters are more polar and can be explained by the way that not all hydroxyl groups of glycerol have reacted with either sebacic acid or dimethyl terephthalate, THF was chosen as solvent instead of chloroform. Precipitation occurred therefore in pentane, because it is very nonpolar in contrast to methanol. Table 13 features results regarding molecular weight of copolyesters obtained via these methods.

Table 13: Summary of molecular weights from P-(GS-co-GT) with varied reaction conditions

Sample	M_w [g.mol^{-1}]	M_n [g.mol^{-1}]	PDI
P-(GS-co-30-GT)+vacuum	2069	1375	1.51
P-(GS-co-44-GT)+vacuum	4247	1857	2.29
P-(GS-co-20-GT)-24h	5436	2158	2.52
P-(GS-co-30-GT)-24h	8411	2557	3.29
P-(GS-co-40-GT)-24h	-*	-*	-*

**: not measured*

The application of vacuum has no influence on average molecular weight and polydispersity, but thermal properties are shifted to higher temperatures, especially of P-(GS-co-44-GT) (see Table 14). There, onset temperature of thermal degradation is moved from 156.7 °C up to 220.7 °C, also T_{max} is shifted from 189.7 °C to 424.3 °C. Melting temperatures are nearly the same, but crystallinity has changed.

Longer reaction times however, tend to result in higher average molecular weights than those samples terminated after 8.25 hours show, although lower concentrations of dimethyl terephthalate were used, e.g. P-(GS-co-44-GT)+¼ glycerol shows a molecular weight of 4833 $g.mol^{-1}$ and P-(GS-co-20-GT) has 5436 $g.mol^{-1}$. This is in accordance with overall principal of polycondensation which declares that high molecular weights can only be achieved, when monomers conversion is above 99%. P-(GS-co-40-GT)-24h was not soluble in any solvent anymore which is explained by a high degree of crosslinking. Also thermal properties are acceptable, results from thermogravimetric analysis show an onset temperature of P-(GS-co-20-GT)-24h at 252.1 °C and a maximum degradation temperature of 428.8 °C. Melting temperatures and melting enthalpies are in the same range as for previous discussed samples.

Table 14: Overview of results from thermal analysis of P-(GS-co-GT) with varied reaction conditions

	TG		**DSC**				
Sample	**T_0 [°C]**	**T_{max} [°C]**	**T_{m1} [°C]**	**ΔH_{m1} [J/g]**	**T_{m2} [°C]**	**ΔH_{m2} [J/g]**	**$\Sigma \Delta H_m$ [J/g]**
P-(GS-co-30-GT)+vacuum	176.1	427.5	74.7	18.3	114.7	122.9	141.2
P-(GS-co-44-GT)+vacuum	220.7	424.3	84.9	0.6	109.2	1.0	1.6
P-(GS-co-20-GT)-24h	252.1	428.8	72.7	8.1	108.7	31.7	39.8
P-(GS-co-30-GT)-24h	263.5	428.2	69.1	2.1	95.2	2.9	5
P-(GS-co-40-GT)-24h	281.9	427.0	60.4	0.8	-	-	0.8

To have a practical application for these copolyesters, their ability of film formation was tested. Whereas the two samples synthesised with vacuum as well as P-(GS-co-30-GT)-24h and P-(GS-co-20-GT)-24h did not lead on to a film formation, the trial with P-(GS-co-40-GT)-24h was quite successful. It showed elastic properties and seems to be homogenous. Among prepared films of the P-(GS-co-GT) series it is by far the best one.

Figure 36 and 37 illustrate ^{1}H-NMR spectra of samples with varied reaction conditions.

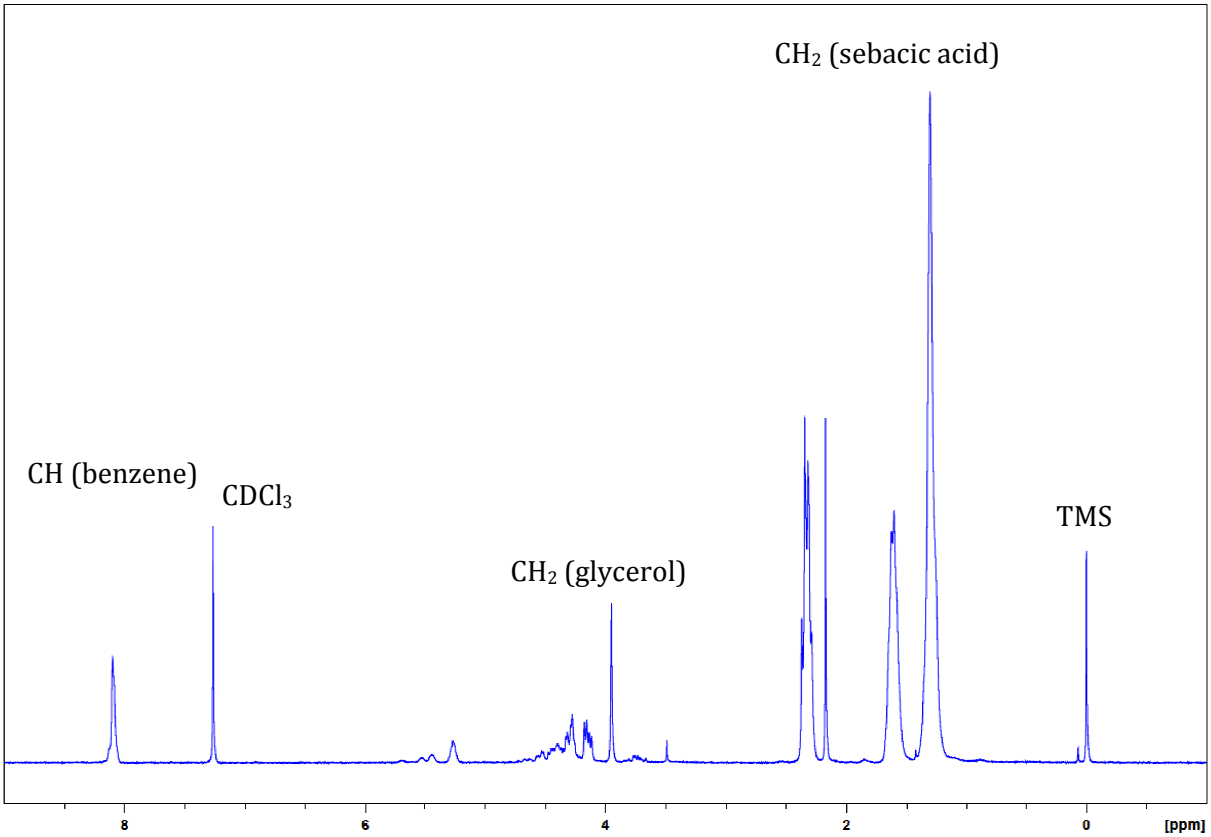

Figure 36: ^{1}H-NMR of P-(GS-co-44-GT) synthesised with vacuum

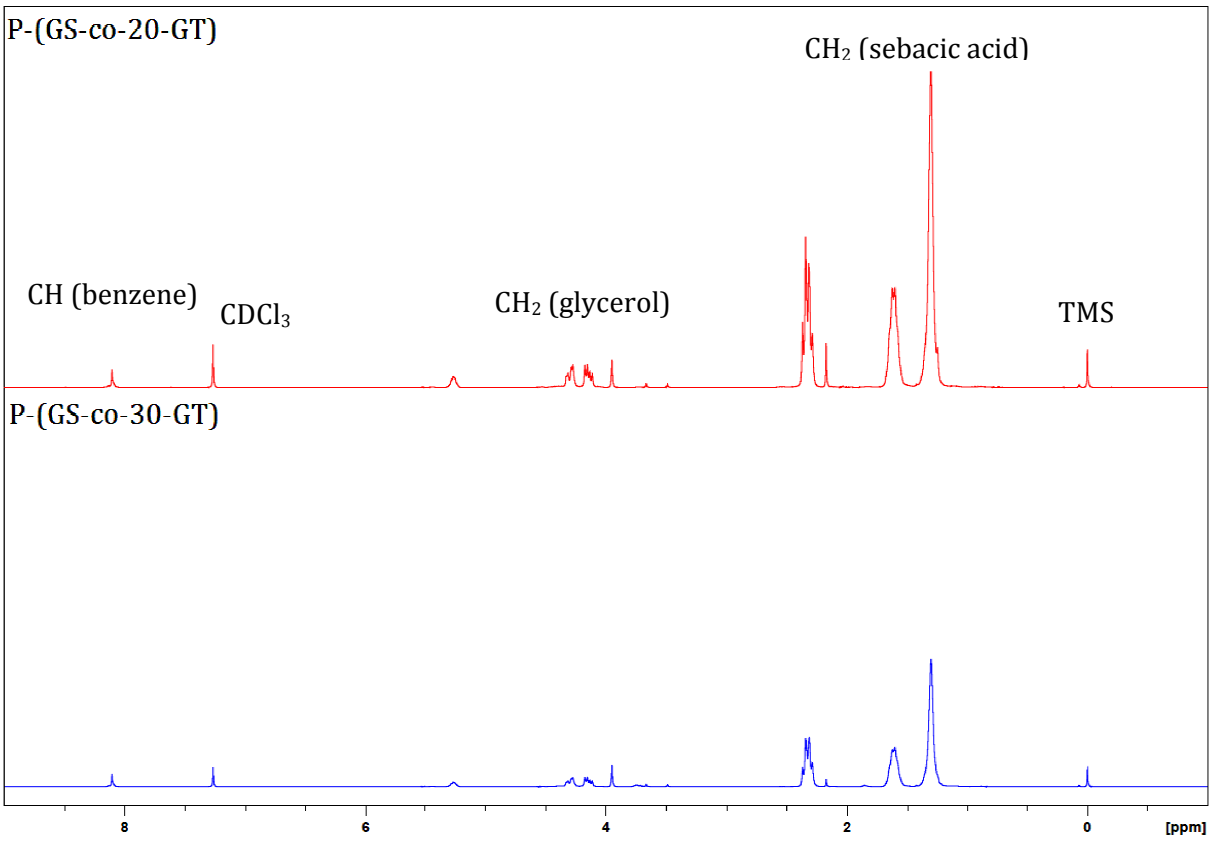

Figure 37: Comparison of ^{1}H-NMRs of P-(GS-co-20-GT) and P-(GS-co-30-GT)

ATR – IR spectra of samples with a reaction time of 24 hours are featured in Figure 38. The most important characteristic peaks are marked in the spectra, a precisely description is given in Table 5. Noticeable is the distinct second vibration band of the carbonyl group of P-(GS-co-30-GT)-24h and P-(GS-co-40-GT)-24h.

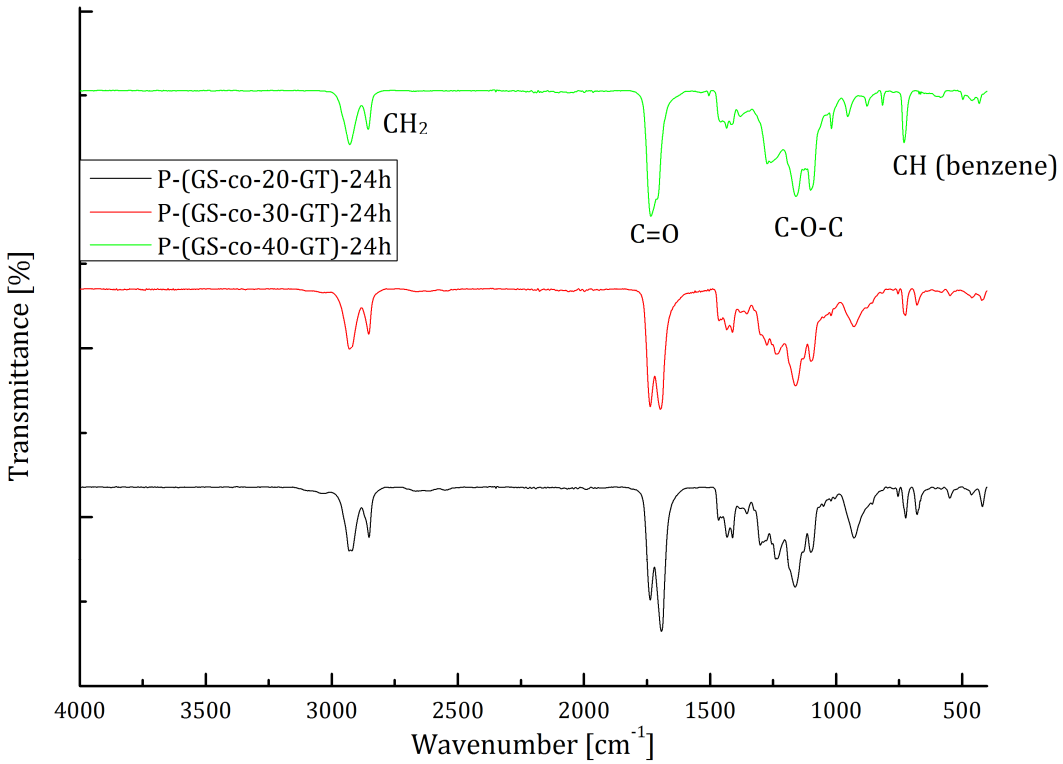

Figure 38: ATR-IR spectra of P-(GS-co-GT)-24h

Summary

In general it can be concluded that the task to synthesise thermoplastic copolyesters based on dimethyl terephthalate, sebacic acid and glycerol could be fulfilled. Furthermore, through varying several parameters including reaction time and conditions as well as glycerol concentration and DMT / SA ratios, also soluble polymers could be prepared which are suitable for the application as polymer matrix materials for drug encapsulation systems (see chapter 4.2). The best results with an overall glycerol concentration of ⅙ could be obtained with a DMT / SA ratio = 44 / 56 which is indicated by the highest molecular weight obtained. A longer reaction time and a higher glycerol concentration (¼) tend to lead to higher molecular weights. P-(GS-co-30-GT)-24h showed a molecular weight of 8411 g.mol^{-1}. The exact structure of prepared copolyesters could not be determined, but NMR and IR analyses proved that polycondensation worked and resulting products are random copolyesters. Thermal analysis revealed that synthesised copolyesters are semi – crystalline and thermoplastic.

4.2 Microparticles

The application of microparticles as drug encapsulation systems represents one of the most important topics in medicine concerning controlled drug delivery. Its aim is to ensure a tailored release of active substances during a defined period of time and therefore to simplify intake of pills. PLA is a very well investigated polymer matrix material for those particles and hence the following chapter deals with the comparison of particle size, encapsulation efficiency and drug release of different types of PLA and synthesised copolyesters.

Microparticles were prepared by a modified double emulsion method ($w_1/o/w_2$) similar to a procedure described by Rosca et al. [53] and a single emulsion technique (w/o) referring to Stloukal et al. [25]. As polymer materials for encapsulation with former method, 6 different kinds of PLA as well as 4 P-(GS-co-GT) copolyesters were accomplished. The second procedure was used for encapsulation with P-(BA-co-BT) as matrix material. Coumarin, a fragrant aromatic compound which is derived from many plants, e.g. from the tonka bean, was used as a model substance because it is UV-active. Furthermore triclosan, an antioxidant, was chosen as an example for a practical application and was therefore encapsulated by two of the most promising candidates of PLA.

4.2.1 Preparation technique

At the beginning five different single and double emulsion preparation techniques were tested and results of particle size distributions and images achieved by optical light microscopy were compared. It turned out, that different procedures provided best data depending on the polymer type used. So the two above described emulsion methods were chosen and work was continued with these procedures. The yields of obtained particles are given in Table 15.

Table 15: Overview of obtained particle yield

Sample	Yield [%]	Sample	Yield [%]
PLA	57	P-(GS-co-40-GT)	30
PLA 3051D	60	P-(GS-co-30-GT)	57
PLA/SA	40	P-(GS-co-50-GT)	42
PLA/SA/CY	78	P-(GS-co-44-GT)	36
PLA_0	79	P-(BA-co-30-BT)	44
PLA_5400	50	P-(BA-co-30-BT)+PEG	49
PLA-triclosan	47	P-(BA-co-30-BT)+TAIC	50
PLA/SA/CY-triclosan	35		

4.2.2 Size distribution

The size distribution of prepared microparticles was determined with static and dynamic light scattering, whereas dynamic light scattering was primarily used for smaller particles (< 1µm), especially P-(GS-co-GT) samples, and static light scattering for microparticles with bigger mean diameters (> 1 µm). In Table 16 is an overview of the particle size distributions of the prepared microparticles given.

Table 16: Overview of the obtained particle size distributions

	Dynamic light scattering		**Static light scattering**
Sample	**Mean diameter [nm]**	**PDI**	**Mean diameter in 90% volume [nm]**
PLA_0 [a]	3138	0.33	10634
PLA 3051D_0[a]	4772	0.23	13671
PLA/SA_0 [a]	4465	0.56	9435
PLA/SA/CY_0 [a]	923	0.77	8566
PLA [b]	-	-	7003 ± 1070
PLA 3051D [b]	-	-	7591 ± 165
PLA/SA [b]	-	-	5551 ± 156
PLA/SA/CY [a]	2398	0.46	4093
PLA_0 [b]	2080	0.31	-
PLA_5400 [a]	3736	0.84	10683
P-(GS-co-40-GT)[c]	655	0.68	-
P-(GS-co-30-GT) [c]	474	0.58	-
P-(GS-co-50-GT) [c]	222	0.28	-
P-(GS-co-44-GT) [c]	209	0.24	-
PLA-triclosan [b]	-	-	5415 ± 147
PLA/SA/CY-triclosan [b]	-	-	2541 ± 3
P-(BA-co-30-BT) [b]	-	-	15268 ± 2715
P-(BA-co-30-BT)+PEG [b]	-	-	16049 ± 456
P-(BA-co-30-BT)+1% TAIC [b]	-	-	35774 ± 3509

[a]: samples were measured with dynamic and static light scattering; [b]: samples only measured with static light scattering; [c]: samples only measured with dynamic light scattering

It is obvious that there are big differences between data analysed with both techniques, static and dynamic light scattering, whereas former method shows particle sizes which are at least twice as much as data achieved by latter one. One reason for this fact is that samples analysed

with dynamic light scattering were ultrasonicated for half an hour before the measurement. It is proven that ultrasonication leads to smaller particle sizes. Stloukal et al. [25] [54] reported in two of their papers that ultrasonication has the biggest impact on particle size distribution. They showed data deviations in the same range as in Table 16 presented. Other less important parameters are stirring speed and the addition of surfactants to the emulsion [25] [54] as well as the type of solvent evaporation. [29]

Furthermore Table 16 maintains the hypothesis that the encapsulated drug has no influence on the particle size, microparticles without coumarin or triclosan have nearly the same size as encapsulated ones. This phenomenon is already proven by several research groups, e.g. Stloukal et al. [25].

Another crucial fact regarding particle size distribution is the type of polymer used for microparticle preparation as well as the molecular weight. Particles prepared with P-(GS-co-GT) have by far the smallest mean diameter in a range between 200 and 655 nm. The main reason for that fact is the low molecular weight, which is between 1990 – 8370 $g.mol^{-1}$. However, PLA features particle sizes between 1 µm and 10 µm. There is no significant difference regarding size among the various types of PLA. P-(BA-co-BT) microparticles indeed exhibit by far the biggest mean diameters in a range of 15 – 35.8 µm. This can be explained on the one hand by the different preparation technique used and on the other hand by the notably higher molecular weight. The biggest mean diameters (35 µm) show microparticles prepared with P-(BA-co-30-BT)+1% TAIC copolyester, which is attributable to its higher crosslinking degree and a M_w of 40000 $g.mol^{-1}$. C.T. Brunner et al. [34] also investigated microparticles with P-(BA-co-BT) as polymer material and reached particle sizes in the same range as above presented whereas Stloukal et al. [54] published data of drug encapsulation systems based on commercial available "Ecoflex®" which are slightly lower between 2 – 9.5 µm.

Moreover images obtained by optical light microscopy (see Figure 39) ensure above presented data. It is also evident by means of these photos that prepared samples do not have a homogenous size distribution, there are always some bigger particles. This may be a result of employment of the high speed homogeniser. [28] Furthermore, some of them also tend to deploy aggregates, e.g. P-(GS-co-30-GT). However, optical light microscopy was also used to get a first impression and information of morphology of these microparticles. All of them feature desired spherical shape. For further characterisation concerning morphology some samples were investigated with scanning electron microscopy (see chapter 4.2.4).

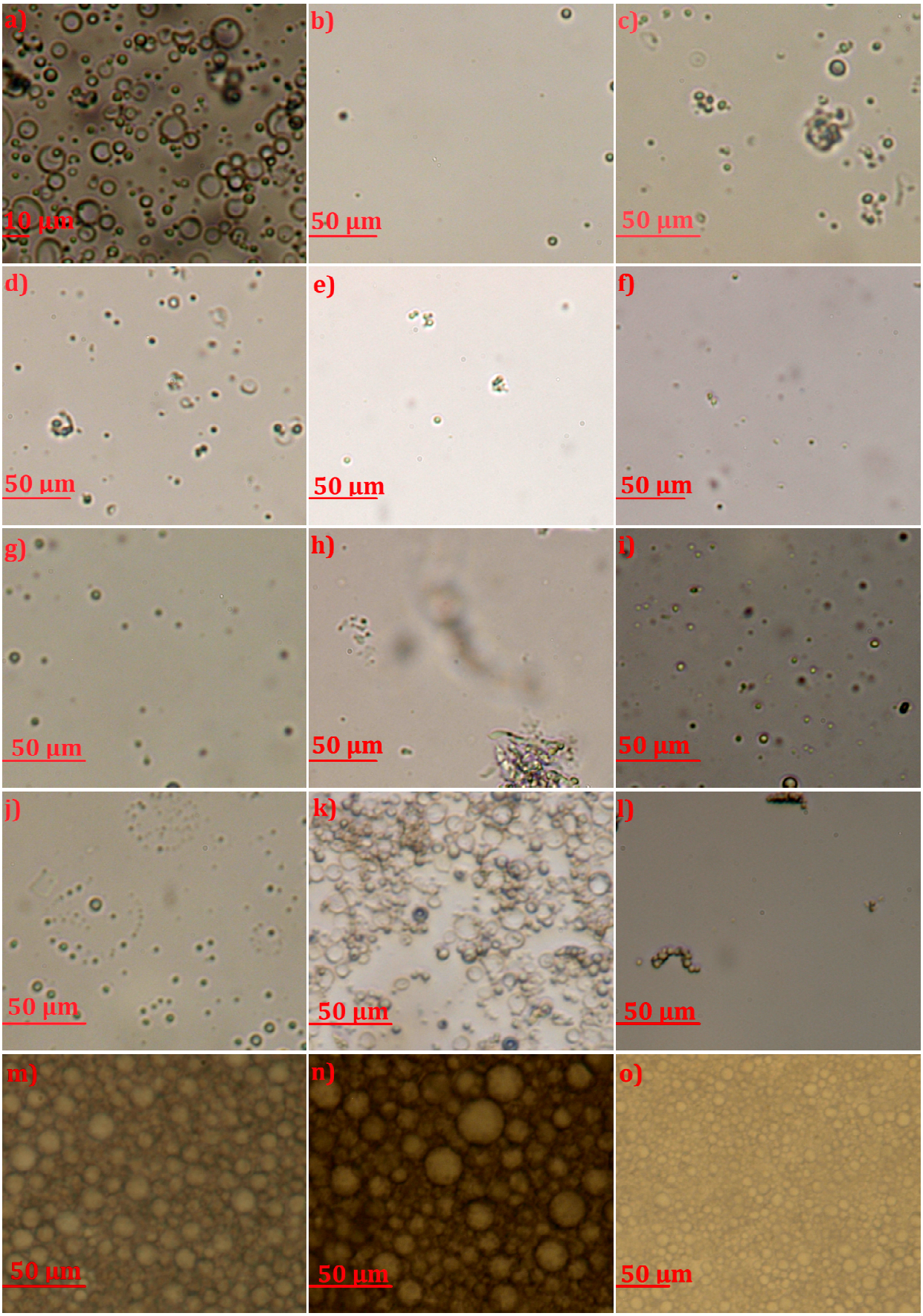

Figure 39: Images of prepared microparticles obtained from optical light microscopy
a) PLA; b) PLA-high molecular; c) PLA-COOH; d) PLA-COOH-SH; e) PLA-amorphous; f) PLA-crystalline; g) P-(GS-40-GT); h) P-(GS-co-30-GT); i) P-(GS-co-50-GT); j) P-(GS-co-44-GT); k) PLA-triclosan; l) PLA-COOH-SH-triclosan; m) P-(BA-co-30-BT); n) P-(BA-co-30-BT)+PEG; o) P-(BA-co-30-BT)+1% TAIC

4.2.3 Encapsulation efficiency

In Table 17 and 18 is an overview of the obtained encapsulation efficiencies (EE) measured by UV – VIS spectroscopy given. It is calculated according to equation 7.

$$EE = \frac{amount\ of\ encapsulated\ drug\ [g]}{amount\ of\ inital\ drug\ [g]} * 100\% \qquad (7)$$

Table 17: Encapsulation efficiency of microparticles including coumarin

Sample	Encapsulation efficiency [%]	Sample	Encapsulation efficiency [%]
P-(GS-co-40-GT)	63	PLA	63
P-(GS-co-30-GT)	54	PLA 3051D	65
P-(GS-co-50-GT)	58	PLA/SA	59
P-(GS-co-44-GT)	60	PLA/SA/CY	65
P-(BA-co-30-BT)	48	PLA_0	68
P-(BA-co-30-BT)+PEG	52	PLA_5400	54
P-(BA-co-30-BT)+TAIC	29		

Table 18: Encapsulation efficiency of microparticles including triclosan

Sample	Encapsulation efficiency [%]
PLA	53
PLA/SA/CY	39

Except three samples, all prepared microparticles show encapsulation efficiencies between 50 and 68 % which is in accordance with data published in literature for PLA as well as for P-(BA-co-BT). [25] [34] [55] It is obvious that encapsulation efficiency is in this case not influenced by the molecular weight in contrast to before discussed particle sizes and drug release. P-(GS-co-GT) microparticles with their rather low molecular weight show also efficiencies between 53 and 62 %. Due to the fact that entrapped drugs are very hydrophobic, triclosan even more than coumarin, active substances do not tend to get to the polar PVA – solution. [28] [34] Furthermore the evaporation of the organic solvent has a great impact on encapsulation efficiency. Removal under reduced pressure leads on the one hand to smaller particle sizes but on the other hand to lower encapsulation efficiencies while the evaporation at room temperature under mild stirring conditions (e.g. 500 rpm) shows the vice versa effect. [29]

4.2.4 Drug release

Drug release studies were performed in phosphate buffer saline solution, PBS, (pH = 7.4) at 37 °C under continuous stirring. These conditions were chosen to simulate bloodstream in human body. Measurements were performed using UV – VIS spectroscopy.

Following Figure 40 – 43 illustrate kinetic measurements of encapsulated coumarin respectively triclosan from various polymer matrix materials. The graphs are displayed as cumulative percentages of released drugs, whereas 100 % represents always the highest concentration of released substance. Process parameters were kept the same for all samples (double emulsion method), except P-(BA-co-BT) microparticles which were prepared by a simple emulsion method.

PLA belongs to the most used and investigated types of polymers concerning drug delivery systems. The reason for that is its proven biocompatibility regarding the human body and its ability of fast degradation. One of the main parameters concerning drug release from the microparticles is molecular weight beside the structural architecture of the used polymer. So we considered to investigate the influence of different kinds of end groups (COOH and COOH – SH) as well as the impact of crystallinity in relation with kinetic behaviour.

Figure 40 compares kinetics of different types of PLA. It can be observed from the plot that after one week 80 % of the overall released amount of drug is diffused into solution, except untreated high molecular PLA 3051D and amorphous PLA_0 which feature a value of about 60 %. Microparticles prepared with unmodified PLA (M_w = 35600 g.mol^{-1}) show a slower release behaviour than the two functionalised samples, whereas PLA/SA exhibits a burst release, because it vacates 43 % of the total amount of coumarin released during the first 24 hours. In comparison, the second modified sample, PLA/SA/CY (M_w = 10200 g.mol^{-1}), does not show such a great burst release, it even frees coumarin in the first 48 hours slower than normal PLA. This can be explained by the higher polarity of carboxyl groups in comparison to thiol end groups. It also seems that crystalline PLA_5400 produces a kind of burst release because it submits 60 % within the first 24 hours into the PBS solution. Hence it can be concluded that a higher crystallinity leads to a faster drug release compared with amorphous and untreated high molecular PLA 3051D. Izumikawa et al.[57] explained this phenomenon by the assumption that crystalline particles have a rougher surface and therefore a larger surface area than amorphous particles which implies a simplified diffusion of the drug into the surrounding medium. Nevertheless these results are quite controversial, because there are also investigations which show that amorphous PLA samples feature a faster drug release than crystalline ones. [28]

The phenomenon that most of the drug is released in the beginning of measurements (mostly during the first 100 hours) can be explained by the assumption that active substance located in the boundary layers of the particles is able to diffuse quite easily into the solution. The transportation into the boundary layers is performed by the organic solvent during the evaporation step. Because the solvent with the dissolved drug acts as a driving force towards water phase, the active substance is therefore carried to the particles boundary layer respectively its surface or the PVA – solution. [28] Since drug residues at the particles surface should be mostly removed by the methanol / water solution used during the washing steps, burst release has to occur due to high concentrations of the drug in the boundary layers.

In general, drug release is performed in a process consisting of three main steps. [28] [35] In the beginning above described burst release takes place which is followed by a lag phase and finally concluded by an again accelerated process due to polymer degradation. During the lag phase release of the active substance is decelerated by the diffusion through the polymer which needs of course more time than release from the outer spheres. This step is mainly depending on molecular weight as well as on the type of end groups of the polymer. Considering this assumptions lag phases can be avoided by using low molecular weight polymers or polymer blends. The third step, the degradation process, is controlled by erosion. However, until this mechanism occurs it takes a period of time which is again dependent on molecular weight and structural properties of the polymer. [28]

In the case of present studies lag phases are not clearly visible from the plotted graphs, but more or less all samples show time periods, were the slope is flatter and then again steeper, so the rate of diffusion is not constant. The third step of above described process could not be reached for all samples, since polymer degradation takes in particular cases more time than samples were measured.

Particle size has no influence concerning differences in drug release between various PLA samples, because all of them have nearly the same size (7 – 13 µm). Usually smaller particles show faster release behaviour than bigger particles, since the drug does not have to diffuse so far through the polymer until it reaches the surrounding medium. [25] [28] [35] [56]

Another crucial parameter is porosity of the polymer. The more pores the surface of a microparticle has, the easier the surrounding solution can get into the particle and therefore drug release is accelerated. [28] [35]

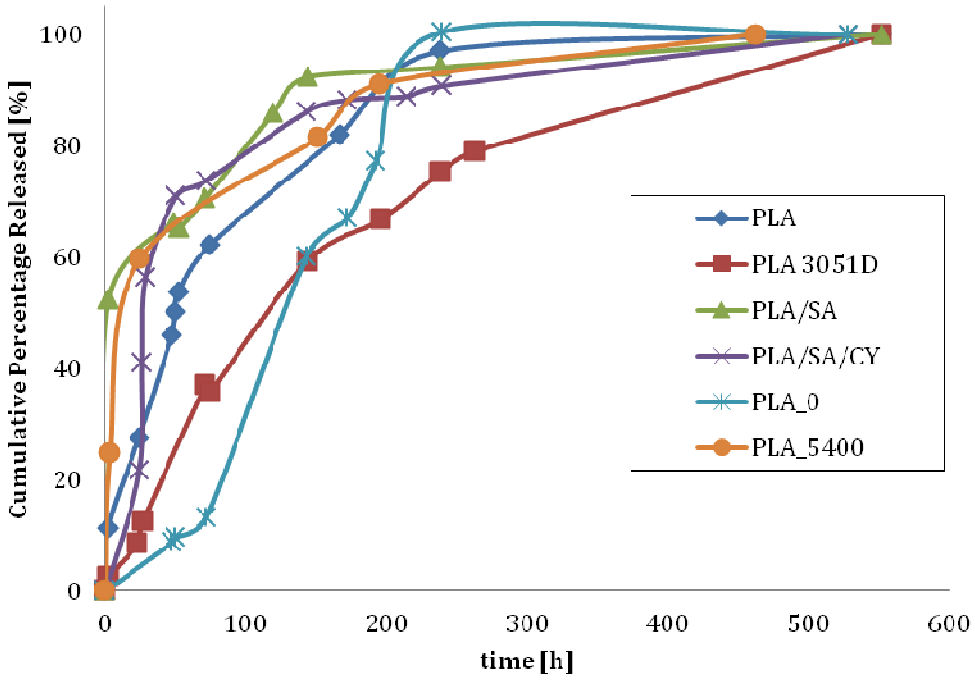

Figure 40: Drug release of different types of PLA with encapsulated coumarin in PBS (pH=7.4) at 37 °C

Figure 41 shows the drug release as a function of time for microparticles prepared with P-(GS-co-GT) copolyesters as polymer material and coumarin as encapsulated drug. The four samples exhibit quite different kinetics. Whereas in P-(GS-co-44-GT) coumarin diffusion is completed after 200 hours, P-(GS-co-40-GT) needs plenty more time, although it shows the biggest burst release of P-(GS-co-GT) particles. The slowest drug release can be afforded with P-(GS-co-30-GT). In general, microparticles with these copolyesters show more or less the same release behaviour as PLA samples, although they have a completely different molecular architecture, molecular weight and particle size distribution.

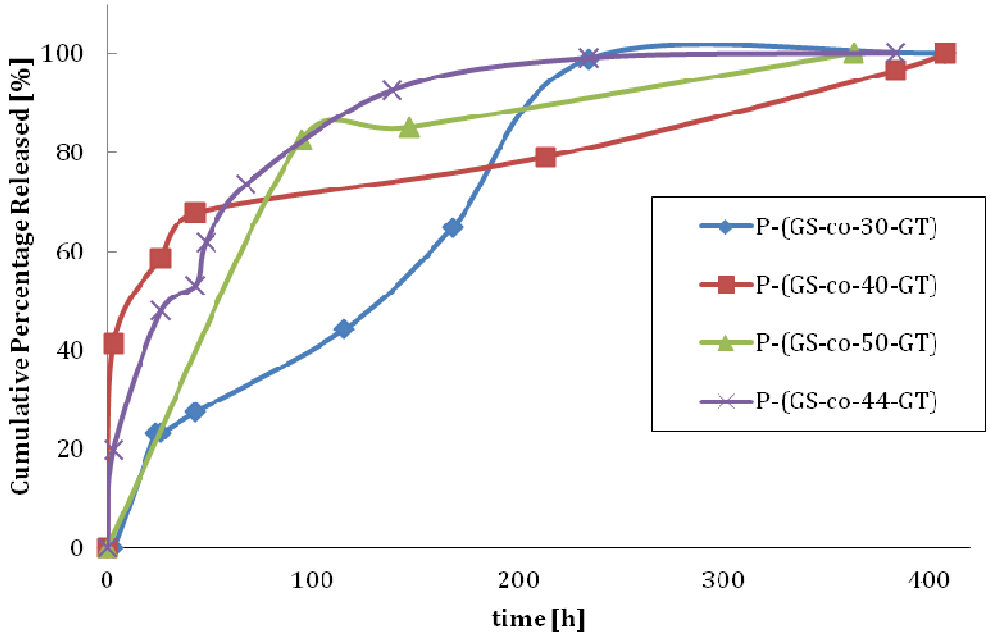

Figure 41: Drug release of P-(GS-co-GT) microparticles with encapsulated coumarin in PBS (pH=7.4) at 37°C

Drug release kinetics of P-(BA-co-BT) microparticles are shown in Figure 42. It is obvious that the release of P-(BA-co-30-BT)+PEG is in the beginning about twice as high as of unmodified P-(BA-co-30-BT). For both samples a burst release can be evidenced, with a loss of active substance of 65 % respectively 55 % during the first 100 hours. Hence the improvement of the degree of hydrolysis due to the implementation of PEG into the polymer is successfully proven. The shape of release kinetics is for both samples the same. In contrast to that, crosslinked P-(BA-co-30-BT) + TAIC microparticles only release about 25 % of the drug in the beginning. These values are comparable to P-(GS-co-30-GT) and P-(GS-co-50-GT). Only amorphous PLA and PLA-3051D reveal a slower release in the initial stage. But in comparison to them the following release progress is completely different. Following the little burst release a kind of lag phase occurs which lasts for about 12 days. Subsequently at the end of the second week of measurements there is a steep increase in drug release observable and after 16 days about 80 % of overall released substance is diffused into the surrounding medium. This can be explained by the beginning of polymer degradation and hence drug release is no longer only driven by diffusion but also by erosion. In contrast, all other samples have more or less completed their release after two weeks. So P-(BA-co-30-BT)+ TAIC is a possible matrix material for drug encapsulation systems working over a long period of time.

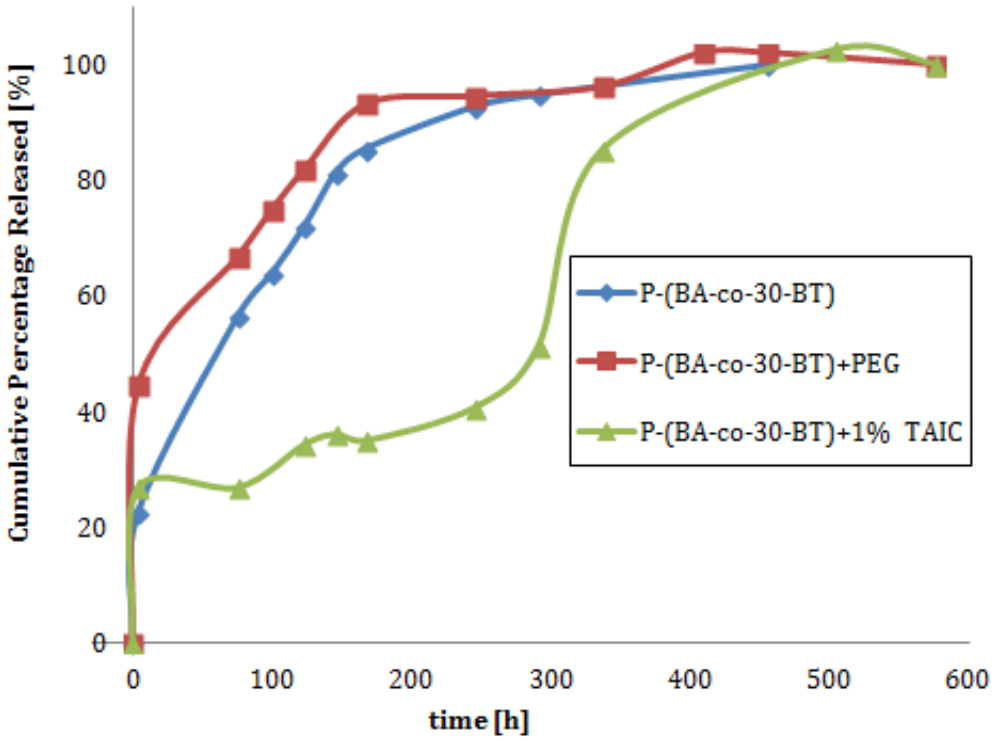

Figure 42: Drug release of P-(BA-co-BT) microparticles with encapsulated coumarin in PBS (pH=7.4) at 37 °C

Kinetics of encapsulated triclosan, an even more hydrophobic drug than coumarin, with PLA and thiol end capped PLA as polymer materials are shown in Figure 43. After one week PLA/SA/CY has released about 80 % of triclosan whereas for PLA a value of about 65 % can be observed. Hence, it can be concluded that PLA with encapsulated triclosan frees the active substance a little bit slower than with coumarin, whereas for thiol end capped PLA no significant differences could be observed. However, it could be indicated, that the polarity of encapsulated drug has at least a little impact on release kinetics. Berkland et al. [55] also revealed the same results in their studies.

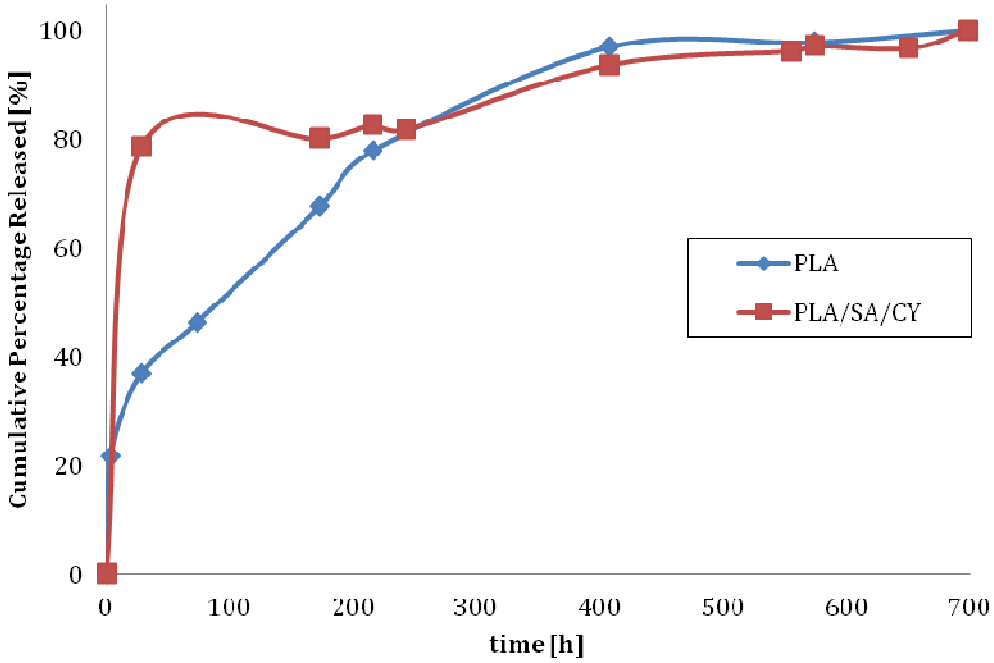

Figure 43: Drug release of PLA and PLA-COOH-SH microparticles with encapsulated triclosan in PBS (pH=7.4) at 37 °C

In Figure 44 are pictures made by scanning electron microscopy of PLA microparticles shown. Thereby the spherical shape of the particles, which was already determined by images taken with the optical light microscope, is proven. Moreover, indicated particle sizes can be confirmed as well. Nevertheless it is not possible to make a clear statement concerning porosity of the particles. It seems that there are little pores on the surface which would also explain the fast release in the beginning of kinetics measurements, but this cannot be claimed explicitly.

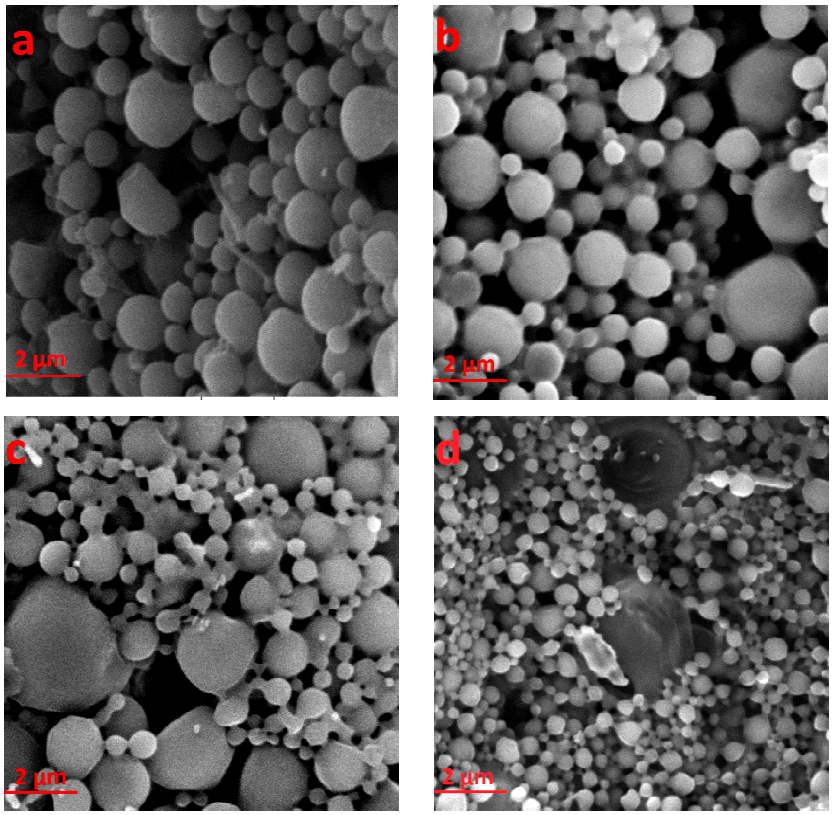

Figure 44: SEM images of microparticles with different types of PLA
a) PLA; b) PLA-high molecular; c) PLA-COOH; d) PLA-COOH-SH

Summary

From presented results it can be concluded that encapsulation of coumarin and triclosan with PLA, P-(BA-co-30-BT) and P-(GS-co-GT) worked. All particles have a spherical shape which could be determined by optical light microscopy and SEM. Moreover, particle size distributions are strongly dependent on the type of polymer used. P-(GS-co-GT) microparticles showed the lowest mean diameters (200 – 655 nm), followed by PLA particles (1 – 10 µm) and P-(BA-co-30-BT) copolyesters (15 – 35.8 µm), whereas latter ones were prepared with a simple emulsion method and former ones by a double emulsion technique. Encapsulation efficiencies are adequate with values of most samples of 50 – 68 % in comparison to data published in the literature. Besides, drug releases show that most of the active substance diffuses at the beginning of measurements into surrounding PBS solution. All samples feature at least a slight burst release. After 200 hours, most samples have released about 80 % of overall substance. Altogether it can be summarised that the purpose of the study has been fulfilled to create encapsulation systems with various polymer types for different requirements concerning drug release profiles.

5. Conclusion

The aim of this master thesis was to synthesise and modify new copolyesters via polycondensation reactions. Poly (butylene adipate – co – butylene terephthalate) copolyesters were successfully synthesised with an amount of 30 mol% BT. These copolyesters were altered with PEG to achieve a more hydrolysable polymer and with triallyl isocyanurate to establish a crosslinked network. Moreover, crosslinking resulted in an increase of molecular weight of about ⅓, whereas there is no significant influence regarding thermal properties. Structural analysis confirms the synthesis of random copolyesters.

Poly (glycerol sebacate – co – glycerol terephthalate) copolyesters were synthesised under various reaction conditions and with different ratios of DMT / SA and glycerol. The aim to prepare soluble and thermoplastic copolyesters could be succeeded through the reduction of the amount of glycerol and hence molecular weights obtained were not exceeding 8400 $g.mol^{-1}$. Prolongation of the reaction time tend to result in higher average molecular weights, although theoretical glycerol-terephthalate fraction was lower, e.g. P-(GS-co-44-GT)+¼ glycerol showed a M_w of 4830 $g.mol^{-1}$ and P-(GS-co-20-GT)-24h a M_w of 5440 $g.mol^{-1}$. Furthermore, thermal analysis confirmed semi – crystalline polymers and an increase of maximal degradation temperature of about 200 °C when syntheses were carried out under reduced pressure or with a prolonged reaction time. Molecular structure of these copolyesters could not be determined in detail, but NMR and IR analysis indicated that random copolyesters were successfully synthesised.

Furthermore, prepared microparticles showed that the particle size distribution is strongly dependent on the molecular architecture and the molecular weight of the used polymer matrix material. Investigations revealed a trend that mean diameters of P-(GS-co-GT) < PLA < P-(BA-co-BT). Besides, drug encapsulation efficiencies are in a range of 50 – 68 % for all particles. Studies concerning the behaviour of drug release show, that all samples undergo a burst release, whereas this phenomenon is significantly more distinct in microparticles with a lower molecular weight or more polar functional end groups of the polymer. Moreover, drug release can be decelerated by the usage of crosslinked polymers. Most samples have about 80 % of overall amount of drug released within 200 hours. The greatest deviations concerning drug release can be attributed to crystallinity differences of PLA. Aside from that, it is impossible to say which preparation method is the best in this case, because both reveal the same results. In general, it depends on the type of application and required properties to select an ideal polymer for the preparation of drug encapsulation systems.

Highlights

- Comparison of drug encapsulation systems of common PLA with P-(BA-co-BT) and P-(GS-co-GT) to invent new possibilities of polymer matrix materials for an even better controlled and exact drug release

- Synthesis of copolyester with a higher hydrophilicity based on P-(BA-co-BT) to achieve a faster drug release

- Synthesis of crosslinked copolyesters based on P-(BA-co-BT) to gain better mechanical properties and a decelerated drug release

- Synthesis of new P-(GS-co-GT) copolyesters with thermoplastic properties and the ability of dissolution in several solvents. Therefore it could be confirmed, that this type of copolyesters represents an interesting alternative to PLA as polymer matrix material for a controlled drug release.

- Drug release depends not only on functional groups present in polyesters but also on other parameters such as molecular weight properties and the degree of crystallinity

- Particle size distribution of prepared microparticles is mainly dependent on molecular weight and the type of emulsion method used

6. Appendix

6.1 List of abbreviations

$CHCl_3$	Chloroform
DMSO	Dimethyl sulfoxide
DMT	Dimethyl terephthalate
DSC	Differential Scanning Calorimetry
GPC	Gel permeation chromatography
IR	Infrared spectroscopy
M_n	Number average molar mass
M_w	Mass average molar mass
NMR	Nuclear magnetic resonance
P-(BA-co-BT)	Poly (butylene adipate – co – butylene terephthalate)
P-(GS-co-GT)	Poly (glycerol sebacate – co – glycerol terephthalate)
PDI	Polydispersity index
PEG	Polyethylene glycol
PLA	Poly (lactic acid)
PVA	Polyvinyl alcohol
SA	Sebacic acid
SEM	Scanning electron microscopy
STA	Simultaneous Thermal Analysis
TAIC	Triallyl isocyanurate
TBOT	Tetrabutyl orthotitanate
T_m	Melting temperature
TG	Thermogravimetric analysis
THF	Tetrahydrofuran

6.2 List of figures

6.3 List of tables

6.4 References

[1] Gan, Z.; Kuwabara, K.; Yamamoto, M.; Abe, H.; Doi, Y. *Polym. Degrad. Stab.* **2004**, 83, 289-300

[2] Siegenthaler, K. O.; Künkel, A.; Skupin, G.; Yamamoto, M. In *Advances in Polymer Science: Synthetic Biodegradable Polymers*; Rieger, B.; Künkel, A.; Coates, G. W.; Reichardt, K.; Dinjus, E.; Zevaco, T. A. Eds.; Springer Verlag: Berlin Heidelberg, **2012**; pp 517-532

[3] Vroman, I.; Tighzert, L; *Materials* **2009**, 2, 307-344

[4] Witt, U.; Yamamoto, M.; Seeliger U.; Müller, R.-J.; Warzelhan, V. *Angew. Chem. Int. Ed.* **1999**, 38, 1438-1441

[5] Edlund, U.; Albertsson A.-C. *Adv. Drug Del. Reviews* **2003**, 55, 585-609

[6] Okada, M. *Prog. Polym. Sci.* **2002**, 27, 87-133

[7] Drobny, J. G. *Handbook of Thermoplastic Elastomers*; Elsevier Inc., **2007**; pp 215-234

[8] Drobny, J. G. *Handbook of Thermoplastic Elastomers*; Elsevier Inc., **2007;** pp 249-264

[9] Witt, U.; Müller R.-J.; Deckwer W.-D. *J. Environ. Polym. Deg.* **1997**, 5, 81-89

[10] Lim, L.-T.; Auras, R.; Rubino, M. *Prog. Polym. Sci.* **2008**, 33, 820-852

[11] Avérous, L. In *Monomers, Polymers and Composites from Renewable Resources;* Belgacem M.N.; Gandini, A., Eds.; Elsevier Inc., **2008**; pp 433-450

[12] Maharana, T.; Mohanty, B.; Negi, Y. S. *Prog. Polym. Sci.* **2009**, 34, 99-124

[13] Jiang, L.; Zhang, J. In *Handbook of Biopolymers and Biodegradable Plastics;* Ebnesajjad, S., Ed.; Elsevier Inc., **2013**; pp 109-128

[14] Rudnik, E. In *Handbook of Biopolymers and Biodegradable Plastics;* Ebnesajjad, S., Ed; Elsevier Inc., **2013**; pp 189-211

[15] Pavelkova, A.; Kucharczyk, P.; Stloukal, P.; Koutny, M.; Sedlarik, V. *Polym. Adv. Technol.* **2014**, 25, 595-604

[16] Philip, S.; Keshavarz, T.; Roy, I. *J. Chem. Technol. Biotechnol.* **2007**, 82, 233-247

[17] Chodak, I. In *Monomers, Polymers and Composites from Renewable Resource;* Belgacem M.N.; Gandini, A., Eds.; Elsevier Inc. **2008**; pp 451-477

[18] Fujimaki, T. *Polym. Degrad. Stab.* **1998**, 59, 209-214

[19] Chandra, R.; Rustgi, R. *Prog. Polym. Sci.* **1998**, 23, 1273-1335

[20] Müller, R.-J.; Kleeberg, I.; Deckwer, W.-D. *J. Biotechnol.* **2001**, 86, 87-95

[21] Lucas, N.; Bienaime, C.; Belloy, C.; Queneudec, M.; Silvestre, F.; Nava-Saucedo, J.-E. *Chemosphere* **2008**, 73, 429-442

[22] Witt, U.; Einig, T.; Yamamoto, M.; Kleeberg, I.; Deckwer, W.-D.; Müller, R.-J. *Chemosphere* **2001**, 44, 289-299

[23] http://www.oecd-ilibrary.org/environment/test-no-207-earthworm-acute-toxicity-tests_9789264070042-en [accessed Oct 24, 2014]

[24] Lassalle, V.; Ferreira, M. L. *Macromol. Biosci.* **2007**, 7, 767-783

[25] Stloukal, P.; Kucharczyk, P.; Sedlarik, V.; Bazant, P.; Koutny, M. *J. Agric. Food Chem.* **2012**, 60, 4111-4119

[26] Chattopadhyay, P.; Huff, R.; Shekunov, B.Y. *J. Pharm. Sci.* **2006**, 95, 667-679

[27] Dong, W.; Bodmeier, R. *Int. J. Pharm.* **2006**, 326, 128-138

[28] Wischke, C.; Schwendemann, S.P. *Int. J. Pharm.* **2008**, 348, 298-327

[29] Hnaien, M.; Ruffin, E.; Bordes, C.; Marcillat, O.; Lagarde, F.; Jaffrezic-renault, N.; Briancon, S. *Eur. J. Pharm. Biopharm.* **2011**, 78, 298-305

[30] Mathiowitz, E. *Encyclopaedia of Controlled Drug Release*; Wiley and Sons, **1999**, 1-2, pp 494

[31] Phromsopha, T.; Baimark, Y. *Int. J. Biomat.* **2014**, 1-6

[32] Rouzes, C.; Leonard, M.; Durand, A.; Dellacherie, E. *Colloids Surf., B.* **2003**, 32, 125-135

[33] Papadimitriou, S.; Bikiaris, D. *J. Controlled Release* **2009**, 138, 177-184

[34] Brunner, C. T.; Baran, E. T.; Duarate Pinho, E.; Reis, R. L.; Neves, N. M. *Colloids Surf., B.* **2011**, 84, 498-507

[35] Freiberg, S.; Zhu, X. X. *Int. J. Pharm.* **2004**, 282, 1-18

[36] Müller, R. H.; Jacobs, C.; Kayser, O. *Adv. Drug Delievery Reviews* **2001**, 47, 3-19

[37] Jäger, A.; Gromadzki, D.; Jäger, E.; Giacomelli, F. C.; Kozlowska, A.; Kobera, L.; Brus, J.; Rihova, B.; Fray, M. E.; Ulbrich, K.; Stepanek, P. *Soft Matter* **2012**, 8, 4343-4354

[38] Gao, H.; Wang, Y.; Fan, Y.; Ma, J. *J. Controlled Release* **2005**, 107, 158

[39] Bilati, U.; Allémann, E.; Doelker, E. *AAPS Pharm. Sci. Technol.* **2005**, 6, 594

[40] Berger, K.; Gregorova, A. *J. Appl. Polym. Sci.* **2014**, 131, 41105

[41] Milinkovic, A.; Pastorek, M.; Gregorova, A. *Thermochim. Acta* **2014**, 590, 40-50

[42] http://www.micchem.com/triallyl_isocyanurate.html [accessed: Oct. 29, 2014]

[43] Yang, S.-L.; Wu, Z.-H.; Yang, W.; Yang, M.-B. *Polym. Test.* **2008**, 27, 957-963

[44] Elias, H.-G. *Makromoleküle Band 1*, Wiley-VCH: Weinheim, **1999**; pp 417-486

[45] Hesse, M.; Meier, H.; Zeeh, B. *Spektroskopische Methoden in der organischen Chemie*; G. Thieme Vlg.: Stuttgart, **1997**;

[46] Socrates, G. *Infrared Characteristic Group Frequencies*; Wiley Verlag: Chichester, **1998**;

[47] Weng, Y.-X.; Jin, Y.-J.; Meng, Q.-Y.; Wang, L.; Zhang, M.; Wang, Y.-Z. *Polym. Test.* **2013**, 32, 918-926

[48] Kijchavengkul, T.; Auras, R.; Rubino, M. *Polym. Test.* **2008**, 27, 55-60

[49] Elias, H.-G. *Makromoleküle Band 2*; Wiley-VCH: Weinheim, **2001;** pp 517-532

[50] Wang, Y.; Ameer, G. A.; Sheppard, B. J.; Langer, R. *Nature Biotechnol.* **2002**, 20, 602-606

[51] Nagata, T. *J. Appl. Polym. Sci.* **1969**, 13, 2601-2619

[52] Poth, U. *Polyester und Alkydharze*; Vincentz Network GmbH: Hannover, **2005;** pp 102-104

[53] Rosca, I. D.; Watari, F.; Uo, M. *J. Controlled Release* **2004**, 99, 271-280

[54] Stloukal, P.; Sedlarik, V.; Husarova, L.; Kasparkova, V.; Koutny, M. Int. Conference on Developement, Energy, Environment, Economics **2010**, 122-127

[55] Berkland, C.; King, M.; Cox, A.; Kim, K.; Pack, D.-W. *J. Controlled Release* **2002**, 82, 137-147

[56] Berkland, C.; Kipper, M. J.; Narasimhan, B.; Kim, K.; Pack, D.-W. *J. Controlled Release* **2004**, 94, 129-141

[57] Izumikawa, S.; Yoshioka, S.; Aso, Y.; Takeda, Y. *J. Controlled Release* **1991**, 15, 133-140

[58] Tang, J.; Mohan, T.; Verkade, J. G. *J. Org. Chem.* **1994**, 59, 4931-4938

[59] Herrera, R.; Franco, L.; Rodríguez-Galán, A.; Puigallí, J. *J. Polym. Sci. Pol. Chem.* **2002**, 40, 4141-4157

[60] Matsumoto, A.; Hirao, C.; Miyata, K.; Aota, H.; Takayama, Y.; Toridome, H. *Polym. J.* **2002**, 34, 558-561