Gel permeation chromatography methods in the analysis of lactide-based polymers

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ABSTRACT

In the literary part of this Master's thesis, the basics of polymer chemistry are discussed. Especially, the lactide-based polymers and their manufacturing routes are emphasized together with the applications and analyzing methods. These polymers are commonly used in the medical field and, for example, the degradation properties of the polymer are important to know to have an approval to use the material in medical devices. The follow-up of the degradation of those polymers can be performed by using gel permeation chromatography (GPC) device. The principles of the GPC are covered and also different ways to analyze the GPC data (the conventional calibration, triple detection, and universal calibration I method) are discussed. Additionally, the basics of viscometry are outlined.

In the experimental part of this thesis, lactide-based polymer samples (PLLA, PLGA, and PLCL) with different inherent viscosities and thus with different molar masses were studied with the help of GPC. The target was to find out how the analyzing method affects the results. The measured average molar masses (M_n, M_p, and M_w) and polydispersity (PD) of the samples were determined with the universal calibration I, conventional calibration, and triple detection methods. In the universal calibration I method, the Mark-Houwink (M-H) parameters from the literature were utilized. Also, the material-specific M-H parameters together with the dn/dc values were calculated using the triple detection method. These parameters were generally comparable to the literature values and, with them, the comparison between the materials was performed. In addition, the inherent viscosities of the samples were measured with microviscometer and the results were in the ranges informed by the manufacturers. The correlation between molar mass and inherent viscosity was formed.

When comparing the molar mass average and PD results, it could be seen that there was a notable difference in the results analyzed with different methods. For this reason, the results obtained from different methods were not necessarily comparable. There was also a notable difference observed when the results were analyzed twice with the universal calibration I method but with different M-H parameters. Therefore, the M-H parameters greatly affected the results. Also, a correlation between the inherent viscosity and some measured values (such the M-H α parameters) was observed. Thus, convention to inform only one value for the material ignoring the inherent viscosity is questionable. This is noteworthy, especially when the degradation of the polymers is on focus.

TIIVISTELMÄ

Tutkielman kirjallisuusosiossa tutustutaan polymeerikemian perusteisiin sekä erityisesti laktidipohjaisiin polymeereihin, niiden valmistamiseen, käyttökohteisiin ja analyysimenetelmiin. Kyseisiä polymeereja hyödynnetään esimerkiksi erilaisissa lääketieteellisissä sovelluksissa, mikä asettaa polymeereille monia vaatimuksia esimerkiksi niiden hajoamisen suhteen. Polymeeriketjujen pilkkoutumista voidaan seurata esimerkiksi geelipermeaatiokromatografilaitteistolla (GPC), jonka toimintaperiaate esitetään. GPC:llä on mahdollista käyttää erilaisia tulosten analyysimenetelmiä, joita myös tarkastellaan (konventionaalinen kalibrointi, universaali kalibrointi ja triple detection –menetelmä). Lisäksi kirjallisuusosiossa tutustutaan viskometrian perusteisiin.

Tutkielman kokeellisessa osassa (erikoistyö) tutkittiin GPC-laitteistolla laktidipohjaisia polymeerinäytteitä (PLLA, PLGA ja PLCL), jotka erosivat sisäisten viskositeettiensa ja näin myös moolimassojensa suhteen. Tarkoituksena oli tutkia, vaikuttiko analyysimenetelmä saatuihin tuloksiin. GPC:llä määritettiin näytteiden keskimääräiset molekyylimassat (M_n, M_p ja M_w) sekä polydispersiteetti (*PD*) ja tulokset analysoitiin kolmella eri menetelmällä (konventionaalinen kalibrointi, universaali kalibrointi I ja triple detection –menetelmä). Universaalissa kalibroinnissa käytettiin kirjallisuuden Mark-Houwinkin vakioita. Samalla selvitettiin triple detection -menetelmällä materiaalille ominaisia Mark-Houwinkin vakioita ja *dn/dc*-arvoja. Saadut tulokset olivat yleisesti verrattavissa kirjallisuusarvoihin ja niitä hyödynnettiin myös materiaalien keskinäisessä vertailussa. Lisäksi näytteiden sisäiset viskositeetit mitattiin mikroviskometrilla ja saadut tulokset olivat valmistajien ilmoittamien arvojen rajoissa. Samalla muodostettiin korrelaatio moolimassan ja sisäisen viskositeetin välille.

Vertailtaessa eri menetelmillä analysoituja keskimääräisiä moolimassoja ja polydispersiteettejä havaittiin, että tuloksissa oli huomattavia eroja. Tästä syystä eri menetelmällä analysoidut GPC-tulokset eivät välttämättä olleet vertailukelpoisia. Lisäksi havaittiin, että universaalissa kalibroinnissa käytetyillä Mark-Houwinkin vakioilla oli suuri vaikutus saatuihin tuloksiin. Tuloksista huomattiin myös, että osa mitatuista parametreistä (muun muassa α-parametri) muuttui näytteiden sisäisen viskositeetin muuttuessa, joten tapa ilmoittaa vain yksi parametrin arvo riippumatta näytteen sisäisestä viskositeetista on kyseenalainen. Tämä on merkittävä huomio etenkin silloin, kun halutaan seurata polymeerin hajoamista.

PREFACE

This thesis was done during the spring and summer 2018. It was carried out in Tampere at Arctic Biomaterials Oy premises. The thesis was supervised by Dr. Niina Ahola and M.Sc. Heikki Siistonen from the Arctic Biomaterials Oy, and professor Raimo Alén from the University of Jyväskylä, Department of Chemistry.

The most of the journal references were found with the help of Google Scholar and Google – search engines. The information about the devices (GPC and microviscometer) together with their functioning was found from the internet websites of several manufacturers (Agilent Technologies, Anton Paar, Malvern, Waters, PSS, Phenomenex, Vornia, Polyanalytik, Polymerlabs, Schott, WGE Dr Bures). The information was collected with the Google search engine. Also different webinars (PSS, Malvern) were utilized. When drawing the figures, ChemSketch and Microsoft Office Powerpoint were exploited.

I would like to thank my supervisors Niina, Heikki, and Raimo for an interesting research subject and helpful advice. Your expertise was priceless not only in writing but also in the laboratory. I also want to thank the whole team in ABM for an inspiring atmosphere in which it was pleasant to work. I want to thank my friends and I am also grateful to my family, especially to my mum and dad Leena and Veikko, who have always supported me. Last, I want to thank Lauri for love and encouragement.

TABLE OF CONTENTS

ABBREVIATIONS

ABM	Arctic Biomaterials Oy
ATP	Adenosine triphosphate
CCD	Chemical composition distribution
CL	ε-Caprolactone (2-oxepanone)
DLS	Dynamic light scattering
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
dn/dc	Refractive index increment
DP	Differential pressure or degree of polymerization
FDA	US Food and Drug Administration
FTD	Functionality type distribution
GA	Glycolic acid
GFC	Gel filtration chromatography
GPC	Gel permeation chromatography
GRAS	Generally recognized as safe
HMDI	Hexamethylene diisocyanate
IP	Inlet pressure
IUPAC	International Union of Pure and Applied Chemistry
LAB	Lactic acid bacteria
LALS	Low-angle light scattering
LS	Light scattering
LVN	Limiting viscosity number
MALS	Multi-angle light scattering
MEK	Butanone; methyl ethyl ketone
M-H	Mark-Houwink
MMD	Molar mass distribution
M_n	Number average molar mass
M_p	Molar mass of the highest peak
M_v	Viscosity average molar mass
M_w	Weight average molar mass
MWD	Molecular weight distribution
M_z	Z-average molar mass
PA	Polyamide

PBAT	Polybutylene adipate terephthalate
PBS	Polybutylene succinate
PCL	Poly(ε-caprolactone)
PCTFE	Polychlorotrifluoroethylene
PD	Polydispersity
PDLA	Poly(D-lactide)
PDLLA	Poly(D,L-lactide)
PE	Polyethylene
PET	Polyethylene terephalate
PGA	Polyglycolide or poly(glycolic acid)
PHA	Poly(hydroxyalkanoate)
PLA	Polylactide or poly(lactic acid)
PLCL	Poly(L-lactide-co-ε-caprolactone)
PLGA	Poly(lactide-co-glycolide) or poly(lactic-co-glycolic)acid
PLLA	Poly(L-lactide)
PMA	Premarket Approval
PP	Polypropylene
PS	Polystyrene
PTT	Polytrimethylene terephthalate
RALS	Right-angle light scattering
RI	Refraction index
ROP	Ring-opening polymerization
RSD	Relative standard deviation
SEC	Size-exclusion chromatography
SLS	Static light scattering
TCB	Trichlorobenzene
T_g	Glass transition temperature
THF	Tetrahydrofuran (oxolane)
USA	United States of America
VS	Viscometer

LITERARY PART

1 INTRODUCTION

There are constantly different polymers developed worldwide and at the same time, the ones already found, improved. Especially, the focus has been in finding alternatives that are less harmful for the environment. For example, lactide-based polymers (PLA) and their copolymers (for example, PLGA and PLCL) have been under a lot of research because of their practical properties, such as biodegradability.[1,2] There are several ways to produce those polymers and the production method determines the properties of the polymer. Usually, PLA is produced from lactide rings with ring opening polymerization (ROP). The basic material for lactide rings is naturally existing lactic acid and it can be produced, for example, by bacteria from glucose or other sugar containing substances.

There are plenty of different applications for the lactide-based polymers and, for example, many medical applications, such as tissue engineering scaffolds, orthopedic implants, and controlled drug release.[3,4] Copolymerizing and blending of the polymers also increases the possibilities to tailor the properties of the polymers.

Products manufactured for different applications, especially for medical use, are strictly regulated by different organizations.[5] For example, in case of the medical devices that bioabsorb in the human body, the manufacturer should be conscious not only of the properties of the polymer but also of the properties of the degradation. The degradation rate of the polymers can be followed with different ways, such as by gel permeation chromatography (GPC).[6] By this technique, different molar mass averages of the polymer can be measured.[7,8] In this method, the different-sized polymers are separated in the column full of porous gel based on their hydrodynamic radius. The calibration and data analysis of the measurements depend on the properties of the device (for example, the number of different detectors) and can be carried out with different methods including triple detection, universal calibration, or conventional calibration.

Additionally, the inherent viscosity of the polymer can be followed during the degradation and with it the molar mass of the polymer can be indicated in dilute solutions.[6] Inherent viscosity can be utilized to determine the intrinsic viscosity.[9,10] Intrinsic viscosity describes the molecular density of the polymer and it also indicates how the polymer affects the viscosity of the solution.

With intrinsic viscosity, the weight-average molar mass of the polymer sample can be determined by exploiting the Mark-Houwink equation.[11] Different viscosities can be measured, for example, with the help of a microviscometer or Ubbelohde-viscometer.

2 POLYMERS

Polymers are long-chained molecular compounds and they consist of repeated subunits called monomers.[12] If the molecule is made up from two monomers, it is called a dimer and if the molecule that consist of monomers is quite short (intermediate relative molar mass) it is called an oligomer. The length of the polymer is described with the term DP (degree of polymerization) that indicates how many monomers are attached to each other in the polymerization.[13] The structure of the polymers can be categorized based on the type and order of the monomers, but also based on their branching for example for linear, star-shaped or cross-linked polymers.[1]

Polymers can be found from the nature (for example, cellulose, Figure 1) but they can be also synthesized.[13] Generally, polymers can be divided into three different groups based on their origin; natural polymers, modified natural polymers (also known as semi-synthetic polymers), and synthetic polymers. The origin of the polymer does not suggest is the polymer biodegradable but the structure determines the properties of the polymer.[14]

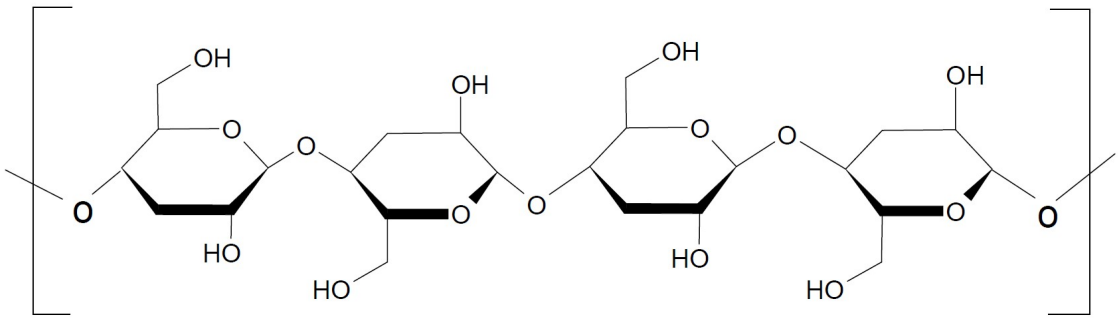


Figure 1. Part of a cellulose chain, a natural polymer which consist of repeated D-glucose monomers.[5]

Industrially used polymers are usually called plastics.[13] Plastics are macromolecular polymers which are deformable in some part of their processing and their final state is in solid form. They

often need some additives to work as desired and also sometimes different stabilizers and colorants are added during the production.[14] Plastic polymers can be mixtures of many monomers, synthetic or biobased and by changing the ratio between the monomers in the mixture, the wanted properties may be achieved. The term bioplastics is also commonly used referring to the plastics which are normally either biobased or biodegradable.[15] Bioplastics can be also both biobased and biodegradable and good example of this kind of bioplastic is PLA, polylactide. Examples of different ordinary petroleum-based plastics and bioplastics are represented in Figure 2.

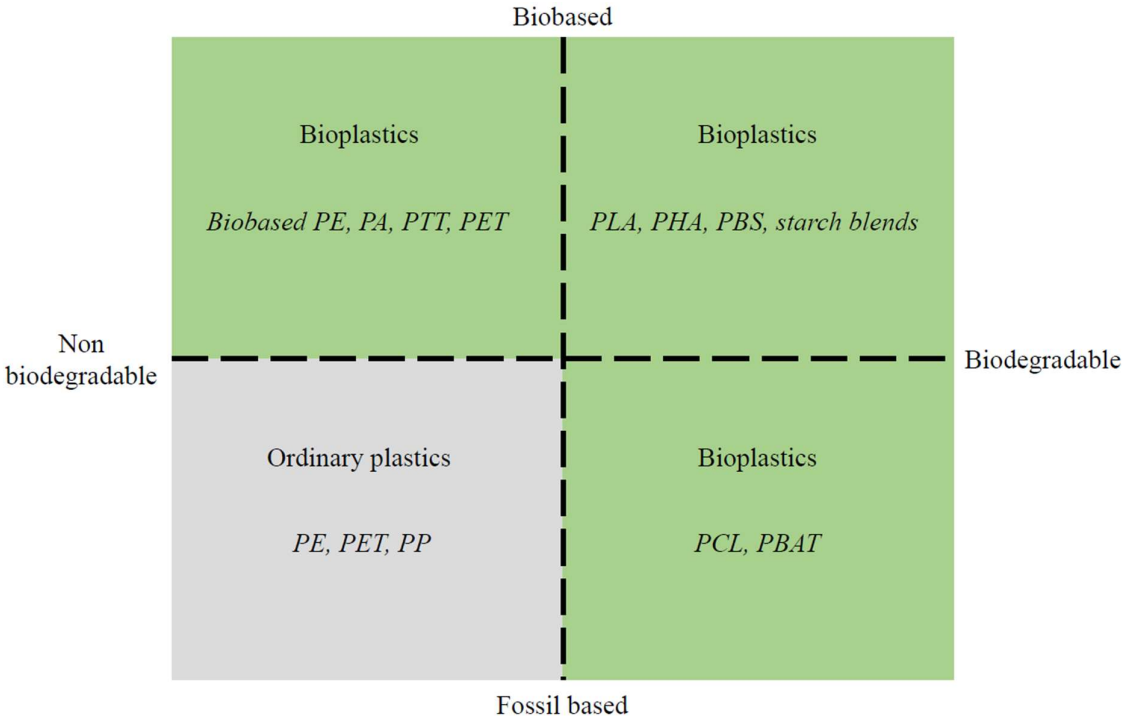


Figure 2. Division of bioplastics and conventional plastics with some example materials.[15] The abbreviations mean different plastics, PE = polyethylene, PA = polyamide, PTT = polytrimethylene terephthalate, PET = polyethylene terephthalate, PLA = polylactide, PHA = poly(hydroxyalkanoate), PBS = polybutylene succinate, PP = polypropylene, PCL = poly(ε-caprolactone), and PBAT = polybutylene adipate terephthalate.

If the polymer consists of only one type of monomers, it is called a homopolymer.[13] Homopolymers can be branched, straight or crosslinked. At the same time, if the polymer consists of two or more different type of monomers, it is called a copolymer. There are different kind of copolymers depending on how the different monomers are located in the polymer chain. For example, monomers can be linked in the chain in random order, in regular order or the same

type of monomers can form uniform segments in the polymer chain (block copolymers). These uniform monomer segments can also form individual branches to the polymer, and this kind of polymers are called graft copolymers.

If two or more different type of polymers are mixed together, it is called blending.[16] In blending, the structures of the polymers do not change like in copolymerization but the properties of the mixture can be different comparing to the initial polymers. Polymer blends are macroscopically homogeneous and they can be divided into different groups based on their ability to blend; miscible and immiscible polymer blends. Furthermore, miscible polymer blends can be separated to homologous and heterogeneous blends. Homologous blends are blends of the same polymer with different fractions. These fractions have different molar mass distributions. In heterogeneous blends there are different polymers forming the blend.

Polymers are usually formed by condensation reaction (condensation polymers, also known as step-growth polymers) or by addition reaction (addition polymers, also known as chain-growth polymers).[17] In condensation reaction, a small molecule, usually water, is split during the reaction and a new molecule, polymer, is formed. On the other hand, in the addition reaction the polymers are formed from unsaturated molecules by radical reaction or via ionic (anionic or cationic) intermediates. Sometimes the term polycondensation is used when referring to condensation polymerization and in proportion, the terms ionic polymerization and radical polymerization are used when referring to reaction routes of the addition polymers.[2,17]

If the monomers are monosubstituted alkenes (CH_2=CHZ), the formed polymer can be isotactic, syndiotactic or atactic based on its configuration.[13,17] In an isotactic polymer, all the Z-groups are on the same side of the carbon chain whereas in a syndiotactic polymer the Z-groups change regularly the side of the carbon chain. In an atactic polymer, the orientation of the Z-groups changes randomly. The crystallinity of the polymer depends on the configuration of the polymer chain and usually the isotactic and syndiotactic configurations can be crystallized, but atactic configuration stays amorphous.

During the polymerization reaction, a large quantity of polymers of different lengths are formed.[18] These different sized polymer chains with different molar masses form a certain molar mass distribution (MMD). MMD is also known as MWD (molecular weight distribution) because formerly the term molecular weight was used instead of molecular mass. Nowadays IUPAC (International Union of Pure and Applied Chemistry) recommends to use the term

molar mass instead of molecular weight but both terms are generally utilized. MMD can be sometimes predictable and sometimes not (nonequilibrium processes) and the number and the lengths of the formed polymer molecules depend on the reaction mechanisms and conditions of the reaction.[7] The MMD and different molar masses of the polymer molecules can be measured, for example, with GPC, which is discussed later in the thesis.

3 BIODEGRADATION

The term biodegradation usually refers to an event in which the material degrades gradually to small and non-toxic compounds employed by biological activity.[1,5,19] Biodegradation can be initiated and sustained by microorganisms or different enzymes and the formed compounds can be eliminated metabolically. The origin of the biodegradable material can be either synthetic or natural and the rate of the biodegradation is depending on the humidity, temperature, and the variety of microbes.[4,14] Other factors which affect to the degradation are pH, salinity, crystallinity and purity of the degrading material, amount of oxygen, and level of nutrients.[20]

Another term which is closely related to the biodegradation is bioresorption which means the degradation of the material under physiological surroundings.[5] The degradation products formed in bioresorption dissolve in the tissue and excrete from the system metabolically. This is called bioabsorption. The degradation of the polymer can be occurred on the surface of the polymer (heterogeneous erosion) or in all over the polymer (homogeneous erosion, also known as bulk erosion).[21] The chemical erosion mechanism depends on the polymer's physical properties and usually these erosion mechanisms happen simultaneously.

Usually, the biodegradable plastics are also compostable meaning that they are biodegradable and the degradation happens in a quite short time.[14] During the degradation of compostable materials, dangerous compounds may not be produced and the compostable product must not change the quality of the compost. In industrial composting, it takes normally approximately 6 to 12 weeks to convert bioplastics into water, CO_2, and biomass.

Biodegradation of plastic materials has been a hot topic as long as plastics have been produced. Nowadays, non-biodegradable petroleum-based plastics are ruling the plastic industry and only a small amount of generated plastic is recycled (under 10 % in USA during the years 2005-2014, Table 1).[22–31] Nevertheless, as can be seen from Table 1, this recycled amount of plastic has been gradually growing and has increased from 5.7 % to 9.5% during the years 2005-2014.

However, if the production of synthetic and bioplastics are compared in annual level, the share of the bioplastics produced is under 1 % of the production of the synthetic plastics.[20]

Table 1. Recycling procent of plastic generated in the USA during the years 2005-2014.[22–31]

Year	Recycling procent of plastic generated in USA (%)
2005	5.7
2006	6.9
2007	6.8
2008	7.1
2009	7.1
2010	8.2
2011	8.3
2012	8.8
2013	9.2
2014	9.5

Crude oil and natural gas resources are limited and it is not sustainable for the planet to use non-biodegradable materials on such a vast scale. It has led to many ecological problems. This is one reason why researchers are constantly looking for biodegradable alternatives for the petroleum-based plastics. Another important circumstance that should be noticed, in addition to the material's biodegradability, is the production of the material from renewable sources.[3] In terms of these, biodegradable plastics could be one possible alternative for petroleum-based plastics. Good examples for bioplastics already in use are PLA, PGA, PCL, and their copolymers (see sections 4.1-4.3). Most of the biodegradable plastics are polyesters.[20]

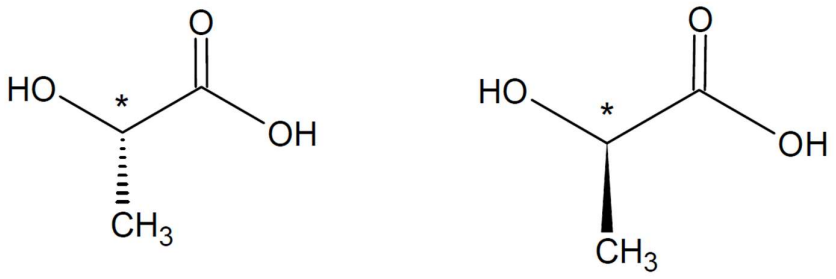
4 LACTIDE-BASED/ LACTIC ACID -BASED POLYMERS

Lactide-based polymers and lactic acid -based polymers often mean the same polymers in the literature.[1] These kinds of polymers differ only on their polymerization reaction mechanisms. Polymers formed from lactic acid by polycondensation are called poly(lactic acid)s (PLAs) and polymers formed from cyclic lactides by ROP are called poly(lactides) (also PLA). Cyclic

lactides are produced from lactic acids, so the original feedstock for the both reactions is the same, lactic acid.

Lactic acid (2-hydroxypropanoic acid) is a chiral molecule and it exists in two different three-dimensional structures, usually assigned with prefixes L- or D-.[2,5,32,33] With these prefixes, different 3D-structures can be separated and these stereoisomers are called enantiomers. The constitutional formulas of lactic acid enantiomers are presented in Figure 3. L- and D-prefixes are older notations for S- and R- prefixes which are nowadays recommended to use according to IUPAC.[34] However, with some compounds the L- and D- prefixes are still actively in use. Usually the prefix L- corresponds to the prefix S- and the prefix D- corresponds to the R- when considering the structure of the enantiomers.

Initially the L- and D- prefixes have separated the enantiomers according to the direction of optical rotation (L- counterclockwise (-) and D- clockwise (+)) the enantiomer causes to the plane-polarized light.[17,34] However, it has been noticed that, for example, L-lactide can cause both positive and negative optical rotation depending on its concentration.[33] That is why the (-)- and (+)- prefixes are avoided. If there is an equal amount of two enantiomers in the mixture, the mixture is called racemic.[17]



L- Lactic acid D- Lactic acid

Figure 3. Constitutional formulas of L- and D-lactic acids.[32]

Lactic acid is usually synthesized from glucose under anaerobic conditions in a reaction catalyzed with lactic acid dehydrogenase.[1] In this fermentative reaction, glucose turns to intermediate (pyruvate) and after that to lactic acid.[33] This reaction chain releases some chemical energy (ATP) and is naturally used as one of a power production methods with the living organisms. The synthesis of lactic acid can be also performed chemically by hydrolysis

of lactonitrile (2-hydroxypropanenitrile) with a strong acid and this way the racemic mixture of lactic acid is produced.[2]

In mammalian systems, lactic acid naturally exist in L-form, but in bacterial systems both L- and D-forms are found.[1,5] That is why a certain enantiomer of lactic acid can be produced via fermentation reaction with microbes (bacteria, fungi or yeasts). Bacterial fermentation is industrially the most common way of lactic acid production and about 90 % of the production is performed by bacterial fermentation.[2] It is also more inexpensive way to produce lactic acid than synthetic production.[2,32] This is one reason why the bacterial fermentation is more often utilized in industrial scale.

The bacteria used to produce lactic acid via fermentation are referred to lactic acid bacteria (LAB) and they can be divided into homofermentative or heterofermentative bacteria based on the reaction products.[5,33] Theoretically, homofermentative bacteria produce from 1 mol of glucose 2 mol of lactic acid, whereas heterofermentative bacteria from 1 mol of glucose 1 mol of lactic acid and lots of different by-products.[5] Lactic acid can be produced also, for example, from sucrose, lactose, and sugar-containing substances, such as molasses.[2] The yield depends on the bacteria utilized and also the material used in fermentation.[20] The homofermentative lactic acid bacteria are more industrially utilized because of the effectiveness of the lactic acid production.

Lactic acid molecules often form esters with each other in the solution because they have both hydroxyl and carboxyl groups in their structure.[5] Generally, the formed oligomers of lactic acid are dimers (lactoyllactic acid) and trimers (lactoyllactoyllactic acid) but also other forms exist. Lactic acid is hygroscopic compound and exists in a balance between water and its oligomers.

4.1 PLA

Poly(lactic acid)/ poly(lactide) (both PLA) is the first biopolymer produced on large scale.[33] It is a homopolymer consisting of several lactic acid molecules coupled together. As a material, PLA is compostable and it is generally produced from renewable sources, such as from starch and sugar.[3] It can be used in many different applications, such as in different packaging materials, films, fibers, and foams. It is also used in the medical field including tissue scaffolds, sutures and implant devices. PLA is also thermoplastic meaning that it is remoldable with heat and pressure.[2,13]

PLA can be produced with different methods and direct polymerization (such as azeotropic dehydration) together with ROP are the most generally exploited methods.[2] Of these two, the ROP method is more utilized.[35] In all of the common PLA production routes, lactic acid is used as the raw material but it is also possible to produce PLA from petrochemical feedstock.[32]

If only the conventional polycondensation is used in the PLA production, the PLA chains obtained are quite short and have a low molar mass.[2] This is because the reaction is an equilibrium reaction and the equilibrium does not favor the polymers with high molar masses.[35] PLA with low molar mass does not have sufficient properties for many applications but if the chains are longer and the formed polymers have high molar masses (M_n > 100 000 g/mol),[5] they have better mechanical properties.[1,5] That is why it is profitable to try to produce high molar mass PLA.

There are several different reaction routes to produce high molar mass PLA but these routes demand accurate reaction conditions (pH, pressure, and temperature).[2] That is why the production of a high molar mass PLA is not so straightforward. Three different production routes are presented in Figure 4.

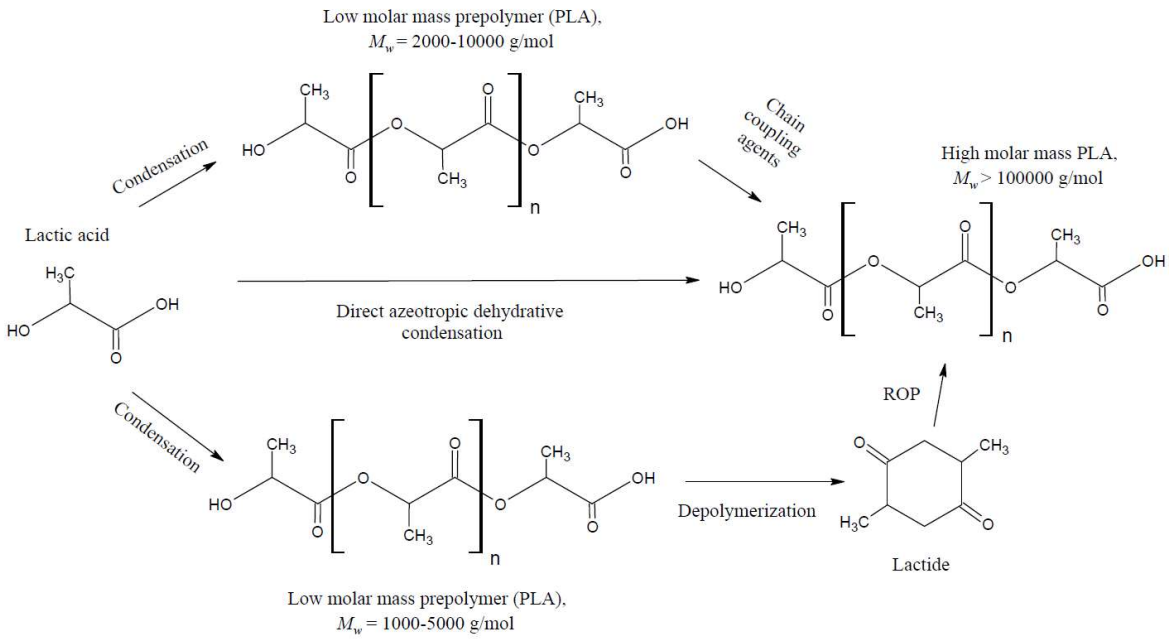


Figure 4. Three different reaction routes to produce a high molar mass PLA.[32]

One way to produce a long chain PLA is to utilize chain coupling agents in the reaction chain.[5] At the beginning, PLA with a low molar mass (prepolymer) is produced using conventional lactic acid condensation. Then, these short PLA chains are linked with each other using coupling agents (for example, 1,6-hexamethylene diisocyanate)[2] to form a high molar mass PLA.

Another way to produce a high molar mass PLA is azeotropic dehydrative condensation.[20] In this process, the water formed in polycondensation is removed with drying agent (often organic solvent) and a high molar mass PLA is produced directly from lactic acid. With this method, the weight average molar masses of the produced PLA can be more than 300000 g/mol.[36]

The third route is the most used on industrial scale and the molar mass of forming PLA can be controlled.[2] First of all, a low molar mass prepolymer (PLA, DP < 100,[1] M_w = 1000-5000 g/mol[32]) from lactic acid is produced by polycondensation.[33] After this, the prepolymer is depolymerized and the depolymerization yields a product called lactide (3,6-dimethyl-1,4-dioxane-2,5-dione). Lactide is a cyclic dimer of lactic acid and is formed via transesterification by back-biting reaction mechanism.[5] Now, lactide is the monomer for the forming PLA and the ring structure is opened with the help of catalysts (usually tin octoate, $Sn(Oct)_2$, also known as Sn(II)2-ethylhexanoate).[20] When the lactide rings are opened, they join quickly together and form long PLA chains.[5] This step is called ROP. In many points of this route, purification is needed and these purification steps are expensive and complex.[2]

A lactide ring is a chiral molecule with two chiral centres and it has three different optical structures (Figure 5).[33] The structure of the lactide ring can be D (R,R), L (S,S) or *meso* (R,S) and the structure depends on the stereochemistry of the reactant, the lactic acid. If the lactide sample is the mixture of L- and D-lactides, it is called rac-lactide (also known as DL-lactide). Respectively as before, the structures of the reacting lactides affect to the structure of the forming PLA chain. If the lactides are purely L-type, the formed PLA chain is called PLLA and if the lactides are purely D-type, the PLA is called PDLA.[2] If the formed PLA chain consist of either *meso*-lactides or rac-lactides (a mixture of L- and D-lactides), it is called PDLLA. However, normally only PLLA and PDLLA forms are used.[3,37]

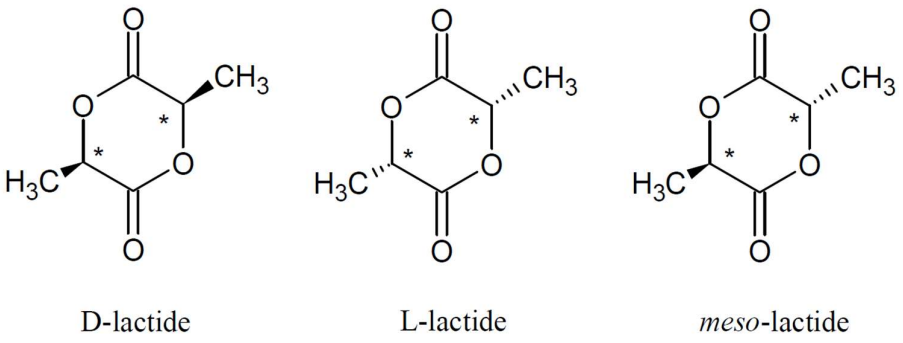


Figure 5. Constitutional formulas of L-, D-, and *meso*-lactides.[33]

PLLA and PDLA have different characteristics and the ratio of the enantiomers (L and D) in their mixtures determines the properties of the material.[3,33] When seeking better mechanical properties, pure PLLA is often used because it is highly crystalline (about 37 %).[38] Pure PLLA has the highest melting point of different PLA grades and adding the D-comonomer to the polymer decreases the melting point. This also causes the mixture to crystallize slower and when the D-content is higher than 12-15 %, the end product turns amorphous.[14,33] However, there is also a downside for resistant PLLA because it degrades very slowly compared to the PDLLA.[1] The poor degradation of PLLA results from the reinforcing crystalline domains formed in the PLLA structure.

The properties of PLA polymers can also be changed by adding different type of monomers into the reaction mixture. In that case, different copolymers of PLA are formed.[2,39] Other ways to alternate the properties of PLA are to change the structure of the PLA polymers by branching the molecules, adding nanoparticles to the PLA to form nanocomposites and coating PLA with high barrier materials.[3,20]

PLA has many advantages from the perspective of many applications and it is used, for example, in suturing materials, in drug delivery, and surgical implants.[40] Formerly, titanium and other metals have been used in different orthopedic screws and plates, but they are not degradable and stay in the body.[2] To remove them, another surgery is needed. There is always a risk in surgeries and, in addition, the extra surgery increases the expenses of the total operation. Due to this, the researchers are interested in bioabsorbable materials utilized in the medical field.

PLA is ecological, deformable, water resistant and in the production energy can be saved compared to the petroleum-based plastics.[39] It has been estimated that it takes about 25-55 % less energy to produce PLA than petroleum-based polymers. Nevertheless, PLA can be brittle and does not endure hard hits. PLA is also comparatively hydrophobic (contact angle with water approximately 80°) and there are not many reactive side-chain groups in this polymer to chemically modify the polymers.

PLA is also generally considered as biocompatible meaning that after implantation to the body, immunological rejection because of the polymer or its degradation products is not observed or observed reaction is much smaller than the advantage gained from the implant.[41–43] Additionally, the biocompatible implants may not be toxic for the system and the degradation products must be eliminated from the system without traces. However, in some studies, reactions with PLA and tissue have been reported.[41,44] It has been discovered that the long degradation times and high crystallinity of the material (especially with pure PLLA) can induce some inflammatory reactions. However, these properties can be changed by copolymerizing the D-form PLA among the L-form and because the reaction has been observed only in few studies PLA is considered as viable for medical use.[3,45]

4.2 PLGA

PLGA (poly(lactide-co-glycolide), also known as poly(lactic-co-glycolic)acid, Figure 6) is a synthetic copolymer of lactide and glycolide rings and it occurs in many different compositions.[4,38,46] If only cyclic glycolide (1,4-dioxane-2,5-dione) monomers are utilized in the polymerization, PGA polymers (polyglycolide, also known as poly(glycolic acid)) are formed by ROP.[38] When adding the lactides among the glycolides, the copolymer PLGA can be formed by the same method, ROP.[46] PLGA belongs to the group of poly-α-hydroxy acids and is soluble in many commonly used solvents.[4] Often the abbreviation PLGA accurately refers to poly (D,L-lactide-co-glycolide), in which the ratio between D- and L- lactic acid forms is equal.

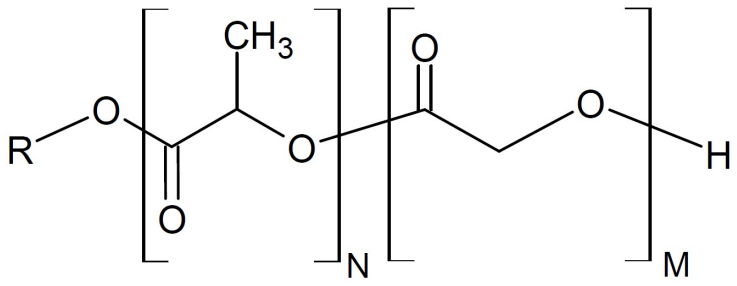


Figure 6. Poly(lactide-co-glycolide).[4] N is the number of lactide units and M is the number of glycolide acid units.

As a material, PLGA is biodegradable and biocompatible.[4] PLGA is a strong material and its mechanical properties can be modified by changing the ratio of lactides and glycolide used in the polymerization. This ratio also has an effect to the erosion times of PLGA.

Because of the special properties, PLGA has been under a lot of interest and it is highly researched. Especially, PLGA has been exploited in controlled drug release.[4,46] In controlled drug release, the drug is released to the body with the steady rate during the certain period of time either from a implanted polymer object or from injected small polymer particles.[5] With the polymer implants, the drug can be targeted to the desired part of the body and the other parts of the body are not harmed because of the drug. PLGA is also used, for example, as resorbable sutures and in bone fixation devices (screws, nails, pins, plates, and clips) because of its good biocompatibility with bone.[19,32,44] It has also been reported that PLGA-copolymers can increase the bone formation rate.[44]

During the years 1966-1994 several different animal, human, and *in vitro* studies have been done for PLA, PGA, and their copolymers. The results of these studies have been gathered in the review by Athanasiou *et al.*[44] From this review it can be seen that in 42 different animal tests there were only minor or no inflammatory responses caused by PLA, PGA or their copolymers. Only exception was the study made in 1991 (meniscal repair in dogs) where a chronic inflammation symptoms were noticed.

From the same review[44] it can be noticed that in twelve human studies during 1974-1994 the inflammatory reactions of the body were not observed. The same results were obtained also in eight *in vitro* –studies. Only exception in *in vitro* –studies was observed in 1992, when

Daniels *et al.*[47] reported about toxic solutions caused by copolymers of lactide and glycolide. The toxicity was probably caused by accumulation of the acidic degradation products.

4.3 PLCL

PLCL (poly(lactide-co-ε-caprolactone), Figure 7) is a copolymer of lactide and ε-caprolactone (CL, 2-oxepanone).[1] This copolymer can be produced by different ways and these production routes form polymer chains with different structures. For example, if PLCL is produced by direct polycondensation or by polycondensation and chain extension with the chain coupling agents (HMDI; hexamethylene diisocyanate), the formed molecule is a linear copolymer with a high molar mass (> 70 000 g/mol). If PLCL is produced by ring-opening polymerization, random and block linear copolymers with a high molar mass (> 70 000 g/mol) are formed. However, generally only the ROP route is utilized in production.[37]

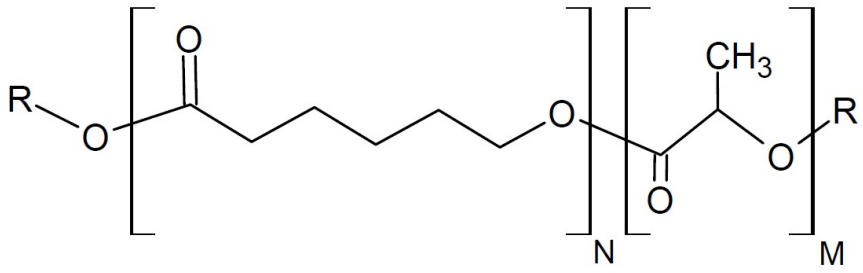


Figure 7. Poly(lactide-co-ε-caprolactone).[39] N is the number of ε-caprolactone units and M is the number of lactide units.

According to Hiljanen-Vainio *et al.*[37] the PLCL copolymers consisting of 80 wt.% of ε-caprolactone were able to crystallize, whereas the copolymers consisting of 40 to 60 wt.% of ε-caprolactone were amorphous. Slow hardening of the PLCL was also observed over the time because of the post-crystallization of the homopolymeric parts of the polymer at room temperature.

PLCL is biodegradable material and the flexibility makes it useful in many different applications. PLCL is studied and utilized particularly in the medical field, for example, in tissue engineering scaffolds and bioresorbable stents.[48–51] Also, PLCL is also utilized in

controlled drug release and it is suitable for 3D-printing, drug encapsulation, and nanoparticle preparation. Furthermore, PLCL dissolves well in common solvents.

In some *in vitro* tests it has been observed that the tensile strength and pliability of PLA diminishes of the course of time.[52] This can be prevented with blending PLA with PCL (poly(ε-caprolactone)). Blending of these two polymers enhances especially the elasticity of PLA. Pure PLA can be rigid but when PCL is added, the material turns a lot more flexible. This blending also lowers the T_g (glass transition temperature) of the material.[39]

4.4 BIODEGRADATION OF PLA, PLGA, AND PLCL

PLA, PGA, PCL, and their copolymers are biodegradable materials and their degradation naturally occurs by ester bond hydrolysis (Figure 8).[1,2] When the molar mass of the polymer is low enough because of the randomly occurring chain scission, the short residual polymers are metabolically removed from the system. Thus, in the degradation of the medical PLA devices the added enzymes or substances which could cause inflammatory reactions in the tissue are not needed. In industrial compost conditions, also the natural enzymes (esterases, lipases, and proteases) excreted by different microorganisms take part in the degradation of PLA.[20] There are differences between the degradation rates between the materials and the degradation rate of the PCL is nearly three times slower than PLA's.[21]

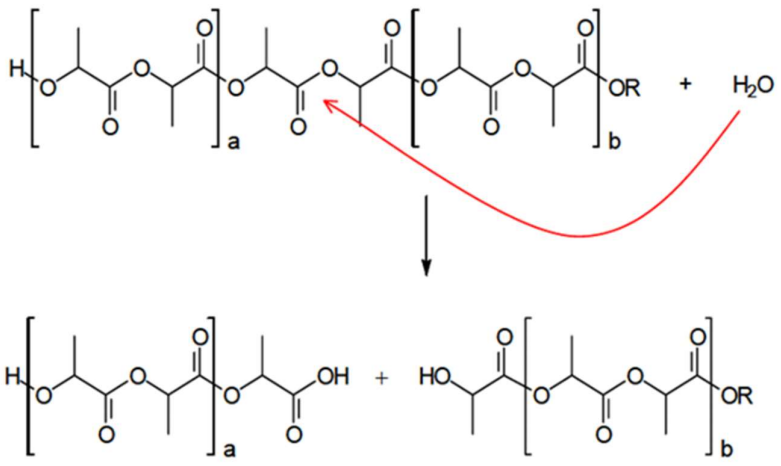


Figure 8. Hydrolysis of PLA molecule.

The degradation products of the PLA (lactic acid), PLGA (lactic acid and glycolic acid), PGA (glycolic acid), PLCL (lactic acid and ε-caprolactone), and PCL (ε-caprolactone) are relatively safe for the environment and are excreted naturally from the system.[2] For example, lactic acid and glycolic acid are eliminated in the natural tricarboxylic acid cycle (also known as citric acid cycle) in the body.[38,43,44] Finally, they can be mainly excreted with the respiration as CO_2. Glycolic acid can be also excreted in the urine.

It is possible that if the degradation of the PLA happens too quickly the acidic degradation products can be accumulated and the local pH of the surrounding tissue may drop.[53] Nevertheless, this is not considered as a threat because in studies the acidic degradation products have not been accumulated in the body.[45] Anyway, this kind of acidosis could be in normal cases harmful only for the infants because the infants have a limited ability to metabolize D-type lactic acid.[40] However, lactic acid is not unfamiliar compound in the nature because it is naturally produced by plants, animals and microorganisms.[1] PLA is also "generally recognized as safe" (GRAS) when using it in contact with food, for example, as food containers.[14,40]

5 MEDICAL DEVICE REGULATION

Before new biomaterials and drugs can be penetrated to the market, they need to pass different regulatory requirements to make sure they are safe and reliable in the use.[5] Generally, there are expensive and long-lasting clinical studies among these requirements.[51] In the USA, the authority that oversees the manufacturing, performance and safety of medical devices is FDA (US Food and Drug Administration). With biomaterials, the awareness of the components of the new materials impacts significantly for getting approval to use the material. Nevertheless, it is not enough to know about the components the new material consist of but also the new product itself has to pass all the tests.

There are two different paths to have a FDA approval to sell and market medical devices in the USA[38]. First of them is FDA's 510(k) process where new devices must be proved to be as safe and efficient as the current devices. Another is the premarket approval process (PMA) which has strict and same requirements as new drug applications. To have PMA, clinical data of the product has to be included in the application document.

In Europe, European commission has set two different regulations for medical devices to unify the laws related to medical devices in the European Union. These regulations are called Regulation (EU) 2017/745 (medical devices) and Regulation (EU) 2017/746 (in vitro diagnostic medical devices) and they replace the earlier directives and regulations concerning medical devices.[54] In both of these regulations, there is a transitional period ongoing. In the regulation 2017/745, the transition time will end in the year 2020 and, respectively, for the regulation 2017/746, the transition time will end in the year 2022. These regulations take effect in EU member states as they are. There are also authorities that oversees the medical device sector in different nations (such as Valvira in Finland) who carefully follow the decisions of European commission and FDA.

There are different requirements medical biomaterials have to answer.[5] Biomaterials must be biocompatible, mouldable, must have sufficient mechanical properties, and they have to be sterilizable. The standards for biodegradable polymers are a bit stricter; in addition to the standards of the plain biomaterials, they have to be bioresorbable and stable during the processing, storing, and sterilizing. For example, PLGA has got the FDA approval as a biodegradable polymer.[4] Also PLA is approved to use as a biodegradable polymer by FDA.[2]

One very important criterion for degradable biomaterials is that the degradation must happen in a controlled way and not too rapidly.[5] FDA obliges in its draft guidance for bone anchors (2017)[55] to evaluate the degradation of the biodegradable material used in medical devices, for example, by following the molar mass changes during the degradation. Also the changes in the mechanical properties (such as pullout force) should be observed to characterize the degradation as well as possible. Changes in molar mass can be followed by GPC or by measuring the inherent viscosity of the sample.[6]

6 DETERMINING PROPERTIES OF THE POLYMERS

It is very difficult to characterize polymers precisely.[56] During polymerization, even the simplest homopolymers formed in the reaction can differ with respect to their length and structure and they can have different stereochemistry. To find out exactly what kind of polymer is produced, at least three different distributions should be determined: MMD, chemical composition distribution (CCD), and functionality type distribution (FTD). Even with these three separate determinations, it cannot be guaranteed that the structure of the polymer is possible to find out. Nevertheless, it is not usually necessary to know everything about the

structure of the polymer and often only the information about the molar mass distribution is required.

6.1 GPC

GPC is an empirical method used to measure MMD and average molar masses of polymers.[7] Sometimes also term SEC (size-exclusion chromatography) is used in the literature, but actually it is more general concept for both GPC and GFC (gel filtration chromatography). In GPC, organic solvents are used as mobile phase, whereas in GFC aqueous solvents are utilized. The GPC device consist of different components including eluent bottle, pump, injector, column set, and detectors which are shown in Figure 9.

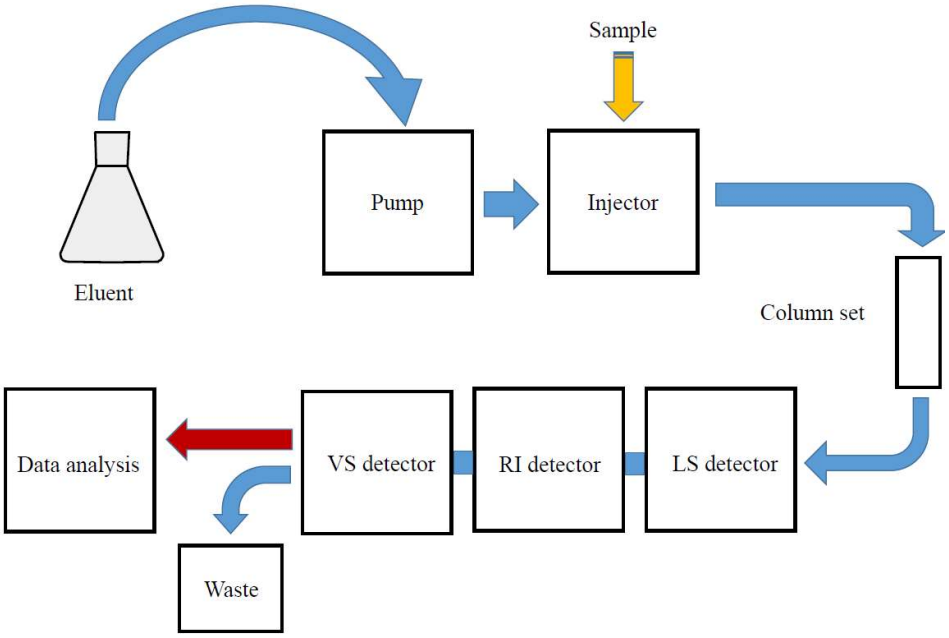


Figure 9. Simplified graph of GPC device with triple detection system.[57] Abbreviations LS, VS, and RI refer to light scattering, refraction index, and viscometer, respectively.

The eluent is transferred from the eluent bottle to the GPC system with a pump and it acts as a mobile phase in this chromatographic system.[57] At the beginning of the sample run, the wanted volume of sample is injected from the sample vial into the eluent flow. Next, the sample moves into the column set and flows through it. In the columns, different sized molecules of the sample are separated. After the column set, the sample proceeds to the detectors and different variables

are measured. The amount and type of detectors is depending on the GPC device and in the triple detection devices there are three different detectors measuring the sample. After the detection, the sample flows to the waste and the results from different detectors can be analyzed. It is also profitable to use a degasser in this chromatographic system, especially when there is a refractive index detector in the device and tetrahydrofuran (THF) is used as a mobile phase.

In GPC, the molecules are separated in the column based on the size of the polymer molecules (hydrodynamic radius, R_h, also known as Stokes radius).[58] The polymers tend to curl to a ball-like shape in the solution (except for the most rigid polymers). Thus, the hydrodynamic radius of the polymer is a radius of a hypothetical sphere which has equivalent hydrodynamic properties as the dissolved sample polymer.[59,60] Hydrodynamic radius of the polymer changes according to the used solvent. This can be seen in Figure 10.

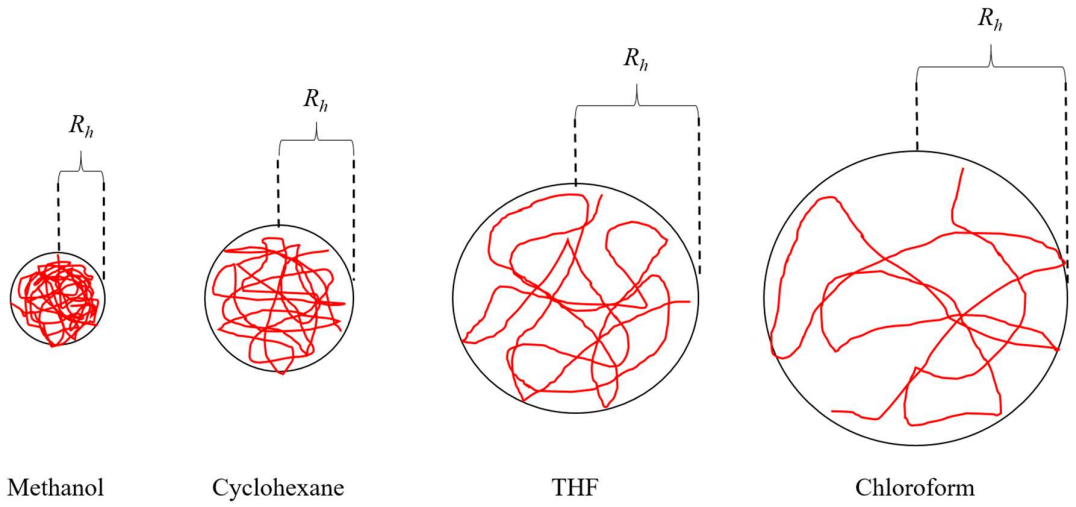


Figure 10. The same polystyrene polymer dissolved in different solvents.[61] The hydrodynamic radius R_h depends greatly on the solvent.

The hydrodynamic radius affects directly to the hydrodynamic volume (V_h) of the sample and V_h can be linked to the molar mass of the polymer with the equation

$$V_h = [\eta] \cdot M, \qquad (1)$$

where $[\eta]$ is the intrinsic viscosity of the polymer (see section 3.2) and M is the molar mass of the polymer (g/mol).[61]

On the other hand, the hydrodynamic radius can be connected to intrinsic viscosity in very dilute concentrations for example with the Einstein's viscosity relation

$$[\eta] = \frac{2.5 \cdot N_A \cdot V_e}{M} = \frac{10 \cdot \pi \cdot N_A \cdot R_h^3}{3 \cdot M}, \quad (2)$$

where N_A is the Avogadro's number ($6{,}022 \cdot 10^{23}\ \frac{1}{\text{mol}}$), M is the molar mass of the polymer (g/mol) and the V_e is the volume of the equivalent spherical particle for the dissolved polymer (cm^3), $V_e = \frac{4 \cdot \pi \cdot R_h^3}{3}$.[8,62,63] If the dissolved polymer molecules are soft, this equation must be modified.[63]

The GPC columns are full of heteroporous, solvent swollen gel beads and this gel functions as a stationary phase.[7,18] The polymer molecules under interest are dissolved to some organic solvent and this solution is controlled to flow through the column usually with a pump. The pump keeps the flow of the eluent constant. In the column, the smaller molecules from the sample can be stuck in the porous stationary phase gel and it takes more time to flow through the column for the smaller molecules than the large-scale molecules (Figure 11). Thus, the polymers in the sample can be separated and studied with different detectors. The GPC method is a fast and reliable way to get information of the polymers average molar masses and molar mass distribution and it has been used since 1960s.[64]

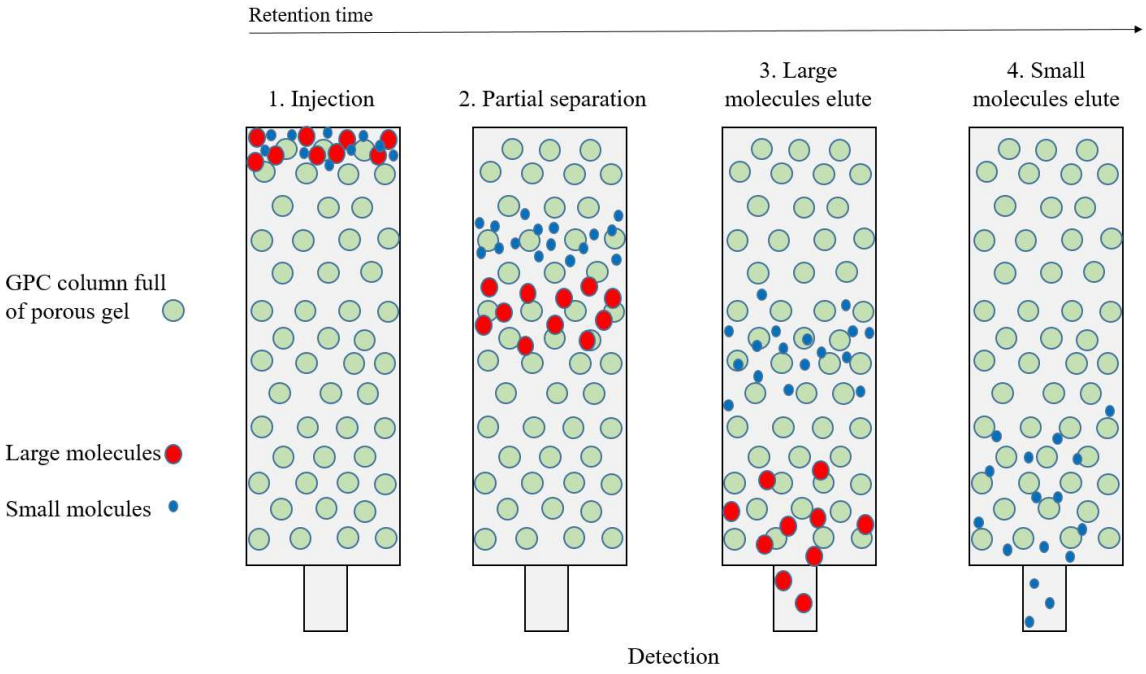


Figure 11. Operational principle of the GPC column.[7,65] The molecules are separated according to the size of the molecules.

Solvent used in GPC must dissolve the sample polymer completely.[7] If this does not happen, the undissolved particles have an impact on the results and may plug the column or cause problems with the detectors. However, the solvent may not decompose the sample polymers because also in this case, the results are distorted and are not equivalent to the sample polymers. When choosing the solvent it must be also taken into account that the solvent does not corrode the device.

There are several different molar mass averages that can be analyzed from the sample and the unit for the molar mass *M* is often [g/mol].[58] Sometimes also corresponding unit, Dalton (Da), is used. These molar mass averages help to describe the polymers and the lengths of the molecules. Usually, the average molar masses are quite the same for different samples of the same polymer. However, the distribution of the polymer chain lengths can vary between the same average molar massed samples depending on the polymer production method. That is why it is important to find out not only the average molar masses of the polymer sample but also the molar mass distribution of the samples.

M_w refers to the weight average molar mass that can be defined as

$$M_w = \frac{\sum_{i=1}^{\infty} n_i \, M_i^2}{\sum_{i=1}^{\infty} n_i \, M_i} = \frac{\sum_{i=1}^{\infty} w_i \, M_i}{\sum_{i=1}^{\infty} w} , \quad (3)$$

where n_i = number of the polymers that have the molar mass of M_i and $w_i = n_i M_i$.[13]

The M_w value may tell for example about the melt viscosity of the polymer and the values for M_w are not dependent on the solvent-temperature conditions.[7,58] The M_w value of the polymer can be determined with the method based on light scattering (see section 3.1.2.1).[13]

Another average molar mass is called number average molar mass M_n and it can be defined as

$$M_n = \frac{\sum_{i=1}^{\infty} n_i \, M_i}{\sum_{i=1}^{\infty} n_i} , \quad (4)$$

where n_i = number of the polymers that have the molar mass of M_i.[13]

Most of the thermodynamical properties of the polymers, such as specific heat capacity, depend on M_n.[18] It has been also noticed that M_n is always smaller than M_w apart from the uniform polymers. Uniform polymers consist of molecules with the same relative molecular mass and constitution.[66] The values for M_n do not depend on the solvent-temperature conditions and M_n can be measured for example by osmometry.[7,58]

There is also average molar mass called z-average molar mass (M_z), that can be defined as

$$M_z = \frac{\sum_{i=1}^{\infty} n_i \, M_i^3}{\sum_{i=1}^{\infty} n_i \, M_i^2} , \quad (5)$$

where n_i = number of the polymers that have the molar mass of M_i.[13]

M_z is related to some viscoelastic properties of the polymer, such as stiffness and the term is not dependent on the solvent-temperature conditions.[7,18] M_z can be found out with sedimentation by an ultracentrifuge.[13]

The final different average molar mass is viscosity average molar mass, M_v, which is defined as

$$M_v = \left[\frac{\sum_{i=1}^{\infty} n_i \ M_i^{a+1}}{\sum_{i=1}^{\infty} n_i \ M_i}\right]^{1/a}, \quad (6)$$

where n_i = number of the polymers that have the molar mass of M_i and a = constant which depends on the combination of polymer-solvent.[13] This constant a is also equivalent to the exponent α used in Mark-Houwink equation (see section 3.1.2.2).[7]

M_v often correlates with the polymer extrudability and molding properties and it depends on the conditions during the measurements.[7] M_v is measured with a viscometer.[13] It is often 10-20 % below M_w and if a = 1, $M_v = M_w$.[67]

To measure MMD and to compare the results, a term called polydispersity (*PD*) has been defined.[13] Polydispersity, $PD = \frac{M_w}{M_n}$, is used as a "measure of the molar mass distribution". This ratio indicates the width of the MMD of the polymer and is 1.0 for the uniform polymers.[7] *PD* has the value of 2 when the distribution follows the Flory most probable distribution and the more cross-linked the polymer is, the larger the value of the *PD* becomes.

If the molar mass results from a polymer sample are put in the graph, a distribution curve is formed. From the highest point of this graph M_p (molar mass of the highest peak) can be found.[68] If all molar mass averages are set in the distribution curve, it can be seen that typically the averages are related so that $M_n < M_p < M_v < M_w < M_z$.[18,58] Also the ratio between $M_n/M_w/M_z$ is 1:2:3 for high molar mass polymers.[18,69] The relative positions of the molar mass averages in the molar mass distribution can be seen in Figure 12.

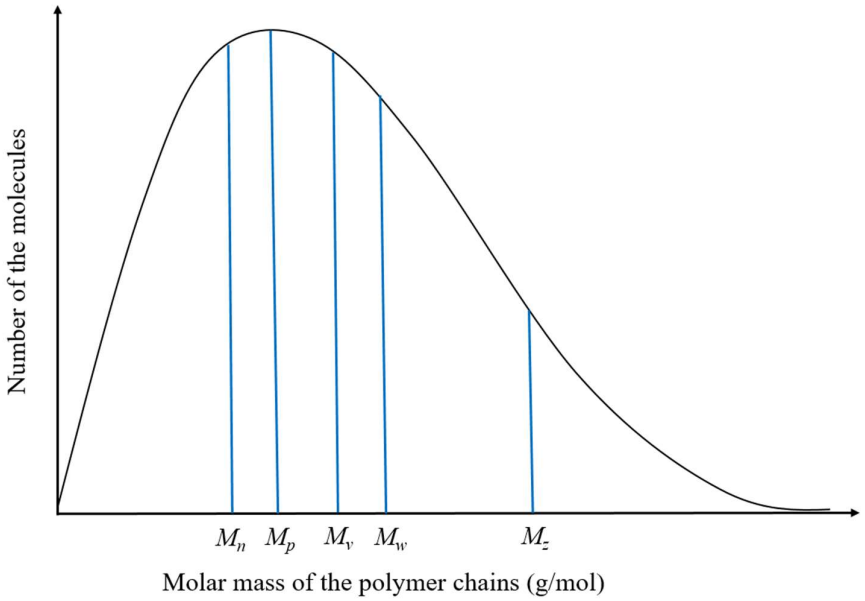


Figure 12. Molar mass averages in the molar mass distribution.[18,58] The locations of the *M* values in the distribution are estimated.

To analyze the results measured with the detectors in specific conditions, the system must be calibrated. In other words, generally in the calibration the results are attached to the general measurement standards.[70] In most cases, the polymer calibrants used in polymer sample calibration are polystyrene (suitable for THF, toluene (methylbenzene), chloroform ($CHCl_3$, trichloromethane), and TCB (trichlorobenzene) as an eluent), polyethylene oxide/glycol (suitable for aqueous eluents and so for DMSO (dimethyl sulfoxide) and DMF (dimethylformamide) as an eluent) and polymethyl methacrylate (suitable for MEK (butanone; methyl ethyl ketone), ethyl acetate, DMF, and acetone as an eluent).[58] The calibration of the GPC can be executed with different type of methods, including the conventional calibration, triple detection method, and universal calibration.[8] The calibration method depends on the properties of the device and these calibration methods are discussed in the following sections.

6.1.1 CONVENTIONAL CALIBRATION

The data from GPC device can be analyzed with different methods. One of the methods is called the conventional calibration. In this method, the GPC uses only a single concentration detector, usually refractive index detector (RI, see section 3.1.2.3), to measure the samples.[71] The detector measures the amount of the material flown through the column and also the time what it takes for molecules with certain size to go through the column. In the calibration, it is

determined how long it takes for standard compounds with known molar masses to elute completely. This time versus mass information from the calibration is compared with the information from the unknown samples to get the results for the average molar masses of the samples. For this reason, conventional calibration is also referred as a relative or comparative calibration.[72]

In the conventional calibration, the calibration is performed with several well-known standards with narrow MMDs and there should be at least 2 to 3 standards for each measuring molar mass decade to obtain a decent calibration curve.[56,73] The results from the standards are fitted to a graph (log *M* as a function of eluting time) and this calibration curve is utilized for analyzing the unknown samples.[71] In GPC calibration, the calibration curve is usually s-shaped as in the Figure 19 in the experimental part.[74] For this curve, a fit function is chosen which is generally a degree of three or higher. There are no recommendations how to do this choice.

In addition, it must be noticed that to have correct values for the samples in the conventional calibration, the standards' chemical structure and chemical properties must be relative to the samples'.[56,72] This is because the GPC separates the molecules according to the size of the molecule and if the molecules dissolve differently to the eluent, the results cannot be compared.[71] There are no suitable standards for all types of samples and this limits the group of polymers that can be reliably analyzed with the conventional calibration method.[56] For example, in the analysis of PLA samples, often different polystyrene (PS) standards are utilized because the properties of PLA and PS are quite similar.[20,32,36,75]

6.1.2 TRIPLE DETECTION

Another analyzing method for GPC results is the triple detection method. Triple detection is a detection technique where three different detectors are used to measure and analyze the samples in the same sample run.[8] This detection system is possible to use both for biopolymers and synthetic polymers. The results from the different detectors are combined to get more accurate molar masses and MMDs for the polymers than what can be achieved with conventional calibration. The alignment of the detector results is performed with one standard.[76]

The detectors used in the triple detection are usually light scattering detector (LS), viscometer detector (VS), and refractive index detector (RI).[8,77] The LS detector focuses to three different issues: measuring MMD, absolute molar masses, and the radius of gyration of the sample

polymers. The VS detector measures intrinsic viscosity (see section 3.2) of the samples and also determines the important Mark-Houwink parameters. The RI detector analyzes the concentration and the change of the refractive index as a function of the concentration (*dn/dc*).

6.1.2.1 LIGHT SCATTERING DETECTOR

In the light scattering detector, a polarized monochromatic laser beam is focused to the sample.[78] When the beam encounters a sample molecule, it scatters in all directions and the intensity of the scattered light is measured. The intensity of the scattered light is not the same in every direction (except if the molecule size is less than 1/20 of the wavelength of the beam) and depends on the size of the sample molecule. The larger the molecule is, the more different parts participating to the scattering there are. With large molecules, destructive interference of the scattered light waves is observed and this reduces the scattering intensity at the higher angles. This is referred as angular dissymmetry. For that reason, the molar masses for large molecules determined by high angles are underestimated. The different intensities measured in different angles at the same time can be utilized to estimate the size of the molecule. This estimate for the size is called radius of gyration, R_g, "the root mean square average distance of the components of the molecule from the centre of gravity". The relationship between R_g and molar mass M can be expressed as

$$R_g = K \cdot M^{\nu}, \tag{7}$$

where the K and ν are constants for specific solvent-polymer combinations. The constant ν can tell about the structure of the polymer and, for example, with the value of 0.5 the polymer is expected to be a random coil in a good solvent. It is generally thought that solvent is good if the hydrodynamic radius of the sample polymer is large in it.[61]

The scattering is fundamentally the result from interaction between the negatively charged electrons of the molecule and oscillating electric field of the light wave.[79,80] The measurement of the scattering can be performed from the cuvette full of sample solution or from a flowing stream of the sample solution and the measuring style depends on the instrumentation. Usually, there is a flowing stream measurement in the GPC.

There are two different types of light scattering techniques: static and dynamic light scattering.[79] In static light scattering (SLS), the intensity of the scattered light is measured many times. The

difference between these measurements is used to determine the radius of gyration (R_g) and the average molar mass of the sample polymer. To determine the molar mass of the polymer completely, also the concentration of the sample is required in this method. SLS is also known as classical light scattering or Rayleigh scattering.

In dynamic light scattering (DLS), the fluctuation of the intensity of the scattered light is measured and from this data, the diffusion coefficient of the molecules in the solution (Brownian motion) is determined.[79] Also, the hydrodynamic radius of the molecule (R_h, see section 3.1) can be determined with the Stokes-Einstein –equation (equation 2). The dynamic light scattering is also known as photocorrelation spectroscopy, beat spectroscopy or quasi-elastic scattering.

There are three different types of light scattering detectors: RALS (right-angle light scattering) that measures the sample at 90° from the incident radiation, MALS (multi-angle light scattering) that measures the sample from two or more angles (typically at 20°-160°), and LALS (low-angle light scattering) that measures the sample at < 10°.[8] RALS ignores the dissymmetry and that is why the accurate molar masses are determined with RALS only if the sample polymers are small enough.[78] LALS measures the intensities in very small angles so the dissymmetry should not affect to the results. However, at the same time the results can be disturbed because of the initial laser beam (0°) and because of the sensitivity of this detection method to contaminants. With MALS, the results can be analyzed with different methods, for example, with partial Zimm analysis.

When using the LS detector, the calibration curve formed from several standards is not needed because this method measures directly the absolute or true molar masses of the samples.[77] That is why the measuring of the sample polymers is easier with the triple detection than with the conventional calibration. However, calibration with one polymer standard is still required and must be performed to define couple of instrument constants.[8] With instrument constants, the signal of the instrument is connected to the molar mass and after that the LS device can give the correct results for the polymers. This calibration can also be executed with pure solvents.

LS detector is very sensitive to particles and the samples must be filtered before the GPC run.[79] Also the solvent used has to be clean (filtered at least with a filter of 0.1 μm, ideally 0.02 μm) because all the extra particles in the solvent or samples may contaminate the LS detector.

6.1.2.2 VISCOMETER DETECTOR

The viscometer measures the viscosity of the eluent with dissolved polymer and compares it to the values of the pure eluent.[78] These solutions are forced to flow through narrow capillaries and the measurement is actually carried out by measuring the pressure drop in the capillaries. This value for the pressure drop can be transformed to the value of viscosity with the help of Poiseuille's law.

Usually, the achitecture of viscometer device is symmetrical 4-capillary bridge with capillaries of 0.25 mm of internal diameter and the bridge has two different flowing paths for the solution (Figure 13).[8,78] These paths flow independently through two capillaries but after that they unite and flow to the waste. In one of these paths, there is a delay column full of glass beads after the first capillary which slows down the flow of the sample molecules and causes pressure differential to the bridge. The pressure difference measured across the centre of the 4-capillary bridge is called differential pressure (*DP*) and the pressure difference measured from the device's inlet to outlet is called inlet pressure (*IP*). These two pressure differences are used to determine for example the specific viscosity of the solution

$$\eta_{sp} = \frac{4DP}{IP-2DP} \qquad (8)$$

and this value can be used to obtain for example intrinsic viscosity of the sample (see section 3.2, equation 15).

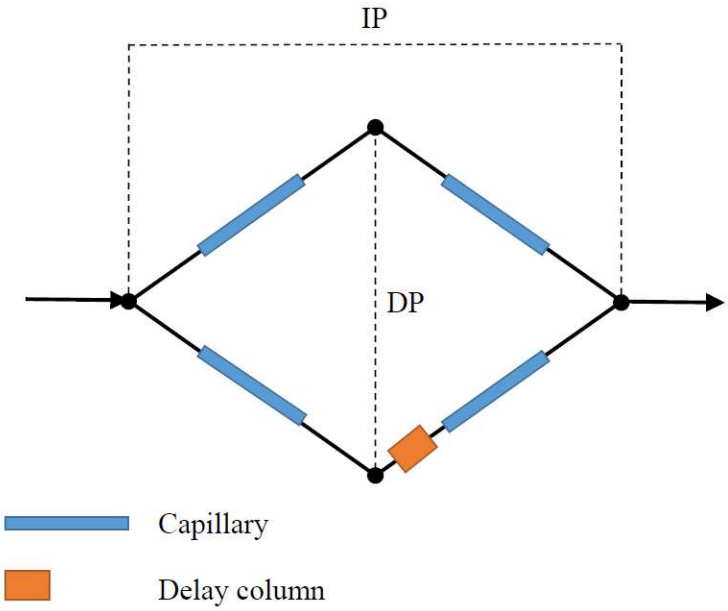


Figure 13. 4-capillary bridge viscometer.[78] The solution flows in the viscometer from the left, is divided to flow via two independent paths, is joined back together and finally is exited to the right.

As noted before, the VS detector determines intrinsic viscosity of the polymer samples. Mark-Houwink parameters (α and K) are constants which are used to determine the relation between the intrinsic viscosity $[\eta]$ and molar mass M.[81] This relation was found by Mark and Houwink in their separate researches (1938 and 1940), and the Mark-Houwink equation is expressed as

$$[\eta] = K \cdot M^{\alpha}. \qquad (9)$$

This equation can be utilized for many different polymers to find out the molar mass of the polymer.[81] The parameters α and K vary a lot between different polymers. Also the measuring temperature and the solvent used to dissolve polymer have a significant influence on the parameters. In addition, the tacticity of the polymer has a slight effect on these constants.[82]

The parameters are determined from the graphic linear curve where natural logarithmic of intrinsic viscosities of the polymer ($\ln [\eta]$) are shown as a function of natural logarithmic of molecular masses ($\ln M$).[81] The curve follows the straight line equation $y = kx + b$,

$$\ln [\eta] = \alpha \cdot \ln M + \ln K, \qquad (10)$$

from which can be seen that the parameter α is the slope of the line and ln K is the intercept of the line with the y-axis (Figure 14).

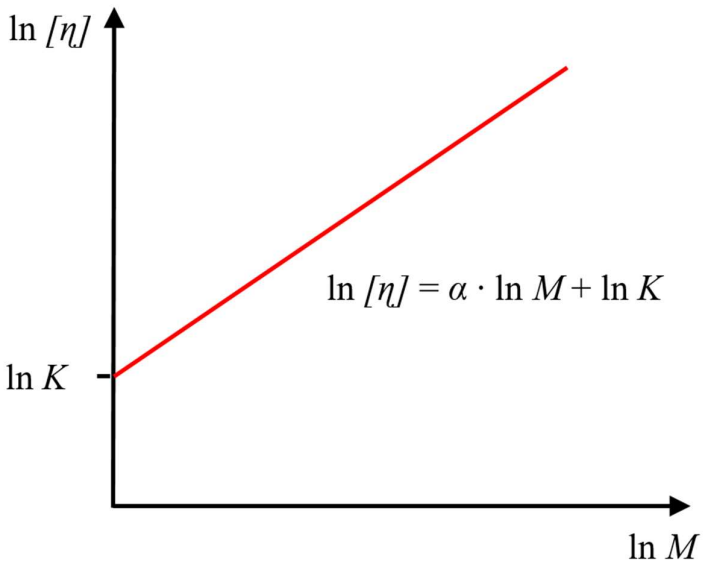


Figure 14. Theoretical Mark-Houwink plot.[67]

The linear curve utilized to determine the parameters can be made experimentally with the help of polymers of known molar masses and their intrinsic viscosities.[81] These polymers must be same type of polymers as the sample polymer. With these data, a graph from ln $[\eta]$ and ln M can be formed. The molar masses of polymers used in forming the straight line graph are generally determined with the help of light scattering method or osmotic pressure.

The values of Mark-Houwink parameters are related to the configuration of the polymer chain.[81] If α has values from 0.0 to 0.5, it is likely that in an ideal solvent the polymer is rigid and spherical. If α has values from 0.5 to 0.8, the polymer structure in a good solvent is a random coil. Good solvent is defined as a solvent which does not generate heat or it cools the mixture when mixing the polymer with the solvent. Also in the good solvent, the intrinsic viscosity is high and the polymer molecule extends loosely. When α has values from 0.8 to 2.0, the polymer chain is stiff and the structure is rigid or rod like.

It is quite laborious to determine the Mark-Houwink parameters without triple detection method and usually these parameters are searched from the literature. However, there are no literature values for α and K for all compounds and materials, and in that case they have to be determined experimentally for example with the iterative method.[81]

Table 2. Mark-Houwink parameters for different structured PLA in different solvents according to Garlotta.[36]

Type of the sample	α	K (dl/g)	Solvent, temperature and molar mass used in Mark-Houwink equation
PLLA	0.73	$5.45 \cdot 10^{-4}$	Chloroform, 25 °C, M_v
PDLLA	0.77	$2.21 \cdot 10^{-4}$	Chloroform, 25 °C, M_v
PDLLA	0.82	$1.29 \cdot 10^{-5}$	Chloroform, 25 °C, M_v
Linear PLLA	0.72	$4.41 \cdot 10^{-4}$	Chloroform, 25 °C, M_w
Star PLLA (six arms)	0.77	$2.04 \cdot 10^{-4}$	Chloroform, 25 °C, M_w
PDLLA	0.689	$2.59 \cdot 10^{-4}$	THF, 35 °C (iterative using GPC), M_v
PDLLA	0.639	$5.50 \cdot 10^{-4}$	THF, 31.15 °C (iterative using GPC), M_v
PLLA (amorphous)	0.68	$6.40 \cdot 10^{-4}$	THF, 30 °C, M_v
PLLA (amorp./semicryst.)	0.66	$8.50 \cdot 10^{-4}$	THF, 30 °C, M_v
PLLA (semicryst.)	0.65	$1.00 \cdot 10^{-3}$	THF, 30 °C, M_v
PDLLA	0.75	$2.27 \cdot 10^{-4}$	Benzene, 30 °C
PDLLA	0.64	$6.06 \cdot 10^{-4}$	Chloroform, 25 °C, M_v
PLLA	0.72	$5.72 \cdot 10^{-4}$	Benzene, 30 °C, M_v
PDLLA	0.78	$1.58 \cdot 10^{-4}$	Ethyl acetate, 25 °C, M_v
PDLLA	0.73	$1.63 \cdot 10^{-4}$	Ethyl acetate, 25 °C, M_w

There are some tabulated Mark-Houwink parameters for PLA in the literature and, for example, Garlotta[36] has collected some values for different structured PLA in his review. These values are presented in Table 2. However, in most cases, in the literature the parameters for PLA are reported without separating them according to the structure. For example, Dorgan *et al.*[83] gave only one general α and K for PLA (Table 3). If Mark-Houwink parameters for PLA samples dissolved in THF at 30 °C are compared between Dorgan *et al.*[83] and Garlotta[36], Garlotta's

values for α range between 0.65 and 0.68, whereas Dorgan *et al.*[83] gave one value for α of 0.736. In proportion, the values for K according to Garlotta[36] are between (6.40 and 10.0) $\cdot$ 10^{-4} dl/g whereas Dorgan *et al.*[83] gave a value of 1.74 $\cdot$ 10^{-4} dl/g for K. There is a lot of variation in the parameters which impacts to the molar masses determined.

Table 3. Mark-Houwink (M-H) parameters for PLA in different solvents according to Dorgan *et al.*[83] at 30 °C. In the M-H equation, the M_v was used.

Solvent	α	K (dl/g)
Chloroform	0.777	1.31 $\cdot$ 10^{-4}
THF	0.736	1.74 $\cdot$ 10^{-4}
CH_3CN and CH_2Cl_2 mixture	0.697	1.87 $\cdot$ 10^{-4}

When searching the Mark-Houwink constants for PLCL in literature, only one set of parameters were found (K = 3.303 $\cdot$ 10^{-3} (no units given) and α = 0.548 for copolymer with 45-55 % lactide content).[11] In case of these values, the solvent was not mentioned and also the units of the parameters are not known, so these values cannot be utilized. However, some values for PCL are found and for example according to Sun *et al.*[84], the Mark-Houwink constants for PCL in chloroform at 30 °C are K = 1.298 $\cdot$ 10^{-4} dl/g and α = 0.828 (M_w). In proportion, according to Iojoiu *et al.*[85], the parameters for PCL are K = 1.395 $\cdot$ 10^{-6} dl/g and α = 0.786. If these values are compared to the constants by Garlotta[36] for PLA in chloroform at 25 °C (K = (1.29-6.06) $\cdot$ 10^{-4} dl/g, α = 0.64-0.82), the PCL's values are almost in the range of PLA except for the K value measured by Iojoiu *et al.*[85]

For PLGA, the Mark-Houwink parameters are found in THF (temperature not informed) and they are K = 1.07 $\cdot$ 10^{-4} dl/g and α = 0.761.[86] Respectively, Mark-Houwink parameters for PLA in THF at 30-35 °C according to Garlotta[36] are in the ranges of K = (1.0-2.59) $\cdot$ 10^{-4} dl/g and α = 0.639-0.689. From these results, it can be noticed that values for K are in the same range for PLA and PLGA but PLGA has larger values for α than PLA has.

There are some differences between the Mark-Houwink parameters reported depending on the used molar mass average. For example, Dorgan *et al.*[83] reported two different Mark-Houwink equations for PLA in chloroform at 30 °C which differ with their molar mass averages:

$[\eta] = 0.0131 M_v^{0.777}$ and $[\eta] = 0.0153 M_w^{0.759}$. However, in this case the values for α and K are quite near to each other.

6.1.2.3 REFRACTIVE INDEX DETECTOR

The refractive index (RI) detector is a concentration sensitive detector and it measures the change in the RI of the solution when the sample flows through the column.[56,87] After measurement, these values are compared to the refraction index of the pure eluent. This detection method suits for the majority of the polymer samples excluding the isorefractive samples.

The RI is a material-specific constant where the speed of light in vacuum is divided with the speed of light in the material.[80] RI depends on the concentration of the solution and data of the measured refractive indices can be plotted against the concentration. This forms a straight line and the slope of this line is referred as *dn/dc* parameter.[8] This parameter is called refractive index increment. The *dn/dc* parameter is sample dependent and also the solvent, temperature, and wavelength have an effect to the results.[88]

The *dn/dc* values for PLA in certain solvents vary a lot in the literature. For example, Dorgan *et al.*[83] determined the *dn/dc* parameter for PLA of 0.081 ml/g when the solvent was a mixture of acetonitrile and dichloromethane (CH_3CN and CH_2Cl_2, respectively). On the other hand, Malmgren *et al.*[75] found out the corresponding *dn/dc* value but in chloroform twice and the results were 0.0237 ± 0.0034 ml/g and 0.0240 ± 0.0049 ml/g. For PLGA, the *dn/dc* value in THF is around 0.05 ml/g.[89] These values are quite small and, for example, for PS samples in chloroform the *dn/dc* value is 0.169 ml/g.[75] Different *dn/dc* values for PLA, PLGA, and PS are collected in Table 4. The corresponding values for PLCL were not found.

Table 4. Different *dn/dc* values for polylactide (PLA) and polystyrene (PS) samples.[77,83-87]

The type of the sample	*dn/dc*, (ml/g)	Solvent
PLLA[90]	0.0558	THF
PLLA[90]	-0.06	Bromobenzene, 85 °C
PLA[91]	0.042	THF
PLA[83]	0.081	CH_3CN/CH_2Cl_2
PLA[75]	0.0237	Chloroform
PLA[75]	0.0240	Chloroform
PS[91]	0.19	THF
PS[75]	0.169	Chloroform
PS[92]	0.185	THF
PLA/PLGA[89]	0.05	THF

The operating principle of the refractive index detector is based on the light beam refraction.[80] In refraction, the direction of the beam changes when it moves from the transparent medium to another. How much the direction of the beam changes depends on the characteristic refractive indices of the media according to the Snell's law of refraction

$$\sin\alpha \cdot n_1 = \sin\beta \cdot n_2 , \qquad (11)$$

where α is the incident angle of the beam of light, β is the refraction angle, n_1 is the refractive index of the medium from which the beam comes to the interface of the two media and n_2 is the refractive index of the medium in which the beam goes after passing the interface.

With this equation, the unknown refractive index can be solved if the rest of the variables are known. When the beam travels from material with lower refractive index into the material with higher refractive index, the beam deflects towards the perpendicular of the surface and in proportion on the other way round.

In the RI detector, the beam goes through a cell with two compartments full of reference solution and sample solution.[8] At first, the light beam travels in the air and encounters the interface of glass of the cell penetrating it. After that the beam gets on to the first compartment

full of reference solution, which has the known refractive index n_0. From the reference solution the beam continues through the glass wall to the next compartment full of sample solution. Finally, the beam travels through the glass back to the air and the total reflection can be measured with two photodiodes. In every interface the beam faces during its journey, the light is refracted (Figure 15) and because all the refractive indices except the sample solution's are known, the sample's RI can be calculated. The RI of the sample changes according to the sample concentration and the refractive index increment *dn/dc* can be determined. It has been noticed that the analysis can be simplified so that the glass interfaces can be ignored. This simplification does not affect significantly the results.

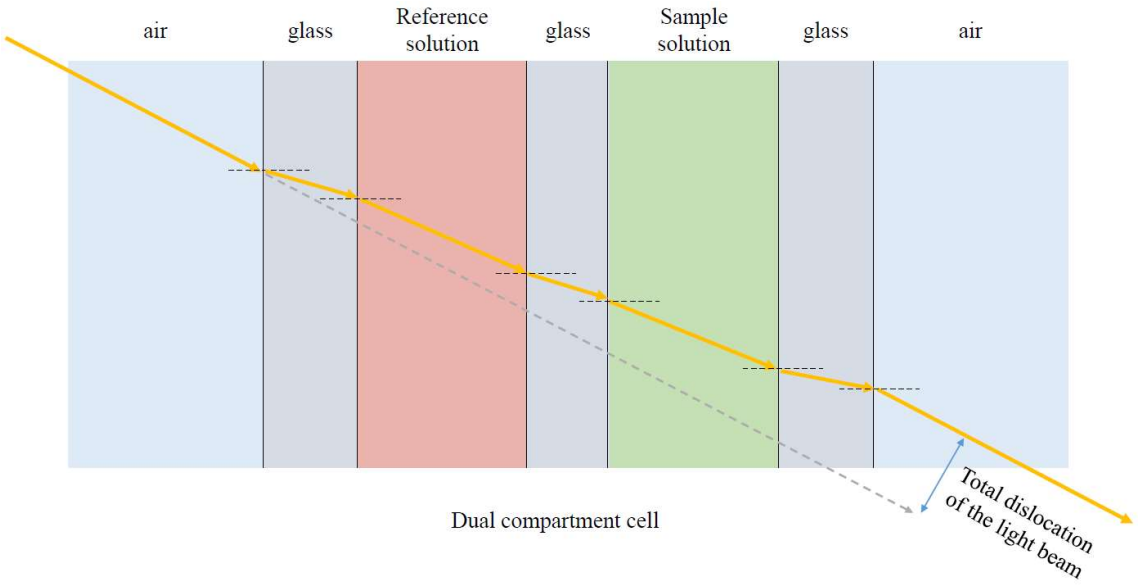


Figure 15. Travel of light beam in the RI detector. The deflection angles are approximate and estimated with the refraction indices for air 1.0, glass 1.5, and for the solutions near 1.3 (water).[80] In reality, especially the refraction indices for the solutions can vary which changes the total dislocation of the beam. The perpendiculars of the interfaces are symbolized with black dashed lines.

6.1.3 UNIVERSAL CALIBRATION

It is also possible to calibrate the GPC system with calibration method called the universal calibration. In this calibration method, the calibration curve is formed with different standards.[72] However, the type of the samples and standards do not have to be similar as those in the conventional calibration. This is because to form a calibration curve, the hydrodynamic volumes (V_h) instead of molar masses of the standard polymers are utilized. In the universal

calibration, log (V_h) is plotted versus retention time and a calibration curve is formed. V_h follows the equation $V_h = [\eta] \cdot M$ as presented in the equation (1). The universal calibration is generally utilized with low molar masses and low *dn/dc* values.[8]

Universal calibration can be divided into two different categories.[74] In the universal calibration I, the calibration is performed with different molar mass standards and also the material specific Mark-Houwink parameters from the literature are utilized during the calibration. In the universal calibration II, the Mark-Houwink parameters are not needed and instead of these parameters, on-line viscometer detection is required. In the experimental part of this thesis, universal calibration I method was utilized.

6.1.4 DIFFERENCES BETWEEN DIFFERENT CALIBRATION METHODS

As can be seen from the previous sections, there are several differences between the conventional calibration, universal calibration I and triple detection methods. The differences are collected in Table 5.

Table 5. Pros (+) and cons (-) of the triple detection, universal calibration I, and conventional calibration systems.

	Triple	Universal I	Conventional
Affordable equipment	-	+	+
Easy to calibrate	+	-	-
Good accuracy	+	-	-
Possibility to measure different types of samples	+	-	-
Information rich method	+	-	-
Need of Mark-Houwink parameters from the literature	-	+	-

A GPC device with conventional calibration system is the most affordable to purchase and for that reason, it is generally used method in companies. However, conventional calibration is a comparative method which requires the measuring polymer and calibrants to have the same chemistry for reliable results.[78] This delimits the polymers which can be measured with this method. This problem is not noticed in triple detection and universal calibration. Also in the conventional calibration and universal calibration methods, there is a need for several different

calibrants to form a calibration curve. With the triple detection system there is no such problem and only one calibrant is needed. The triple detection method also gives much more information of the sample polymers than the conventional and universal calibration methods because of the several different detectors. In addition, because of the several detectors, the triple detection method determines the Mark-Houwink parameters itself and the results do not depend on the used standards or the parameters found from the literature. Correspondingly in the conventional calibration, the results depend on the used standard polymers and in universal calibration, the results depend on the used Mark-Houwink parameters.

6.1.5 DIFFERENT TYPE OF COLUMNS

It is important to choose an appropriate column to the GPC equipment because it determines how the molecules with different sizes are able to be separated from each other during the GPC run.[93] The column must be suitable for the sample's precise and particular molar mass range and this range can be adjusted by altering the pore size and distribution of the column gel. This way, the retention to size –relationship can be modified and better results can be achieved. There are single pore size columns and columns containing gel with different pore sizes (mixed-bed columns) on the market.[94]

One of the main reasons why the careful column selection is important is that if there is an interaction between the column gel and sample material, it affects the results of GPC.[93] If the column gel molecules and sample molecules interact with each other, it takes more time for sample molecules to flow through the column. This extends the retention times. In that case, the differences between the separation times are not only result from the size of the molecules according to the GPC principle and the results are incorrect. For this reason, these interactions which can be caused, for example, by the differences between the polarities of the sample and column gel must be minimized.

There are different types of columns designed for distinct solvent/sample use to minimize the interactions between the column gel and sample material. The column manufacturers have almost similar column selections with different names and, for example, Agilent has columns for GPC called PLgel, PolarGel, and PL aquagel-OH.[93] In the PLgel columns, there are cross-linked polystyrene-divinylbenzene particles in the column gel and these columns are designed so that they can be used especially with many organic solvents. In PolarGel columns there is a unique particle composition and they can be used with polar samples when the polar organics

or water/organic mixtures are utilized as a solvent. In the PL aquagel-OH columns there are hydrophilic particles in the column gel and these columns can be used with water, high-salt buffers and up to 50 % methanol.

In the experimental part of this thesis, the mixed-bed PLgel columns were utilized and that is why they need more accurate examination. There are many different PLgel columns based on their molar mass operating ranges and some of these are referred as MIXED-A, B, C, D, and E columns.[95] Because the columns have different operating ranges, the columns are suitable for different-sized polymers. Additionally, the operating temperature has an influence to the decision which column to use. The molar mass ranges and temperatures for different PLgel MIXED columns are collected in Table 6. The linear molar mass operating range means that the calibration of the column is designed so that the calibration curve is linear in this certain molecular range.

Table 6. Linear molar mass ranges for MIXED-PLgel columns.[96] Also, the operating maximum temperatures for the columns are included.

PLgel column	Linear molar mass operating range (g/mol)	Maximum operating temperature (°C)
MIXED-A	2000 - 40000000	220
MIXED-B	500 - 10000000	220
MIXED-C	200 - 2000000	150
MIXED-D	200 - 400000	150
MIXED-E	up to 25000	110

As can be seen from Table 6, the MIXED-A and MIXED-B columns are suitable for high molar mass polymers, whereas the MIXED-C columns are aimed for mid-range polymers.[95,96] MIXED-E columns are designed for low molar mass polymers and MIXED-D columns are engineered, especially for condensation polymers and resins.

It is possible to couple several columns one after another and most of the column sets are composed of two to four columns.[76] If identical pore-sized columns are coupled, the resolution can be increased and when doubling the column length, the resolution increases by factor of 1.4.[97] On the other hand, if different pore sized columns are coupled, the better molar mass

separation ranges in GPC may be achieved.[98] However, by coupling the single pore size columns, the calibration curve is seldom linear and this may cause a higher chance for errors. For that reason, if the columns are coupled, usually these mixed-bed columns are used.

Generally, there is also a guard column before the proper columns.[99] The guard column can remove possible impurities from the GPC run. In this case, the contaminants do not access the proper columns where the separation takes place. Also this way the operating life of the separating columns are extended.

When coupling the columns, the time of the sample run and also the backpressure in the column increases due to the growth in the total length of the column.[97] Also the solvent consumption increases and if the columns coupled have different pore sizes, there is a possibility of column mismatch which can be seen, for example, as "shoulders" in the analysis results. That is why it is not profitable to set too many columns in series. It is usually considered that the diameter of the columns coupled and the packing type of the columns should be quite the same whereas the order of the columns does not have a clear consensus.[7] For example, in the study made by Kempf *et al.*[98] there was no notable difference observed in the measured M_w values when changing the order of the columns in the GPC run. However, most of the column manufacturers recommend that the columns should be set in the order of decreasing pore size.[7]

6.2 MEASURING VISCOSITY

6.2.1 DIFFERENT VISCOSITIES

The term fluid refers to a material which is in either liquid or gas state.[100] If the fluid experiences any shear stress, there will be motion in the fluid and the motion continues until the shear stress is stopped. This does not happen for solid objects. Fluids have a property called viscosity (η) that is related to the resistance observed when the fluid is flowing.[80] Thus, viscosity corresponds to the friction in kinetics. Viscosity is a sublevel of the branch of science called rheology where the flow behavior and the deformation of the materials is studied.[101]

There are high- and low-viscosity fluids and they are divided based on their ability to resist deformation of the fluid.[101] In the same temperature, the low-viscosity fluids hardly resist the deformation and flow easily whereas high-viscosity fluids resist deformation and can be quite

stiff. That is why flowing of the material takes more time for high-viscosity fluids than for low-viscosity fluids in the same temperature.

Ideally, viscous fluids are referred as Newtonian liquids.[101] They are nonviscous and in Newtonian liquids, the external force does not affect to the internal flow resistance of the fluid. One common example of Newtonian liquids is water. For non-Newtonian liquids, the viscosity changes according to the external shear stress and the apparent viscosity can be determined. All non-Newtonian liquids do not act the same way and, for example, from the change in the shear rate cannot be concluded how the viscosity of the fluid changes. Therefore, a material-specific research or knowledge from the literature is required. Polymer solutions are fundamentally non-Newtonian, but their shear rates are so low that they are considered as Newtonian liquids.

There are three aspects which affect to the flowing of the material: the shear stress, which is caused by external force (such as gravity, pushing, and pulling), the inner structure of the material (tight/loose) and the flowing conditions, such as pressure and temperature.[101] Increasing temperature decreases the viscosity of the material and this relation works for all fluids. In addition, the pressure normally influences the viscosity so that the increasing pressure causes increasing viscosity. The type of the material has an influence on the level of the change in viscosity caused by temperature or pressure.

Viscosity can be divided into different subsections including relative, inherent, specific, and intrinsic viscosity.[101] Relative viscosity η_r is important parameter when analyzing polymer solutions and is related to many other viscosities. It is defined as

$$\eta_r = \frac{\eta}{\eta_0}, \tag{12}$$

where η is the viscosity of the sample solution and η_0 is the viscosity of pure solvent.

Relative viscosity value is used to determine many other parameters which are required when polymers are studied.[101] These kind of parameters are inherent viscosity (also known as logarithmic viscosity number), specific viscosity (also known as relative viscosity increment), intrinsic viscosity (also known as Staudinger function or limiting viscosity number (LVN)), and molar mass.

Inherent viscosity (η_{inh}) is defined with the help of relative viscosity[9] as

$$\eta_{inh} = \frac{\ln \eta_r}{c} \ , \qquad (13)$$

where c is the concentration of the solution.

In proportion, specific viscosity (η_{sp}) can be also defined with relative viscosity[81] and it can be expressed as

$$\eta_{sp} = \eta_r - 1 = \frac{\eta - \eta_0}{\eta_0} \qquad (14)$$

Specific viscosity is used to determine intrinsic viscosity. Intrinsic viscosity ($[\eta]$) can be determined by way of specific viscosity with Huggins equation

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c} \ , \qquad (15)$$

where c is the concentration of the polymer (g/ 100 ml solution).[9] The term $\frac{\eta_{sp}}{c}$ is called reduced viscosity and if reduced viscosity is plotted versus concentration c, the intercept of the straight line gives the value for intrinsic viscosity.[81] Also if $1/c \cdot \ln \eta_r$ is plotted versus c, the intrinsic viscosity is also found from the intercept the line forms.

Intrinsic viscosity can be also determined by using the inherent viscosity of the sample with the Craemer equation[9]

$$[\eta] = \lim_{c \to 0} \eta_{inh} \qquad (16)$$

The term intrinsic viscosity refers to the molecular density of a sample polymer and the polymer's ability to affect to the viscosity of the solution.[9] When the sample polymer is tight and compact, it has relatively low intrinsic viscosity and in proportion if the polymer has loose structure, it has relatively high intrinsic viscosity.[8] The molecule's intrinsic viscosity affects to the viscosity of the whole solution and the viscosity of the solution changes when the concentration of the sample polymer is varied. The intrinsic viscosity of the sample polymer can be calculated by measuring the total viscosity of the diluted solution when changing the

sample polymer amount in the solution. The diluted solutions are utilized because the interactions between the sample molecules are desired to be as weak as possible. According to the Mark-Houwink equation (9) it can be also noted that the larger the molecule (larger molar mass *M*), the larger the intrinsic viscosity. Different intrinsic viscosity values of PLA are gathered in Table 7. From these values, it can be seen that the intrinsic viscosity values can have some variation.

Table 7. Intrinsic viscosities for PLA samples.[90]

The type of the sample	$[\eta]$, dl/g	Solvent and temperature
PLLA	3.8-8.2	Chloroform, 25 °C
PLLA	2.63	Chloroform, 30 °C
PLLA	4.2	Chloroform
PLLA	1.38	Bromobenzene, 85 °C
PLLA	3.2	Dioxane
PDLLA	0.1-1.5	Chloroform, 25 °C

6.2.2 MICROVISCOMETER

Viscosity can be measured with different methods.[10] For instance, there are methods where the sample liquid flows through the glass capillaries based on the gravity but also different pressurized devices to induce flowing are exploited. The pressure can be generated with weight, gas or electrically with a motor. There are also falling-ball and rolling-ball viscometers where a specific ball rolls through the sample solution in inclined capillary. The viscometer is called rolling-type, if the inclination angle is between 10° and 80° and if the angle is more than 80°, the viscometer is falling type. In all of these methods time (liquid's flow time or rolling/falling time of the ball) is measured and utilized to determine different types of viscosities.

In the experimental part of this thesis, the Anton Paar Lovis 2000 M/ME –microviscometer was used to measure the inherent viscosity of the samples. This device can measure intrinsic viscosity of the polymer and is suitable also for transparent samples.[102] The idea of this microviscometer is based on the principle of the Höppler's rolling ball.[10] In this microviscometer, the ball with known dimensions rolls in the wanted angle between 15° and 80° through a micro capillary full of sample liquid and the spent time of the rolling ball

is measured. The rolling time in the fixed distance is directly proportional to the viscosity of the sample. Measuring range and the precision of the measurements are dependent on the ball utilized and during the rolling, the temperature is controlled with the help of liquid bath thermostat.

One significant advantage of the microviscometer is the low need of sample solution and only 100 µl can be enough for measurements.[10] This amount of the sample solution depends on the viscosity of the sample. There are also other advantages. The microcapillaries can be made from glass but also capillaries from the PCTFE (polychlorotrifluoroethylene) are available for the samples which can corrode the glass. The time the ball spends in the capillary is registered with inductive sensors so the manual stop watch is not needed. The microviscometer is best for low-viscosity samples and for higher viscosities for example motor driven viscometers are more suitable.

6.2.3 UBBELOHDE VISCOMETER

As mentioned before, there are also glass capillary viscometers which are resting upon the gravity. One of these kinds of viscometers is called the Ubbelohde viscometer named after a German chemist Leo Ubbelohde.[10] In this viscometer, the sample solution is set in the Ubbelohde viscometer (branch 1, Figure 16) and it is either pressed or sucked in the branch 2 with overpressure.[103] After that, the shifting of the meniscus from the point M_1 to M_2 is followed. At the same time the time is measured. There are manual and automatic gravimetric capillary viscometers and with manual ones, the stop watch is used to measure the sample flow time.[10] However, this is quite impractical and devices with automatization have been developed.

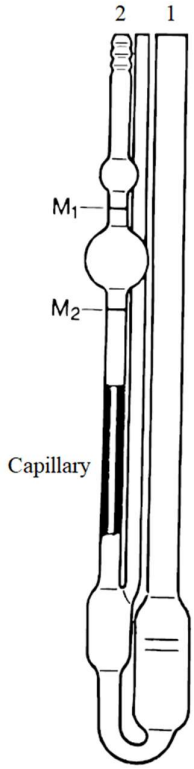


Figure 16. Ubbelohde glass capillary viscometer. Modified from the picture of Wilke *et al.*[103]

Gravity-based viscometers have many benefits.[10] Because the gravity exists worldwide, the method is free of charge as for driving force. Also the gravity does not need separate equipment or maintenance to work. However, there are also downsides for gravity-based devices. The strength of the gravity cannot be adjusted and it is not strong enough for all samples, such as high viscosity samples. To measure a wide range of viscosities, several capillaries with different dimensions are needed. In this thesis' experimental part, the Ubbelohde viscometer was not utilized. However, the used Anton Paar microviscometer was set to correlate the conditions of the Ubbelohde viscometer.

7 SUMMARY OF THE LITERARY PART

Polymers are long-chained molecules consisting of smaller subunits, monomers. The type of monomers determines the properties of polymers and according to the monomer constitution, there are homopolymers and copolymers. Polymers are usually formed either by condensation or addition reactions and they can be divided into natural polymers, natural modified polymers, and synthetic polymers based on their origin.

Polymers are widely used in different applications of which the industrially used polymers, also known as plastics, are probably the most well-known. Traditionally, the plastics have been petroleum-based and non-biodegradable but nowadays, there are also several different bioplastics developed. Bioplastics are biobased and/or biodegradable and one good example of plastic having both of these properties is PLA. The basic material of PLA is chiral lactic acid which can be produced by bacterial fermentation. In polymer construction, the used lactic acid molecules define the three-dimensional structure of the forming PLA that can be either PLLA, PDLA or PDLLA. Usually, only PLLA and PDLLA are found. There are different characteristics with different structures and, for example, PLLA is the most resistant of the PLA structures due to its reinforcing crystalline domains.

PLA is generally produced from lactide rings by ROP. With this way, high molar mass PLA with better properties compared to low molar mass PLA can be manufactured. Also other manufacturing routes exist. To have new properties, PLA can be also blended with other polymers or copolymerized with different comonomers. These comonomers can be, for example, glycolide rings (copolymer PLGA is formed) or ε-caprolactone rings (copolymer PLCL is formed). There are many different applications for these copolymers and they can be used in medical applications in sutures, drug delivery, and medical implants. New possible uses are constantly researched.

The medical use sets some requirements to the materials. PLA and its copolymers are suitable for these kinds of applications because they are biodegradable and during the degradation, toxic compounds are not formed. Also, the degradation products of PLA do not accumulate to the body and can be excreted naturally in tricarboxylic acid cycle. This has been discovered in several animal, human, and *in vitro* tests. The materials used in medical field are strictly supervised by different organizations, including FDA. FDA sets the regulations which should be fulfilled to get an approval to use product in medical applications. For example, the degradation rate of the material should be known.

During the polymerization, polymers with different lengths are formed and they can be characterized with different methods by GPC. By this technique, different average molar masses and *PD* of the polymer sample can be found out and also by this way the degradation of the polymer can be followed. In GPC, the polymer chains are separated in a column full of porous gel based on their hydrodynamic radius. There are different columns for different

purposes (single pore and mixed bed columns) and often there is a couple of columns connected together to get the best result.

There are GPC devices which differ with their detection methods. The amount of detectors affects to the way of calibration and data analysis, and if there is only one detector (usually RI), the conventional calibration method is utilized. In the conventional calibration method, there is a need of several calibrants with known molar masses which are utilized to form a calibration curve. In the universal calibration method, there is a same idea as in the conventional calibration but the calibration curve is formed based on the hydrodynamic volume of the calibrants. In addition, there is also a need of Mark-Houwink parameters or on-line viscometer in the universal calibration unlike in the conventional calibration. The universal calibration method can be divided into two different categories (I and II) based on the need of Mark-Houwink parameters or on-line viscometer detection.

In some GPC devices there are three different detectors (triple detection, typically RI, LS, and VS detectors), and they can measure several different variables from the polymer samples. That is why in triple detection analysis method there is need for only one standard which can be utilized to align the different signals. The RI detector follows the concentration of the sample and at the same time it measures the change in the refractive index (*dn/dc*). In proportion, the LS detector is based on the scattering of the light from the sample and the intensity of the scattered light is measured in different angles. The VS detector measures the viscosity of the sample and compares it to the viscosity of the pure eluent. There are different type of viscosities including relative, inherent, specific and intrinsic viscosities. The viscosity of the sample can be also measured with the microviscometer or the Ubbelohde viscometer. The idea of microviscometer is usually based on the Höppler's rolling ball principle, whereas the Ubbelohde viscometer relies on gravity in its operation principle.

To link the viscosity and molar mass together, the Mark Houwink equation is generally utilized. In this equation, there are two material-characteristic parameters, α and K. These parameters may indicate the configuration of the polymer chains in the sample. The triple detection system can determine these parameter but in conventional calibration, the parameters must be searched from the literature.

EXPERIMENTAL PART

8 INTRODUCTION

Different lactide-based polymer samples (PLLA, PLGA, and PLCL) were measured by GPC (Agilent 1260 Infinity MDS) and analyzed with the triple detection, universal calibration I and conventional calibration methods. The results, especially different molar mass averages and *PD*s, were compared to find out if there is a difference in the results due to the different analyzing methods. Also, the influence of different Mark-Houwink parameters on the results was studied when analyzing PLLA samples with the universal calibration I method. Additionally, the Mark-Houwink parameters and *dn/dc* values of the samples were determined with the triple detection method and they were utilized in the comparison of the materials. The comparison between the measured values and the literature ones was performed. The samples for the GPC measurements were prepared so that they were dissolved completely in chloroform, filtered, and measured with GPC. In the equipment, THF was used as an eluent. The sample preparation and the results are presented in the experimental GPC part.

The inherent viscosities of the samples were determined using an Anton Paar – microviscometer (Lovis 2000 M/ME). Also in these measurements, the samples were first dissolved in chloroform, filtered, and then measured. The results were compared to the values informed by the manufacturers. The sample preparation and the results for these measurements are presented in the experimental inherent viscosity part.

GPC PART

9 EQUIPMENT

The GPC measurements were carried out with an Agilent 1260 Infinity Multi Detector Suite (MDS) device. In this device, there was an Agilent 1260 Infinity Quaternary Pump (G1311B) containing a 4-channel vacuum degasser to pump the eluent into the system. The autosampler was G1329B and the thermostatted column compartment G1316A. The used device consisted of three different detectors (G7800A): a dual light scattering detector (measuring in the angles of 15° and 90°), an RI detector, and a VS-detector. In the measurements and data analysis, an Agilent GPC/SEC Software, version A.02.01 was used. There were two columns in series

(PLgel MIXED-C and PLgel MIXED-D) and before the columns there was also a guard column (Agilent GPC/SEC Guard Column).

In weighing, Mettler Toledo XP205 scales was used. Additionally, the dosing feeder (Brand Dispensette organic) and sample shaker (Stuart orbital shaker SSL1, rate 80 rpm) were used in the sample preparation. The samples were prepared to the vials (Agilent, screw, 2 ml) and closed with caps with septums (Agilent PTFE/WS). The filtering to the vials was carried out with a syringe (Henke Sass Wolf, Norm-Ject, luer lock, 2 ml) equipped with a syringe filter (Agilent Captiva PTFE 0.2 µm). The samples were sucked into the syringes from the measuring bottles with the help of needles (Braun 0.90 x 70 mm BL/LB).

The used solvents in the measurements were chloroform (Honeywell Chromasolv for HPLC, amylene stabilized) and THF (Honeywell Chromasolv plus for HPLC, Riedel-de Haën, ≥ 99.9 % and in one measurement made on 14.2.2018 Merck, EMSURE for analysis).

10 SAMPLES AND STANDARDS

There were three different types of samples (PLLA, PLGA, and PLCL) with different inherent viscosities used in the measurements. The information about the samples is collected in Table 8. The standards used in the measurements were PSs with different M_p values in the range of 575-3187000 g/mol. When analyzing the results with the triple detection method, only the PS standard with M_p of 70500 g/mol was utilized. In the universal and conventional calibration, all of the standards of the standard kit, except for the smallest M_p were used to form a calibration curve.

Table 8. Information about the measured samples. The inherent viscosity of the samples has been informed by the manufacturer.

The sample polymer	Inherent viscosity (dl/g)	Manufacturer	Abbreviation used in the measurements
PLLA	1.0	Corbion Purac	PL10
PLLA	1.8	Corbion Purac	PL18
PLLA	2.4	Corbion Purac	PL24
PLLA	3.2	Corbion Purac	PL32
PLLA	3.8	Corbion Purac	PL38
PLLA	4.9	Corbion Purac	PL49
PLLA	6.5	Corbion Purac	PL65
PLGA	2.2	Evonik Industries	PLG22
PLGA	2.4	Corbion Purac	PLG238
PLGA	3.1	Corbion Purac	PLG311
PLGA	6.2	Evonik Industries	PLG62
PLCL	1.5	Evonik Industries	PLC15

PLLA samples (Purasorb PL, Poly(L-lactide) were produced by Corbion Purac (The Netherlands). The inherent viscosities of the samples were 1.0, 1.8, 2.4, 3.2, 3.8, 4.9, and 6.5 dl/g and the abbreviations PL10, PL18, PL24, PL32, PL38, PL49, and PL65 were used, respectively, for these samples. All of the samples were solid, white in color, and a bit translucent grains (Figure 17). They were packed in double aluminium bags and were stored in a freezer (-60 °C or -20 °C). From these bags, part of the contents was transferred to double plastic bags from which the samples for the analysis were taken. This transferring to the plastic bags was made to avoid the defrosting of the whole sample batch every time when the samples were needed. The samples were hygroscopic and it was important to let the samples set to room temperature before opening the plastic bags.

There were four different PLGA samples measured in the analyses. Two of them were produced by Evonik Industries (Germany, Resomer LG 824 S, 82 % L-lactide and 18 % glycolide content with inherent viscosity of 2.2 dl/g and Resomer LG 857 S, 86 % L-lactide and 14 % glycolide content with inherent viscosity of 6.2 dl/g). The other two samples were made by Corbion Purac (Purasorb PLG 8523 and Purasorb PLG 8531) and the ratio of L-lactides and glycolides in these

samples was 85/15 for both of them. The inherent viscosities of these samples were 2.38 and 3.11 dl/g, respectively. The PLGA samples were marked with abbreviations of PLG22, PLG238, PLG311, and PLG62 according to the inherent viscosities of the samples informed by the manufacturer. All of the samples looked white to off-white translucent solid grains (Figure 17) and they were stored in a freezer (-20 °C) in a double aluminium bags as the PLLA samples. The PLGA samples produced by Purac seemed more yellowish than the Evonik's samples. The samples were let to set to room temperature before the bags were opened. Also with these samples, part of the aluminium bag contents was transferred to the double plastic bags so that the unnecessary defrosting of the whole sample batch was avoided.

There was only one PLCL sample measured. It was produced by Evonik Industries (Resomer LC 703 S) and the ratio of L-lactide and ε-caprolactone in the sample was 68/32. The inherent viscosity of the sample was 1.5 and the sample was marked with the abbreviation of PLC15. Also the PLCL sample looked like white solid grains (Figure 17) and was stored and handled similarly as the PLLA and PLGA samples.

The utilized PS standards were from two Agilent calibration kits (S-M-10). In the first kit (used only in the measurements made on 14.2.2018), there were eight different PS standards with the M_p values of 575, 1320, 4830, 9970, 29150, 70500, 224900, and 990500 g/mol. The expiry date of the first kit was 1.3.2017 so these standards were out of date. However, it did not considerably affect the results. In the second calibration kit which was used in all other measurements there were ten different PS standards with the M_p values of 580, 1230, 4750, 9570, 27810, 70500, 187700, 466300, 1044000, and 3187000 g/mol. The expiry date of the second kit was 4.2.2025. The PS standards were white, solid, and powdery apart from the smallest molar massed standards (M_p = 575 or 580 g/mol). These standards were translucent and sticky, like honey, and they were difficult to weigh. The standards were normally stored in the fridge (4 °C) and before using them they were let to settle at room temperature.

Figure 17. Used materials. From the left, PLLA (inherent viscosity 3.2 dl/g), PLGA (produced by Evonik, inherent viscosity of 2.2 dl/g), PLGA (produced by Purac, inherent viscosity of 3.11 dl/g), PLCL (inherent viscosity of 1.5 dl/g), and polystyrene standard (M_p = 70500 g/mol).

10.1 PREPARATION

The sample preparation and the measurements were carried out at room temperature (21-23 °C). The standards were normally stored in a fridge (4 °C) and the samples in a freezer (-60 °C or -20 °C). Before the measurements they were allowed to settle at room temperature at least for 2 hours.

In the PLLA sample preparation, about 100 mg of each polymer sample was weighed in the 50 ml measuring bottles. However, in two measurements made on 19.4.2018 and 14.6.2018 about 200 mg of samples were weighed in the 100 ml measuring bottles. In the PLGA and PLCL sample preparation, all the weighings were done in the 100 ml measuring bottles in the same way as the PLLA samples. The weighed amount of polymer was carefully written down in the laboratory book for the precise concentration calculation (about 2 mg/ml). After that, the measuring bottles were half filled with chloroform. The filling was executed with a dosing feeder. All the samples were prepared the same way. After adding chloroform, the measuring bottles were put in the shaker and were allowed to dissolve overnight. With the standards, this same protocol was carried out except for weighing: for the standards about 20 mg of the each standard was measured in a 20 ml measuring bottle (concentration about 1 mg/ml).

On the next day, the samples and standards were taken out of the shaker and filled with the chloroform to the containing mark. The filling near to the mark was carried out with dosing feeder and the final filling was performed with the help of a glass Pasteur pipette. After the

filling, the measuring bottles were carefully shaken by hand. Then the samples and standards were filtered with a syringe to the GPC vials so that the vials were ½ - ⅔ full. The first 5-6 drops of the samples pushed through the filter were dropped to the waste beaker. When sucking the samples into the syringe from the long-necked measuring bottles, the syringe attachable needles were used. THF was also filtered to the one vial.

10.2 MEASUREMENTS

In the GPC measurements, the Solvent Enhancement method was used. As mentioned before, the PLA samples were dissolved in chloroform and during the GPC run the samples were shifted to THF which was used as an eluent in the GPC run. This solvent change was carried out because polylactides generally dissolve well in chloroform but not in THF.[2] Also acetonitrile, dioxane, methylene chloride, dichloroacetic acid, and 1,1,2-trichloroethane dissolve polylactides. If THF or, for example, toluene, ethyl benzene, and acetone were used in dissolution, the polylactides would be only partially dissolved (at the temperature under boiling temperatures).

However, if chloroform was used as an eluent in the GPC, the signal of the RI detector would be very weak because of a small *dn/dc* value. This is because the refractive indices of solvent and polymer are near to each other.[104] In THF, the RI signal is much stronger and that is why it is profitable to change the solvent from chloroform to THF in the measurements. Also, the LS signal is stronger when PLA is transferred from chloroform to THF because PLA has a larger refraction index in THF than in chloroform. The solvent change also caused an extra peak of chloroform to the results at a retention time of about 21 min.

There was constantly a slow flow of eluent in the GPC (0.4 ml/min). Before the measurements, the flow of the eluent (THF) was increased slowly from 0.4 ml/min to 1.0 ml/min. This raising was necessary to do at least two hours before the measurements so that the columns had time to stabilize. The most stable signals from the detectors were achieved when increasing of the eluent flow was done already a day before the measurements. THF was used as an eluent in every measurement.

In the method used in the measurements, the temperature of both the thermostatted column compartment and the detectors of the GPC was set to 30 °C. The purge time was 600 s for the viscometer (IP and DP) and RI detectors and the pressure limits for the pump were 0-150 bar.

The analysis stop time was 35 min. The injection volume for the standards and samples was set to 100 µl.

The sample sequence was prepared so that it started with pure THF. After THF, there were the standards and samples and the sequence ended with the pure THF, which was injected from the same vial as the first THF. Before the last THF, there was also a control injection of one of the standards (usually PS with M_p = 70500 g/mol). After the last injection, the eluent flow was lowered back to 0.4 ml/min. In the measurements, there were two separate parallel samples for every sample.

Before the sequence run was started, the RI and viscometer detectors (VS IP and VS DP) were purged in the order of VS IP, VS DP, and RI. Every purge took about ten minutes. After purging the detectors, the sample run was started. In addition, the viscometer detector's inlet pressure was checked during the run because it was needed in the data analysis.

After the GPC run, the results were analyzed. Before the proper sample data analysis, the calibration of the detectors was executed. In the calibration with the triple detection method, first the baseline (dash line under the signal) was set for the standard (M_p = 70500 g/mol) with the help of two blue regions (Figure 18). The baseline was set to answer the signal base as well as possible. This was done similarly to all of the samples so that the results would be comparable. The blue baseline regions were placed quite near the signal peak and the suitability of the baseline regions was checked separately with all of the signals. Especially, the RI signal was paid attention to. When setting the baseline regions, the RI signal was zoomed so that the maximum value for the signal was about 10 mV and this scale was not changed when the other signals were observed. When the baseline setting was ready, the green integration region for the PS standard was set (Figure 18) and also in case of the integration region, especially the RI signal was observed. If there was some shoulders in the standard peaks, they were included in the integration region. During the measurements, the viscometer detector's inlet pressure was 41.1-41.3 kbar and this value was used in the calibration.

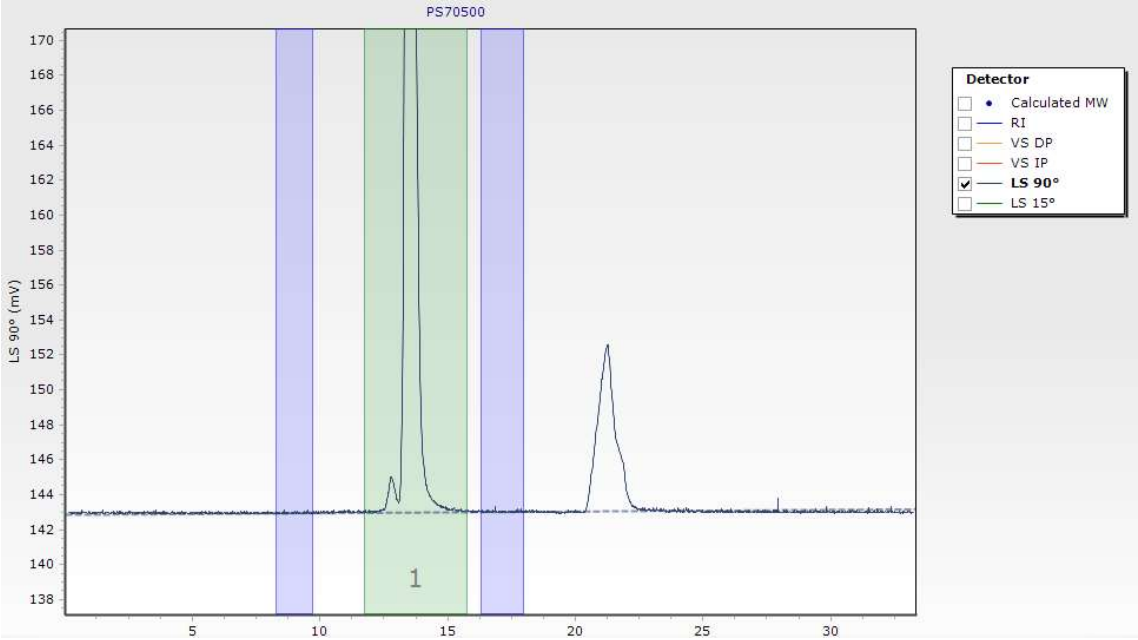


Figure 18. Setting the baseline and integration regions for the standard sample (polystyrene, M_p = 70500 g/mol). The peak on the right (at the retention time ≈ 21 min) is caused by chloroform.

When doing the calibration with the universal calibration I and conventional calibration methods, the calibration was executed with several PS standards to form a calibration curve. The standard with M_p of 580 g/mol was excluded from the calibration due to the small molar mass and weighing difficulties. A new calibration was always created for measurements made on different days. In the calibration, first the baseline and the integration regions were determined for all of the standards and then they were added to the same calibration. When adding the standards to the calibration, the M_w values of the standards and Mark-Houwink parameters of PS were provided to the program. These Mark-Houwink parameters used for standards (PS) in both the universal calibration I and conventional calibration were $\alpha = 0.7$ and $K = 14 \cdot 10^{-5}$ dl/g which were automatically proposed by the program. When all of the standards were applied to the calibration, they formed a curve. The curve fit was set to answer 3rd degree so that the curve responded to the standard dots the best (Figure 19).

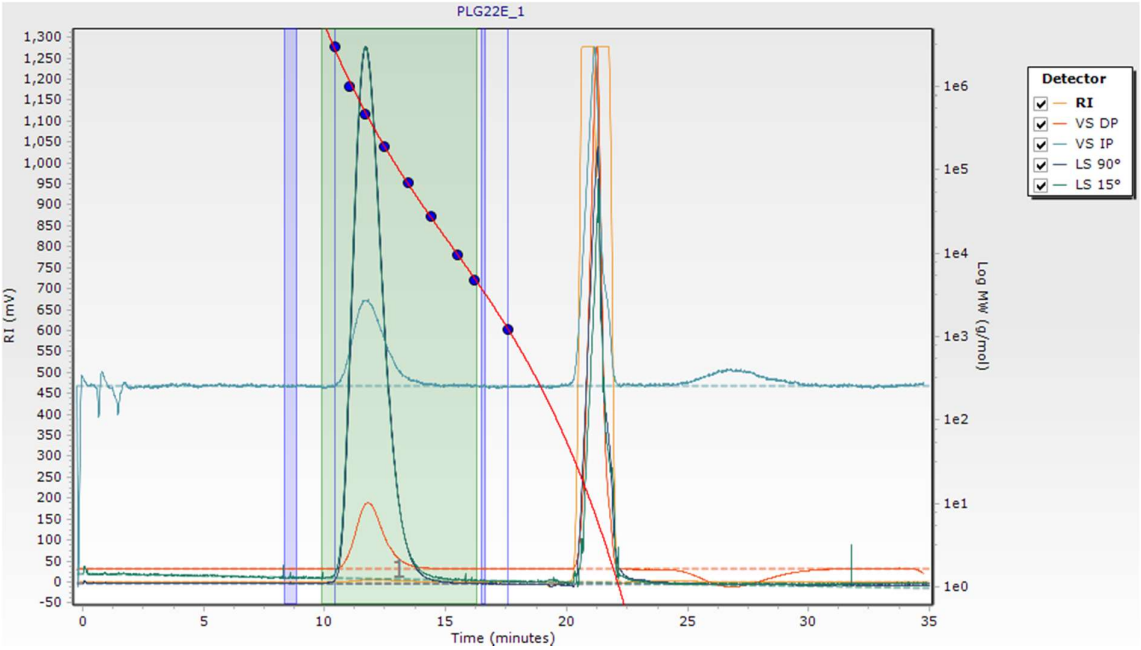


Figure 19. Calibration curve formed in the conventional and universal calibrations with nine different standards (blue dots). The sample in the figure (PLG22_1) was measured on 14.6.2018.

After the calibration, it was time for sample data analysis. In all calibration methods, the baseline was set individually for every sample similarly to as the PS standards. When the baseline of the RI signal seemed good enough, it was checked that the baseline was decent also for the other signals. After that, the integration region was set so that it covered the whole sample signal peak. The baseline and integration regions set were different every time because the signals had from the samples were always individual.

When the baseline and integration regions of the samples were set, the samples could be analyzed. In the triple detection method, the GPC device calculated the results mainly itself with the triple analysis wizard. During data analysis, the data to be included in the result calculations were selected by hand. Good quality data were chosen by a yellow area in which the blue dots (log M_w) responded the red line the best (Figure 20). With this yellow area, it was determined the data which was used in the analysis of the results. The chosen area was tried to set quite wide so that there would be as much data as possible in the analysis. After this, the device was able to calculate the results.

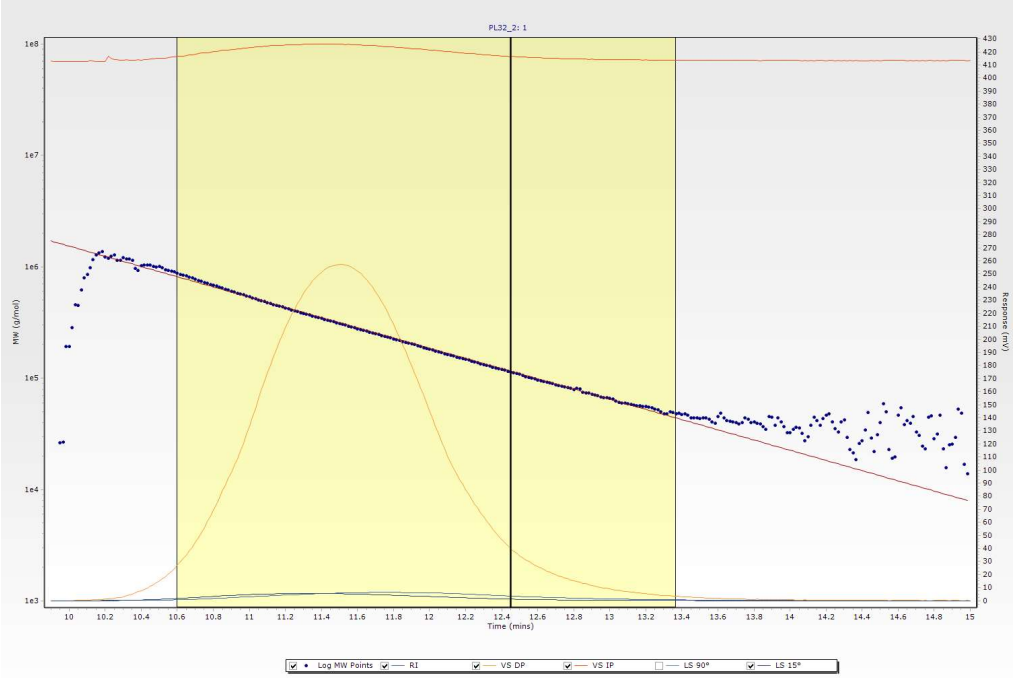


Figure 20. Choosing the yellow area in the triple detection data analysis. The sample (PL32_2) was measured on 23.5.2018.

When analyzing the results with the universal calibration I and the conventional calibration methods, copies of the samples used in the triple detection were utilized. First, the baselines and integration regions were set for all of the samples and then the analysis was started. In the universal calibration I, the material specific Mark-Houwink parameters were set for all of the samples according to the literature values of the polymer in question and then the software was able to calculate the results by exploiting the created calibration curve. In conventional calibration, the same Mark-Houwink parameters for PS (provided by the program) as used to form a calibration curve were utilized for all of the samples.

11 RESULTS (GPC) AND DISCUSSION

11.1 TRIPLE DETECTION

Different molar mass averages (M_p, M_w, and M_n), *PD*s and also the Mark-Houwink parameters K and α were determined by the triple detection method for the PLLA, PLGA, and PLCL samples. Additionally, the material-specific *dn/dc* values were determined. The results are gathered in Appendixes I and II.

Occasionally, the baseline and integration region were quite difficult to set because of the noise of the signal (especially with the signals from LS detectors). This could have unpredictable effects on the results but only to a lesser degree. There was also some pressure problems in the GPC device in the measurements made on 22.5.2018 and 23.5.2018. However, the results were comparable to the results measured earlier so they were utilized for the data analysis. The measurement of the sample PL10_1 (measured on 23.5.2018) did not succeed for an unknown reason.

11.1.1 PLLA SAMPLES

There were seven different sample types which differed in their inherent viscosity. The viscosities of these samples were 1.0, 1.8, 2.4, 3.2, 3.8, 4.9, and 6.5 dl/g. The four separate measurements were carried out (14.2.2018, 19.4.2018, 22.5.2018, and 23.5.2018) and there was a separately weighed parallel sample of every measured sample. From the results, it could be seen that the smaller the polymer (smaller inherent viscosity), the longer the retention time as it should be according to the theory (Figure 21). From the same figure, it could be seen that in case of the samples with inherent viscosity of 1.0, 1.8, and 2.4 dl/g there is a shoulder in the signal peak. This might be possibly caused by impure raw material. Normally, the shoulder in the signal could be also caused by column mismatch but because there was shoulder observed only in case of couple of samples, it is not likely that the shoulder would have been a result of the mismatch.

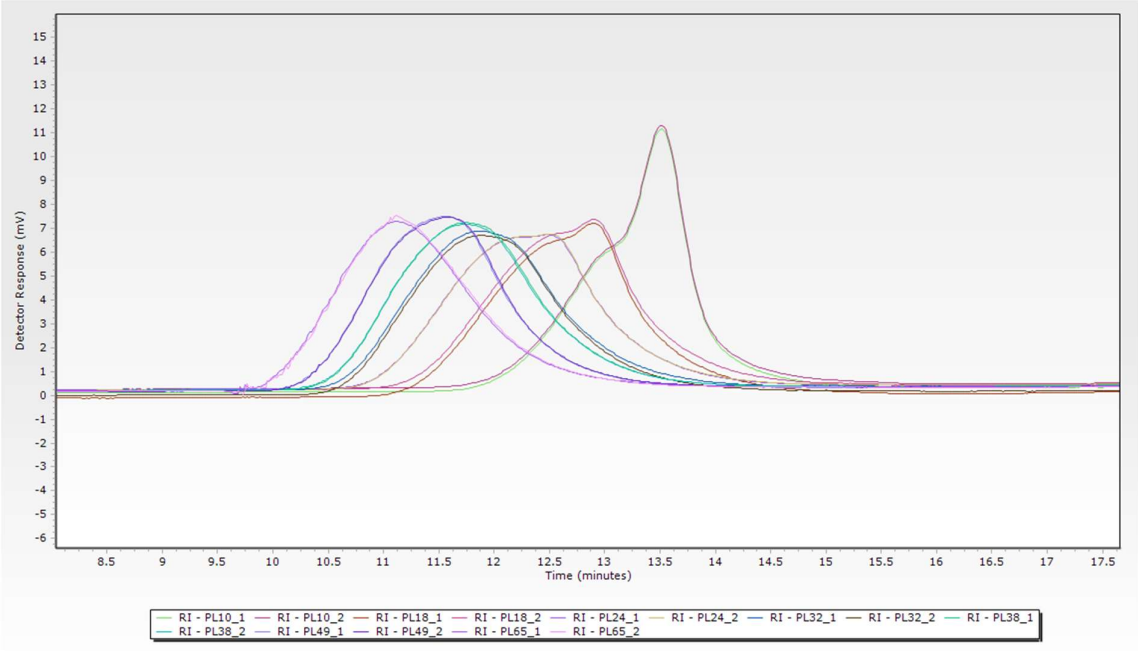


Figure 21. RI signals of the whole PLLA sample series with different inherent viscosities (measured on 19.4.2018). The signals from the left are PLLA (IV 6.5 dl/g), PLLA (IV 4.9 dl/g), PLLA (IV 3.8 dl/g), PLLA (IV 3.2 dl/g), PLLA (IV 2.4 dl/g), PLLA (IV 1.8 dl/g), and PLLA (IV 1.0 dl/g). There have been two parallel samples for every sample and thus two measured signals for every sample.

11.1.1.1 MOLAR MASS AVERAGES AND POLYDISPERSITIES

Different molar mass averages (M_p, M_w, and M_n) were determined using GPC and they are collected in Appendix I in Tables 1-3. Also the *PD*s of the PLLA samples were calculated and these results are gathered in Appendix I in Table 4.

From Appendix I, Table 1 it can be seen that when the inherent viscosity of the PLLA samples increased, also the M_p increased. This followed the theory of the Mark-Houwink equation (9). The values for the parallel samples were almost the same, except for the PL24 samples measured on 23.5.2018. When analyzing the M_p data from the same sample type (same inherent viscosity) measured on different dates, the values did not differ significantly. The biggest differences were found in the sample type PL24. In this sample type, when the difference between the smallest and the largest values was compared to the largest value, the percentage value was about 28.5 %. This difference may be caused, for example, by the differences in the choosing of baseline and integration regions in the analysis. With other sample types (PL10,

PL18, PL32, PL38, PL49, and PL65), the corresponding percentage values were 9.7 %, 6.1 %, 11.6 %, 16.5 %, 24.4 % and 15.7 %, respectively. These percentages were not significantly large except for 24.4 % and therefore, it seemed that the measurements made on the same material on different dates were comparable. If the relative standard deviations (RSD) of the measured M_p values were observed, the RSD varied between 2.1 and 13.4 %. The largest RSD was in the sample type PL24.

Also as the M_p values, the increasing inherent viscosity correlated with the increase in the M_w values (Appendix I, Table 2). All the parallel sample M_w results were on the same range, also with the sample PL24 measured on 23.5.2018 unlike with the M_p values. If the form of the signal peak was observed (Figure 21) it could be seen that the signal peak had a quite wide top. Because of this, the peak point of the signal might change easily and thus, the M_p value might change although the other molar mass averages would stay quite the same. If the difference between the smallest and largest M_w values of the same sample type (same inherent viscosity) was compared to the largest value like it was done with the M_p values, the percentages for PL10, PL18, PL24, PL32, PL38, PL49, and PL65 were 6.6 %, 5.0 %, 8.6 %, 11.2 %, 12.7 %, 14.1 %, and 13.7 %, respectively. Thus, the variation between the M_w results from the same sample type was quite small and the percentages were smaller than the corresponding M_p values. The RSD of M_w values varied between 1.7 and 5.6 % and this range was a bit smaller than the RSDs observed in the case of M_p.

When the measured M_w values were compared to the inherent viscosity values informed by the manufacturer, the calculated average values for M_w results for each sample type were used (Table 2 in Appendix I). The correlation can be seen in Figure 22 where the curve is near linear.

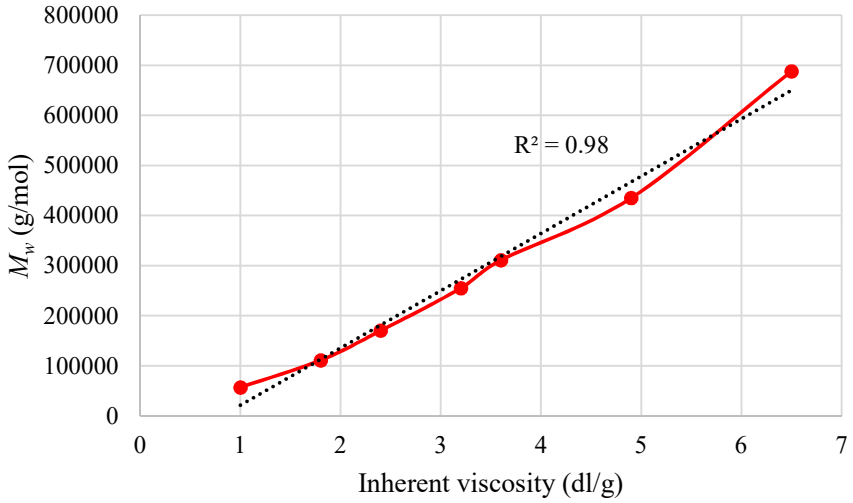


Figure 22. Correlation between the M_w and inherent viscosity of PLLA. The used M_w values are the average values of the measurements (Appendix I, Table 2). The trendline (black dashed line) is also included.

If the M_n averages of the PLLA samples were examined, the same observation could be made as with the M_p and M_w values; if the inherent viscosity of the sample increased, also the M_n increased. Also according to the theory (Figure 12), there should be $M_n < M_p < M_w$ for the results. This agreed with all of the samples, except for the sample PL10. With PL10, the order was $M_p < M_n < M_w$. There was a shoulder in the measured signals in case of PL10 (Figure 21) which could have an effect on the calculated results. This may explain the deviant order of the different molar masses. The measured M_n averages are gathered in Appendix I, Table 3. If the measured values for the same sample type were compared (the difference between the largest and the smallest values divided by the largest value), the percentages for PL10, PL18, PL24, PL32, PL38, PL49, and PL65 were 9.3 %, 7.2 %, 15.1 %, 16.7 %, 17.7 %, 27.4 %, and 29.8 %, respectively. These percentages were bigger than the corresponding percentages of M_w and almost in the same range as the percentages of M_p. When observing the RSD of M_n, the percentages varied between 2.2 and 9.9 % which was quite the same as the RSDs observed with M_p and M_w results.

When considering the *PD*s of samples, the results were in the same range despite of the sample's inherent viscosity (Appendix I, Table 4). The RSD of different type of samples varied between 1.8 and 12.2 % and therefore, the RSD was quite small to all of the sample types. The largest RSD was observed in case of sample type PL65. There was possibly a small increase in the *PD* values when the inherent viscosity of the samples increased. The smallest *PD* values

were measured for the sample PL10 (average 1.34), and the largest for PL65 (average 1.88). If an average from the average values of different sample types was calculated, it would be 1.63. In the literature, the *PD* values for PLA are between 1.5 and 3.79,[105] so the results were in the line of these values except for the PL10 sample. However, the *PD* of the polymer depends on the polymerization conditions[106] and because the preparing conditions of the measured polymers and polymers from the literature were not informed, the comparison was not necessarily worthwhile.

11.1.1.2 MARK-HOUWINK PARAMETERS

During the triple detection data analysis, the selected yellow area had a strong influence to the Mark-Houwink parameters. In the selection of yellow area, the red line was tried to set to respond the blue dots the best (Figure 20). The selected area determines the data utilized for the analysis. If the selected area was narrow (where the blue dots confidently followed a straight red line), the values had for parameter α were over 1.0. If the selected area was as wide as possible (so that the red line still fitted in the group of blue dots), the parameter α was clearly smaller, about 0.6. This is one reason why the determination of the Mark-Houwink parameters can be difficult.

Based on the average values of Appendix I (Table 5), it seemed that the value for the α parameter increased slightly when the inherent viscosity of the PLLA sample increased (Figure 23). The average values from the measured values for α parameter differed between 0.60 and 1.25. The literature values for α are 0.65 according to Garlotta[36] (PLLA semicrystalline in THF 30 °C, M_v) and 0.736 according to Dorgan *et al.*[83] (PLA in THF 30 °C, M_v). If these literature values were compared to the measured results, the literature values were similar to the PLLA samples with inherent viscosities between 1.8 and 2.4 dl/g.

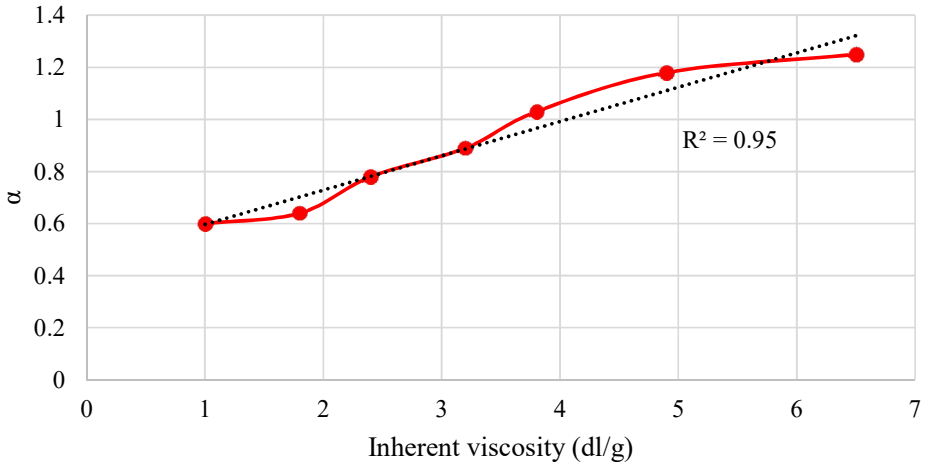


Figure 23. Correlation between the inherent viscosity and Mark-Houwink α parameters of the PLLA samples. The used inherent viscosities are informed by manufacturers and α parameters are the average values from Appendix I, Table 5. The trendline (black dashed line) is also included.

When examining the Mark-Houwink *K* parameter results of the PLLA samples (Appendix I, Table 6), the results did not show a predictable behavior (Figure 24) and they vary between 0 and $360.14 \cdot 10^{-5}$ dl/g. Only correlation which could be possibly seen was that with the samples of larger inherent viscosities, the *K* parameter was quite small. The corresponding literature values for *K* are $1.00 \cdot 10^{-3}$ dl/g according to Garlotta[36] (PLLA semicrystalline in THF 30 °C, M_v) and $1.74 \cdot 10^{-4}$ dl/g according to Dorgan *et al.*[83] (PLA in THF 30 °C, M_v). If these literature values were converted to the same scale than the measured results ($1.00 \cdot 10^{-3}$ dl/g = $100.00 \cdot 10^{-5}$ dl/g and $1.74 \cdot 10^{-4}$ dl/g = $17.40 \cdot 10^{-5}$ dl/g), it could be seen that there is some variation in the literature values. The measured results were in the range of the literature values within the sample types of PL10, PL18, PL24, and PL32; in other words, with the samples of low inherent viscosities.

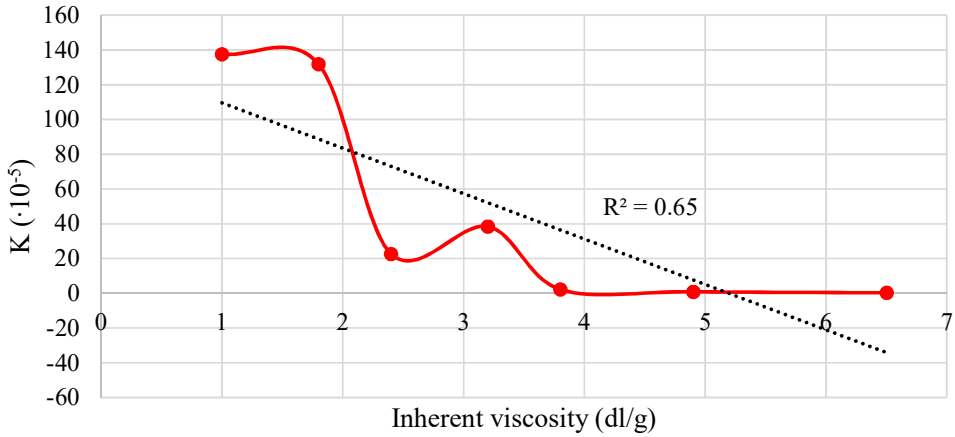


Figure 24. Correlation between the inherent viscosity and Mark-Houwink K parameters of the PLLA samples. The used inherent viscosities are informed by manufacturers and K parameters are the average values from Appendix I, Table 6. The trendline (black dashed line) is also included.

11.1.1.3 *DN/DC* VALUES

The *dn/dc* literature values for PLA (Table 4) have some variation. In THF, the *dn/dc* value of the PLA has been reported to be 0.042 ml/g[91] and according to another reference, the *dn/dc* value for PLLA in THF is 0.0558 ml/g.[90] The measured *dn/dc* values for PLLA were between 0.046 and 0.049 ml/g (Appendix I, Table 7), so they were clearly in the range of literature values. The average of all of the *dn/dc* results was 0.047 ml/g. The *dn/dc* value is material specific and according to the results, the inherent viscosity of the material did not have an effect to the measured values.

11.1.2 PLGA AND PLCL SAMPLES

Four different PLGA samples with inherent viscosities of 2.2, 2.38, 3.11, and 6.2 dl/g were analyzed with the triple detection method. Additionally, in the same measurements, one PLCL sample with inherent viscosity of 1.5 dl/g was measured. The measurements were carried out on 14.6.2018, 19.6.2018, and 20.6.2018 and in these measurements there were always two separately weighed parallel samples for each material tested. When observing the RI signals of the PLGA and PLCL samples (Figure 25), it could be seen that the smaller the inherent viscosity of the sample, the longer the retention time.

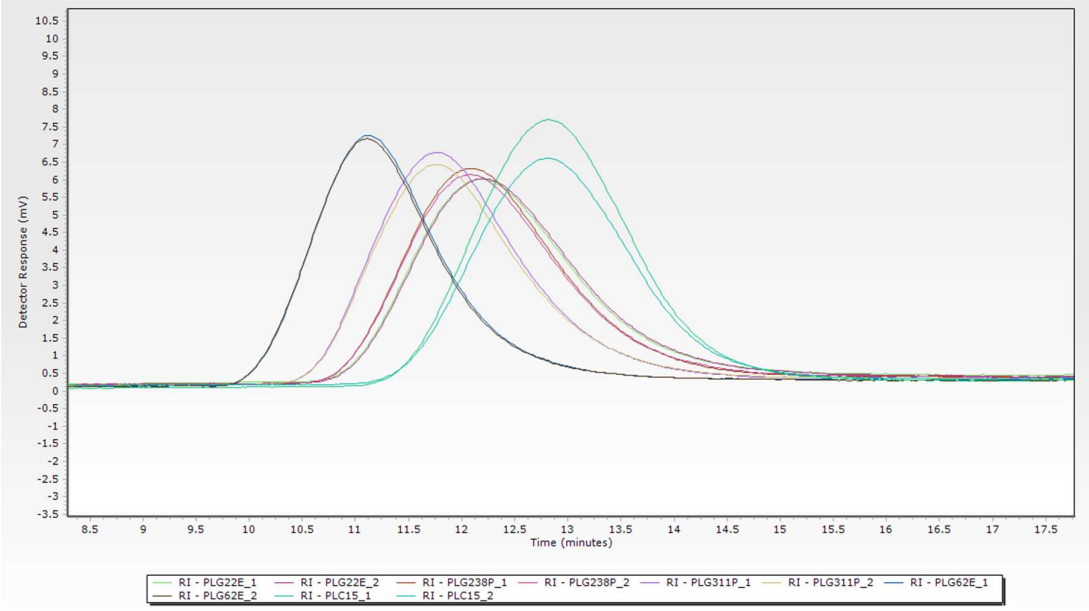


Figure 25. RI signals of the PLGA sample series with different inherent viscosities (measured on 14.6.2018). Also the RI signals of the PLCL samples are included. The signals from the left are PLGA (IV 6.2 dl/g), PLGA (IV 3.11 dl/g), PLGA (IV 2.38 dl/g), PLGA (IV 2.2 dl/g), and PLCL (IV 1.5 dl/g). There is a parallel sample from every sample and thus, two measured signals for every sample.

11.1.2.1 MOLAR MASS AVERAGES AND POLYDISPERSITIES

As for the PLLA samples, the molar mass averages (M_p, M_w, and M_n) were determined from the PLGA and PLCL samples by the triple detection method. The results of both the PLGA and PLCL samples are collected in the Tables 1-3 in Appendix II. From the molar mass average results, it could be seen that with the PLGA samples, the order of magnitude of the different molar mass averages was $M_n < M_w \approx M_p$ for all of the sample types. With the PLCL sample, the corresponding order was $M_n < M_p < M_w$ as it should be according to the theory. In addition, the *PD*s of the samples were found out and these results are gathered in Appendix II, Table 4.

From Table 1 in Appendix II, it can be seen that according to the assumptions, when the inherent viscosity of the PLGA samples increased, the M_p increased. Also, when comparing the results with same inherent viscosity, the measured molar masses were in the same range. The biggest variation between the M_p results could be observed in the results of the sample type PLG22 with a variation of 7.1 % (the difference between the largest and smallest value divided by the largest value). Additionally, when analyzing the PLGA M_w results it could be observed that

when the inherent viscosity of the samples increased, also the M_w increased (Appendix II, Table 2). The variation between the PLGA M_w results measured for the same sample type was quite small. The RSDs of M_p results varied between 1.4 and 2.7 % and in case of M_w results, the variation was between 1.1 and 2.3 %. Thus, the observed RSDs were quite small.

If the M_p and M_w results of PLCL were concerned, the variation was small and there were no deviant measuring results among the results. The RSD in the M_p results of PLCL was 1.4 % and in M_w results, the corresponding value was almost the same 1.2 %.

The inherent viscosities of PLGA and M_w values (average values of the measurements for different sample types) could be connected. The correlation can be seen in Figure 26 which was linear. In case of PLCL, for the inherent viscosity of 1.5 dl/g, the M_w was about 99600 g/mol.

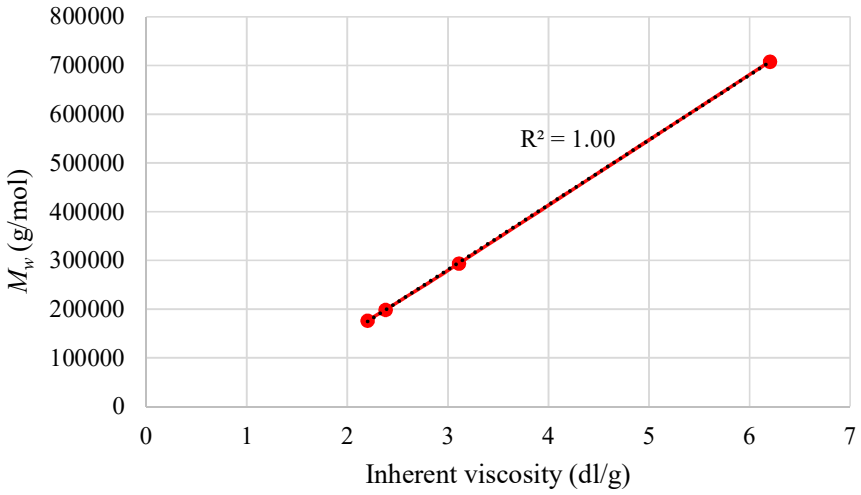


Figure 26. Correlation between the M_w and inherent viscosity of PLGA. The used values M_w values are the average values from the measurements (Appendix II, Table 2). The trendline (black dashed line) is also included.

When analyzing the M_n results for PLGA and PLCL samples, the same trend could be observed. When the inherent viscosity of the samples increased, also the M_n increased (Appendix II, Table 3). The results of the same sample type were quite even and the biggest variation in the PLGA samples could be observed again in the sample type of PLG22. However, this variation was only 16.0 % (the difference between the largest and smallest value divided by the largest value). Because the biggest variation was in every molar mass average in the sample type PLG22, it is possible that there has been happened something in the preparation or analysis of

these samples which increases the variation. However, the variation was not significantly large. RSD of the M_n values of PLGA varied between 3.2 and 6.9 % and these values were a bit larger than in case of the RSDs of M_p and M_w. Respectively, the RSD in the M_n results of PLCL sample was 2.4 %.

When analyzing the *PD*s of the PLGA samples, it seemed that the *PD* value did not greatly depend on the inherent viscosity of the sample (Appendix II, Table 4). The measured values of *PD* for PLGA differed between 1.74 and 2.13 and the averages of the *PD* of different sample types between 1.82 and 1.96. Thus, the variation between the *PD* values was quite small and the RSD varied between 2.4 and 5.4 %. If one average from all of the average values of different sample types was calculated, the *PD* of the PLGA was 1.87. In the literature, the *PD* of PLGA is 1.52 (PLGA 50) and 1.55 (PLGA 85) according to Li *et al.*[107] Therefore, the literature values for PLGA's *PD*s were smaller than those measured in this thesis. However, in the literature measurements, the samples were different to the samples used in this thesis. The inherent viscosities of the literature PLGA samples were 0.55-0.75 dl/g which were much smaller than the inherent viscosities of the PLGA samples used in this thesis. Also, the preparation conditions of the polymers might have been different which impacts to the *PD* of the samples. This can partly explain the observed difference.

When analyzing the *PD*s of measured PLCL sample, the measured values differed between 1.64 and 1.74 and the average of these results was 1.69 (Appendix II, Table 4). The RSD was small, 2.1 %. The *PD* of PLCL in the literature is about 2.0 (PLCL (80/20) = 2.1, PLCL (60/40) = 2.1, and PLCL (40/60) = 2.0) according to Malin *et al.*[21] Thus, the literature value for *PD* of PLCL is bigger than the measured value. Again, the used samples in the literature were not the same as those used in the thesis measurements and the preparation conditions might have been different which could affect the difference observed.

11.1.2.2 MARK-HOUWINK PARAMETERS

The Mark-Houwink parameters (K and α) were also determined for the PLGA and PLCL samples during the triple detection measurement. The results are gathered in Appendix II in Tables 5 and 6. The results on the α parameter were quite consistent. Possibly a slight increase in the α parameters was observed when the inherent viscosity of PLGA increased. This kind of correlation was observed also in the PLLA samples but much stronger. In case of K parameter, the values varied a lot. From the results, it could be seen that when measuring PLGA samples,

α got values between 0.72 and 1.02 and the average value calculated from all of the α averages was 0.80. In case of PLGA's K values, the values differed between (0.85 and 21.34) $\cdot 10^{-5}$ dl/g and the average of the K averages was 13.77 $\cdot 10^{-5}$ dl/g. The Mark-Houwink parameters of PLGA in THF are α = 0,761, $K = 1.07 \cdot 10^{-4}$ dl/g = $10.7 \cdot 10^{-5}$ dl/g according to Kenley *et al.*[86]. Therefore, the measured values of PLGA's α and K were in the same range as the literature values.

When concentrating on the Mark-Houwink parameters measured for PLCL, the α parameter did not differ greatly between the measurements and the average value for α was 0.68. The K values differed between (36.68 and 57.39) $\cdot 10^{-5}$ dl/g and the average of these measurements was 44.84 $\cdot 10^{-5}$ dl/g. There were no Mark-Houwink parameters for PLCL found in the literature, so the measured values could not be compared to them.

11.1.2.3 *DN/DC* VALUES

In the *dn/dc* determination for PLGA and PLCL, it was noticed that the measured values were relatively stable. The measured *dn/dc* values for PLGA varied between 0.047 and 0.049 ml/g and they are collected in Appendix II in Table 7. The *dn/dc* average calculated from all the averages of PLGA samples was 0.048 ml/g. The corresponding value in the literature for PLGA is about 0.05 ml/g[89]. Therefore, the average of the measured values was quite the same as the literature value.

In addition, the measured values for PLCL were always the same apart from one measurement. The average of these measurements was 0.052 ml/g. The corresponding literature values for PLCL were not found.

11.2 UNIVERSAL CALIBRATION I

The results of PLLA, PLGA, and PLCL samples were analyzed with the universal calibration I method and different molar mass averages (M_p, M_w, and M_n) and *PD*s were calculated. The analysis of the PLLA results were carried out twice with two different Mark-Houwink parameter pairs according to Garlotta[36] and Dorgan *et al.*[83] In the analysis of PLGA samples, the Mark-Houwink parameters were set according to Kenley *et al*.[86] and in the case of PLCL, the parameters were set according to the results had from the triple detection measurements. The results are gathered in the Appendixes III, IV, and V.

11.2.1 PLLA SAMPLES

The same samples as in the triple detection (see section 10) were also analyzed with the universal calibration I method. The M_p, M_w, and M_n averages and also *PD* were determined.

11.2.1.1 MOLAR MASS AVERAGES AND POLYDISPERSITIES WITH DIFFERENT MARK-HOUWINK PARAMETERS

The molar mass averages (M_p, M_w, and M_n) and the *PD*s of the PLLA samples were determined by using two different Mark-Houwink parameter pairs found in the literature. First, the parameters determined by Garlotta[36] were used and after that, the parameters determined by Dorgan *et al.*[83] were utilized. The measured data used in the analysis were exactly the same as those used in the triple detection analysis.

When the Mark-Houwink parameters were set according to Garlotta[36] in the universal calibration I (PLLA semicrystalline in THF 30 °C, $\alpha = 0.65$ and $K = 1.00 \cdot 10^{-3}$ dl/g, Table 2), it could be noticed that almost with every sample type the magnitude of the different molar mass averages followed the order $M_n < M_p < M_w$. Only with the sample PL10, the order was $M_p < M_n < M_w$. The same observation was noticed in the triple detection results and it may be caused by the shoulder in the measured signal (Figure 21). The results of the molar mass averages determined with the Garlotta's Mark-Houwink parameters are gathered in Appendix III (Tables 1-3).

From Table 1 in Appendix III it can be seen that when the inherent viscosity of the sample increased, also the M_p increased. The differences between the M_p results of the same sample type were quite small as in the results analyzed with the triple detection. Only exception was the sample PL32_1 measured on 19.4.2018 which was smaller than the equivalent values. Also in case of M_w results, when the inherent viscosity of the PLLA samples increased, the M_w increased (Appendix III, Table 2). As with the M_p results, the M_w results of the same sample type were in the same magnitude except for the PL32_1 -sample measured on 19.4.2018 which was smaller than the other measured values.

The same trend is discovered with the results of M_n compared to the results of M_p and M_w (Appendix III, Table 3). When the inherent viscosity of the PLLA increased, also the M_n of the sample increased. When examining the results, the only noteworthy observation was noticed

with the value of the sample PL32_1 measured on 19.4.2018. It was smaller than the equivalent values of the same sample type. This observation was made with every molar mass averages (M_n, M_p, and M_w) so it is possible that there was something wrong with the data analysis of that certain sample.

When concentrating on the RSDs of the measured molar mass averages, for M_p the RSD lay between 1.9 and 18.5 % (notably larger for the sample types PL24 and PL32 than for the others). For M_w, the corresponding values were 1.0-19.0 % (notably larger for the sample type PL32) and for M_n, the values were between 1.3 and 18.2 % (also notably larger for the sample type PL32 than for the others). Therefore, the range of the RSDs was quite the same for all of the different molar mass averages. As noticed earlier, the sample type PL32 was different to the other values which might be caused by some issues in the sample analysis (for example in the selection of baseline and integration areas).

When observing the average results for *PD*s of the PLLA samples with different inherent viscosities analyzed with the Mark-Houwink parameters of Garlotta[36], the values differed between 1.45 and 2.21. It seemed that when the inherent viscosity of the sample increased, also the *PD* of the sample increased. The results are in Appendix III (Table 4). The RSD of different sample types varied between 2.2 and 3.2 % and was quite small. The measured *PD* was a bit larger for the sample types of larger inherent viscosity apart from some exceptions. The literature values of *PD* for PLA vary between 1.5 and 3.79[105] so the measured values were for the most part in the range of literature values.

When the Mark-Houwink parameters were set according to Dorgan *et al.*[83] (PLA in THF 30 °C, $\alpha = 0.736$ and $K = 1.74 \cdot 10^{-4}$ dl/g, Table 3), the molar mass average results were hand in hand with the results analyzed with parameters of Garlotta[36]. The results for different molar mass averages followed the order $M_n < M_p < M_w$ almost with all the sample types. In the case of sample PL10, the order of the molar mass averages was $M_p < M_n < M_w$. The measured results for these molar mass averages are gathered in Appendix IV (Tables 1-3). When looking for the results for M_p, M_w, and M_n, always when the inherent viscosity of the sample increased, the molar mass of the sample increased. With every molar mass averages (M_p, M_w, and M_p), there was no large variation between the results of the same sample type and the RSDs were for M_p between 1.4 and 14.8 % (notably larger for the sample type PL24 than for the others), for M_w between 1.0 and 2.9 % and for M_n between 1.0 and 3.7 %. The larger RSD in the M_p results for the sample type PL24 was caused by the measurement made on 23.5.2018. In that measurement,

the measured values were notably larger than the results obtained in other measurements (especially, the sample PL24_1). The larger results might be caused by some errors in the measurements or analysis.

The average *PD*s of the PLLA samples analyzed with the universal calibration according to Dorgan *et al.*[83] varied between 1.39 and 2.03 (Appendix IV, Table 4). From the results it could be noticed that when the inherent viscosity of the PLLA sample increased, also the *PD* of the samples increased. The RSD of the sample types lay between 1.2 and 3.1 % and thus the results did not differ significantly from each other. In addition, these values were almost in the range of literature values (for PLA, the range is 1.5-3.79[105]).

11.2.1.2 DIFFERENCES BETWEEN THE PLLA RESULTS ANALYZED WITH DIFFERENT MARK-HOUWINK PARAMETERS

When the PLLA molar mass average results analyzed with the universal calibration I method with different Mark-Houwink parameters were compared, the results obtained with the Mark-Houwink parameters of Garlotta[36] were in every case smaller than the results obtained by using the parameters according to Dorgan *et al.*[83] (Figure 27 and Table 9).

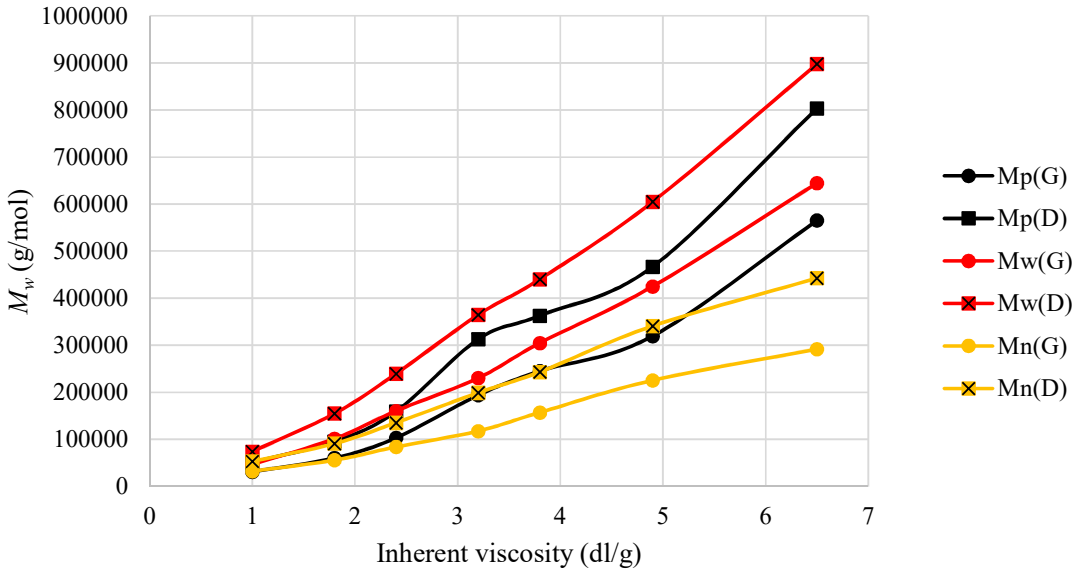


Figure 27. Molar mass averages (M_p, M_w and M_n) analyzed with universal calibration I method with the parameters of Garlotta[36] (G) and Dorgan *et al.*[83] (D) as a function of inherent viscosity. The used molar mass averages are the average values from Appendixes III (Tables 1-3) and IV (Tables 1-3).

If the average values of different M_w measurements for different inherent viscosities were compared ((Dorgan-Garlotta)/Garlotta), the results obtained with the parameters of Dorgan *et al.*[83] were 39.3-59.4 % larger than the results calculated with those of Garlotta[36] (Table 9). In proportion for M_n and M_p measurements, the results calculated with the parameters of Dorgan *et al.*[83] were 51.2-69.2 % and 42.1-64.0 % larger than the results calculated with the parameters determined by Garlotta[36], respectively. Therefore, the Mark-Houwink parameters had a great effect to the determined molar mass averages in the universal calibration I method.

Table 9. Difference between the molar mass average results of the same PLLA samples analyzed with the universal calibration I method with different Mark-Houwink parameters. The Mark-Houwink parameters have been set according to Garlotta[36] (G) and Dorgan *et al.*[83] (D). The *M* values used are the average values of the molar mass averages determined in different measurements (see Tables 1-3 in Appendix III and Tables 1-3 in Appendix IV).

Sample	M-H parameters	M_p	(D-G)/G (%)	M_w	(D-G)/G (%)	M_n	(D-G)/G (%)
PL10	Garlotta	31243	64.0	46553	59.4	32160	66.1
	Dorgan	51233		74219		53410	
PL18	Garlotta	60274	58.7	101412	52.8	55812	63.0
	Dorgan	95674		154930		90977	
PL24	Garlotta	103242	54.5	160487	49.3	83973	61.3
	Dorgan	159484		239681		135457	
PL32	Garlotta	193818	61.4	230303	58.4	117671	69.2
	Dorgan	312837		364819		199111	
PL38	Garlotta	245247	48.1	304727	44.5	157076	54.9
	Dorgan	363137		440379		243323	
PL49	Garlotta	319647	46.1	425201	42.3	225509	51.2
	Dorgan	467067		604950		340994	
PL65	Garlotta	565325	42.1	644624	39.3	291845	51.7
	Dorgan	803156		897659		442796	

When observing the *PD*s of the PLLA samples analyzed with the universal calibration I method with different Mark-Houwink parameters (according to Garlotta[36] and Dorgan *et al.*[83]), it could be seen that the results calculated by using the parameters of Dorgan *et al.*[83] were slightly smaller than the results calculated by using the parameters of Garlotta[36]. The difference was under 10 % in every case (Table 10). Thus, the difference was relatively small and the *PD* values of the samples did not greatly depend on the Mark-Houwink parameters. Generally, the

PD is determined as a ratio of M_w and M_n ($PD = \frac{M_w}{M_n}$) and because both of these variables changed similarly according to the analyzing parameters, the ratio (i.e. *PD*) stayed quite the same.

Table 10. Difference between polydispersities (*PD*s) of the same PLLA samples analyzed with the universal calibration I method with the different Mark-Houwink parameters. The Mark-Houwink parameters have been set according to Garlotta[36] (G) and Dorgan *et al.*[83](D). The *PD* values used are the average values of the molar mass averages determined in different measurements (see Table 4 in Appendix III and Table 4 in Appendix IV).

Sample	M-H parameters	*PD*	(D-G)/G (%)
PL10	Garlotta	1.44	-4.0
	Dorgan	1.39	
PL18	Garlotta	1.82	-6.3
	Dorgan	1.70	
PL24	Garlotta	1.91	-7.4
	Dorgan	1.77	
PL32	Garlotta	1.95	-6.2
	Dorgan	1.83	
PL38	Garlotta	1.94	-6.7
	Dorgan	1.81	
PL49	Garlotta	1.89	-5.9
	Dorgan	1.78	
PL65	Garlotta	2.21	-8.2
	Dorgan	2.03	

11.2.2 PLGA AND PLCL SAMPLES

The same PLGA and PLCL samples as in the triple detection (see section 10) were also analyzed with the universal calibration I method. The molar mass averages and *PD*s of the samples were found out.

The molar mass averages of PLGA and PLCL samples were determined by using different Mark-Houwink parameters. The Mark-Houwink parameters for PLGA samples were set according to Kenley *et al.*[86] ($\alpha = 0.761$, $K = 1.07 \cdot 10^{-4}$ dl/g, PLGA in THF). These molar mass average results are gathered in Appendix V (Tables 1-3). There were no

Mark-Houwink parameters found for PLCL in the literature which would have been utilized in the universal calibration I method. For that reason, the universal calibration I of PLCL was executed with the parameters obtained from the triple detection measurements according to Tables 5 and 6 in Appendix II ($\alpha = 0.68$, $K = 44.84 \cdot 10^{-5}$ dl/g). The results of different molar mass averages for PLCL sample are shown in the same tables as the results of PLGA (Appendix V, Tables 1-3).

From the molar mass average results, it could be seen that with all of the PLGA sample types of different inherent viscosities, the order of different molar mass averages was $M_n < M_p < M_w$ as it should be according to the theory. Also the PLCL sample followed the order of different molar mass averages according to the theory ($M_n < M_p < M_w$).

When observing the results of different average molar masses of PLGA analyzed with the universal calibration I method (Appendix V, Tables 1-3) it could be noticed that when the inherent viscosity increased, the molar mass of the sample increased in every case. When concentrating on the results of the same sample type there was not much variation between the results. Only notable difference was in the results for PLG62-sample measured on 14.6.2018. These results were bigger than the other results for the same sample type. This difference could be noticed in M_p, M_w, and M_n values so it is possible that the sample preparation were not succeeded as well as the other sample preparations. The RSD for M_p results ranged between 0.9 and 9.7 %, for M_w between 0.3 and 10.4 %, and for M_n between 1.9 and 8.3 %. Clearly, the biggest RSD was observed with the sample PLG62 as it was expected on the base of the earlier data analysis.

When concentrating to the molar mass average results of PLCL, the equivalent molar mass average results were in the same line. When observing the RSDs of different molar mass averages, for M_p results the deviation was 0.6 %, for M_w results it was 0.5 % and for M_n results it was 1.9 %. Thus, the deviation was quite small and the parallel sample preparation and analysis was succeeded.

The measured *PD*s of PLGA analyzed with the universal calibration I method (parameters from Kenley *et al.*[86]) varied between 1.91 and 2.27 (Appendix V, Table 4). When the averages of different sample types were observed, it could be noticed that all the results were in the same range (RSD was between 1.9 and 3.3 %). From this, it could be concluded that the inherent viscosity of the PLGA sample did not greatly affect *PD* of the sample. An explanation for this

can be found from the equation of *PD*. When considering the equation of *PD* ($PD = \frac{M_w}{M_n}$) it can be concluded that if the inherent viscosity of a sample increases, also both M_w and M_n increase and thus, the ratio of them stays quite stable.

If the average *PD* from the averages of different PLGA sample types was calculated, the *PD* of the PLGA was 2.05. The corresponding literature value for PLGA is 1.52 (PLGA 50) and 1.55 (PLGA 85) according to Li *et al.*[107], so the measured value was much bigger than the literature value. Also, the conditions of the polymerization have a great effect to the *PD* of the samples so it could cause the difference between the measured and literature values. In the case of PLCL, the measured *PD*s varied between 1.77 and 1.86 and average of these results was 1.82. In the literature, the *PD* of PLCL is about 2.0 (PLCL (80/20) = 2.1, PLCL (60/40) = 2.1, and PLCL (40/60) = 2.0) according to Malin *et al.*[21] so the measured values were smaller than the literature values.

11.3 CONVENTIONAL CALIBRATION

All the results of PLLA, PLGA, and PLCL were also analyzed with the conventional calibration method. As in the case of the triple detection and universal calibration, the molar mass averages (M_p, M_w, and M_n) and *PD*s were determined. In conventional calibration, the Mark-Houwink parameters for PS provided by the analyzing program were utilized ($K = 14.10 \cdot 10^{-5}$ dl/g, $\alpha = 0.70$). The results are gathered in Appendix VI.

11.3.1 PLLA SAMPLES

The same measured data of PLLA as used in the triple detection and universal calibration (see section 10) were also analyzed with conventional calibration method. The three different molar mass averages (M_p, M_w and M_n) and *PD*s of the samples were determined.

When comparing the molar mass averages of PLLA analyzed with the conventional calibration method (Appendix V, Tables 1-3) it could be observed that the order of different molar mass averages followed the theory with an order $M_n < M_p < M_w$, except for the sample PL10. For PL10, the order was $M_p < M_n < M_w$. This same occurrence was noticed also when the results were analyzed with other methods and it might be a results of the shoulder in the signal of PL10 sample (Figure 21).

With all the molar mass averages, when the inherent viscosity of the samples increased, also the molar mass average increased according to the theory. When observing the results, generally the measured values of the same sample type were in the same magnitude. However, some exceptions were also found. The RSD of the M_p results varied between 1.6 and 15.1 % and the biggest RSDs were found in the sample types of PL24 and PL49. The RSD of the M_w results varied between 1.0 and 2.8 % and the respectively for M_n results, the RSD was between 1.4 and 4.6 %. When considering the M_p results, it could be noticed that the samples PL24_1 (measured on 23.5.) and sample PL49_1 (measured on 22.5.) were clearly larger than the other results in that sample type. This variation could be explained with the differences possibly taken place in the sample analysis. Also, the M_p of the sample PL65_2 (measured on 23.5.) was smaller than the other measured values of the sample type PL65. This lower measuring result for the sample PL65_2 could be also observed in the results of M_w and M_n. This rose the question if something was occurred in the sample preparation.

In case of *PD*s, the measured values ranged between 1.39 and 2.11 and the largest values were observed in the samples with biggest inherent viscosities (Appendix VI, Table 4). Thus, it seemed that when the inherent viscosity of the samples increased, also the *PD* increased. If the equation of the *PD* is observed ($PD = \frac{M_w}{M_n}$) it might be concluded that when the inherent viscosity of the sample increased, M_w increased more than M_n. This same observation was noticed also when PLLA samples were analyzed with different analyzing methods (triple detection and universal calibration). However, the polymerization conditions have a large effect to the *PD* of the polymers and because they were not informed, it is not guaranteed that the samples are comparable. When considering the averages of the different sample types, the averages varied between 1.40 and 2.05 and the RSD was between 1.4 and 3.6 %. The literature values for PLLA *PD* values are between 1.5 and 3.79[105], so the measured average values were for the most part in the range of literature values.

11.3.2 PLGA AND PLCL SAMPLES

The same PLGA and PLCL samples which were analyzed earlier with the triple detection and universal calibration I methods (see section 10) were also analyzed with the conventional calibration method. In this analysis, different molar mass averages and *PD*s of the samples were determined. The used Mark-Houwink parameters for PS were provided by the program ($K = 14.10 \cdot 10^{-5}$ dl/g, $\alpha = 0.70$). The results are gathered in Appendix VII.

From the molar mass average results of PLGA (Appendix VII, Tables 1-3) it could be seen the order of different molar mass averages followed the order $M_n < M_p < M_w$ with every sample type. This order followed the theory. Also for PLCL, the order of the molar mass averages was the same. In case of PLGA samples, it could be observed that when the inherent viscosity of the samples increased, also the molar mass averages increased.

When considering the M_p results of PLGA, all the samples in the same sample type were in the same magnitude. The RSD of the M_p results varied 1.0-2.1 % and thus, it was quite small. With the M_w results, also the measured values were relatively the same and the RSD was between 0.4 and 1.1 %. In case of M_n results, there were slightly smaller results in the samples PLG238_2 and PLG62_2 (measured both on 20.6.2018) compared to the other same sample type results. The RSD of M_n results varied between 3.0 and 1.2 %. The observed differences may be caused by the differences in the determination of baseline and integration region in the analysis.

In case of PLCL molar mass average results, there were no notably different measuring values observed among the results. The RSD for M_p results was 1.6 %, for M_w results 0.4 % and for M_n results 1.8 % so the RSDs were quite small in the scale of polymer analysis.

When observing the *PD*s of PLGA analyzed with the conventional calibration it could be seen that the measured values varied between 1.99 and 2.24 (Appendix VII, Table 4). The RSD was between 2.7 and 3.6 % and the averages calculated separately for different sample types varied between 2.07 and 2.15. Thus, the variation was quite small. The literature values for the *PD* of PLGA are 1.52 (PLGA 50) and 1.55 (PLGA 85) according to Li *et al.*[107] and thus the measured values were larger than the literature values. For PLCL, the measured *PD*s analyzed with the conventional calibration method differed between 1.84 and 1.74 and the average of the measurements was 1.78. The RSD was in this case 2.0 %. The literature values for the *PD* of PLCL are about 2.0 (PLCL (80/20) = 2.1, PLCL (60/40) = 2.1, and PLCL (40/60) = 2.0) according to Malin *et al.*[21] and therefore, the literature values were larger than the measured values. However, as noted before, because the polymerization conditions of the compared polymers are not known, the comparison is possibly not profitable. This is because the polymerization conditions affect greatly to the *PD* of the forming polymers. In addition, the correlation between the *PD* and inherent viscosity of the samples could not be observed and the measured values of PD stayed quite the same despite of the inherent viscosity of the sample.

11.4 DIFFERENCES BETWEEN THE RESULTS ANALYZED WITH DIFFERENT METHODS

11.4.1 PLLA SAMPLES

When all the results of molar mass averages of PLLA were observed, it could be noticed that with all of the molar mass averages (M_p, M_w, and M_n) and with all the sample types, the order M(Universal, Garlotta[36]) < M(Triple) < M(Universal, Dorgan *et al.*[83]) < M(Conventional) took place. Therefore, the molar mass average results analyzed with the triple detection system were in between the results analyzed with the universal calibration I. In addition, the results analyzed with the conventional calibration were in every case the largest. These observations can be also seen from the distribution plots of the samples (Figure 28).

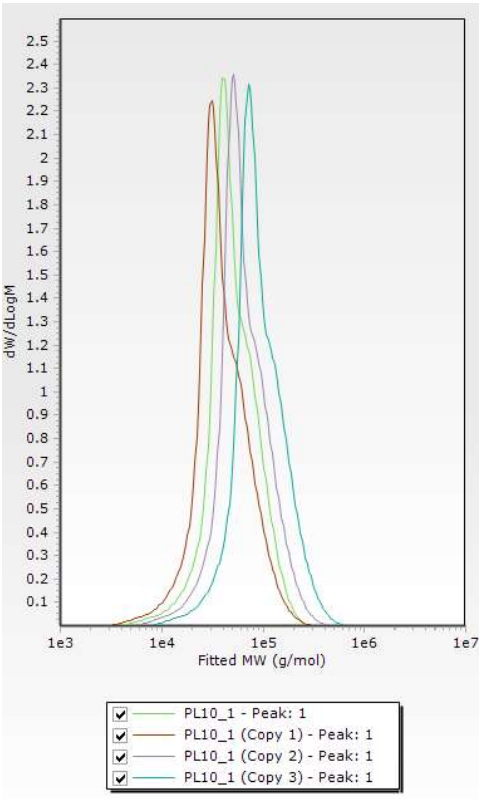


Figure 28. Distribution plots of the same PLLA sample (IV 1.0 dl/g, measured on 19.4.2018) analyzed with the triple detection, universal calibration I (according to Garlotta[36] and Dorgan *et al.*[83]), and conventional calibration. The curves from left to right: Garlotta, triple, Dorgan, and conventional.

Generally, the results from the triple detection method could be assumed to be more realistic than the results analyzed with other analyzing methods. That is because in the triple detection system, there were more detectors and more data obtained for calculations. That is why the results analyzed with the conventional and universal calibration methods were compared to the triple detection results. The percentual differences between the molar mass average results analyzed with the universal or conventional calibration compared to the triple detection results are gathered in Table 11. From this table, it could be seen that the results of universal calibration analyzed with the Mark-Houwink parameters of Garlotta[36] were always smaller than the results analyzed with the triple detection. The maximum observed difference between the results analyzed with the parameters of Garlotta[36] and the triple detection method was 23.9 % and generally, the difference was about 20 %. However, when concentrating to the M_w results, the percentual differences between the Garlotta's universal calibration results and triple detection results were mostly under 10 %. This difference was relatively small.

When concentrating on the molar mass average results analyzed with the Mark-Houwink parameters of Dorgan *et al.*[83], the biggest difference observed between the universal calibration and triple detection method results was 42.9 %. The molar mass average results analyzed with parameters of Dorgan *et al.*[83] were always bigger than those of the triple detection method. Generally, the difference was 20-30 % but in case of the M_w results, the difference was even larger. For this reason, it seemed that the results analyzed with the universal calibration with parameters of Garlotta[36] instead of parameters of Dorgan *et al.*[83] were nearer to the results analyzed with the triple detection. Because of this, the results of molar mass averages of PLLA analyzed with the universal calibration with Garlotta's parameters were possibly more trustworthy than the results analyzed with parameters of Dorgan *et al.*[83] Also from Table 11 it can be seen that in PLLA samples, the difference between the results (analyzed with the triple detection and universal calibration method) was fairly the same regardless of the inherent viscosity of the samples.

Table 11. PLLA molar mass averages determined with the triple detection (T), universal calibration I (U), and conventional calibration (C) with comparison to the triple detection. Term X means C or U. In the universal calibration, different Mark-Houwink parameters were utilized according to Garlotta[36] and Dorgan *et al*.[83] The *M* values used are the average values determined in different measurements (see Tables 1-3 in Appendix I, Tables 1-3 in Appendix III, Tables 1-3 in Appendix IV, and Tables 1-3 in Appendix VI).

Sample	Analyzing method	M_p (g/mol)	(X-T)/T (%)	M_w (g/mol)	(X-T)/T (%)	M_n (g/mol)	(X-T)/T (%)
PL10	T	40836		56693		42253	
	C	72947	78.6	106276	87.5	75963	79.8
	U, Garlotta	31243	-23.5	46553	-17.9	32160	-23.9
	U, Dorgan	51233	25.5	74219	30.9	53410	26.4
PL18	T	74208		111096		68790	
	C	138036	86.0	227338	104.6	130605	89.9
	U, Garlotta	60274	-18.8	101412	-8.7	55812	-18.9
	U, Dorgan	95674	28.9	154930	39.5	90977	32.3
PL24	T	132425		170235		103133	
	C	232654	75.7	355481	108.8	200932	94.8
	U, Garlotta	103242	-22.0	160487	-5.7	83973	-18.6
	U, Dorgan	159484	20.4	239681	40.8	135457	31.3
PL32	T	235686		255365		151379	
	C	464403	97.0	543709	112.9	285794	88.8
	U, Garlotta	193818	-17.8	230303	-9.8	117671	-22.3
	C, Dorgan	312837	32.7	364819	42.9	199111	31.5
PL38	T	277950		311494		190660	
	C	538939	93.9	659452	111.7	351614	84.4
	U, Garlotta	245247	-11.8	304727	-2.2	157076	-17.6
	U, Dorgan	363137	30.6	440379	41.4	243323	27.6
PL49	T	370792		435320		277511	
	C	696927	88.0	911865	109.5	497731	79.4
	U, Garlotta	319647	-13.8	425201	-2.3	225509	-18.7
	U, Dorgan	467067	26.0	604950	39.0	340994	22.9
PL65	T	654597		687988		370112	
	C	1212184	85.2	1367084	98.7	668218	80.5
	U, Garlotta	565325	-13.6	644624	-6.3	291845	-21.1
	U, Dorgan	803156	22.7	897659	30.5	442796	19.6

If the results of the conventional calibration were compared to those of the universal calibration and triple detection, it could be seen that the results of the conventional calibration were always larger than the results obtained from other methods. When the results of the conventional calibration were compared to those of the triple detection method, the difference varied between 75.7 and 112.9 %. Therefore, the difference was significant and much larger than the difference between the results of universal calibration and triple detection. This can be also seen from Figure 29 where differently analyzed M_ws of PLLA are set in the graph as a function of inherent viscosity. Thus, it seemed that the results analyzed with the universal calibration I method with parameters of Garlotta[36] corresponded the results of the triple detection the best. In proportion, the results of the conventional calibration corresponded the results of the triple detection the worst. This might be a result of a fact that in conventional calibration, the used Mark-Houwink parameters did not answer the parameters of PLLA but the standard material, PS. Correspondingly, in the universal calibration, the parameters were set according to the analyzed material and therefore, the results were nearer to the triple detection results than the universal calibration results. In addition, from Figure 29 it can be noticed that the difference between the molar masses analyzed with different methods increased when the inherent viscosity of the samples increased.

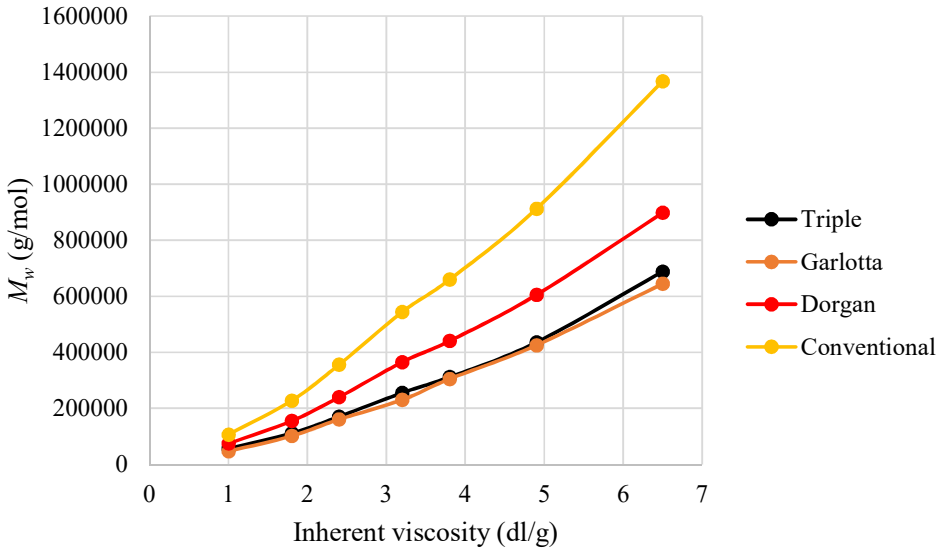


Figure 29. The correlation of inherent viscosity and M_w of the PLLA samples analyzed with different methods. In the universal calibration, the parameters of Garlotta[36] and Dorgan *et al.*[83] are used.

When concentrating on the measured *PD*s analyzed with the triple detection, conventional calibration and universal calibration I methods (using the Mark-Houwink parameters of Garlotta[36] and Dorgan *et al.*[83]), it could be seen that in every case the *PD*s of the triple detection were the smallest (Table 12). The difference between the results of the triple detection and universal calibration when using the parameters of Garlotta[36] was between 7.9 and 19.8 %, whereas the equivalent difference when utilizing the parameters of Dorgan *et al.*[83] was 3.5-12.7 %. Therefore, the results of the universal calibration were nearer to the results of the triple detection method when utilizing the parameters of Dorgan *et al.*[83] than the results analyzed with the parameters of Garlotta[36].

Correspondingly, in the case of *PD* results analyzed with the conventional calibration, the difference to the results analyzed with the triple detection method was between 4.2 and 16.4 %. Thus, the observed differences were not large and they were in the same magnitude. From Table 12 it can be seen that the *PD*s analyzed with the universal calibration by using the parameters of Garlotta[36] were the largest of all the analyzed results. In addition, the *PD*s analyzed with the parameters of Dorgan *et al.*[83] and the conventional calibration were in the same range. On the whole, the order of magnitude of *PD*s analyzed differently was *PD*(Triple) < *PD*(Dorgan *et al*.[83]) ≈ PD(Conventional) < *PD*(Garlotta[36]).

Table 12. PLLA polydispersities (*PD*s) determined with the triple detection (T), universal calibration I (U), and conventional calibration (C) with comparison to the triple detection. Term X means C or U. In conventional calibration, different Mark-Houwink parameters are utilized according to Garlotta[36] and Dorgan *et al*.[83] The *PD* values used are the average values of the molar mass averages determined in different measurements (see Table 4 in Appendix I, Table 4 in Appendix III, Table 4 in Appendix IV, and Table 4 in Appendix VI).

Sample	Analyzing method	*PD*	(X-T)/T (%)
PL10	T	1.34	
	C	1.40	4.2
	U, Garlotta	1.45	7.9
	U, Dorgan	1.39	3.5
PL18	T	1.62	
	C	1.74	7.8
	U, Garlotta	1.82	12.5
	U, Dorgan	1.70	5.5
PL24	T	1.65	
	C	1.77	7.0
	U, Garlotta	1.91	15.7
	U, Dorgan	1.77	7.1
PL32	T	1.69	
	C	1.90	12.7
	U, Garlotta	1.95	15.6
	U, Dorgan	1.83	8.5
PL38	T	1.64	
	C	1.88	14.7
	U, Garlotta	1.94	18.7
	U, Dorgan	1.81	10.7
PL49	T	1.58	
	C	1.83	16.4
	U, Garlotta	1.89	19.8
	U, Dorgan	1.78	12.7
PL65	T	1.88	
	C	2.05	9.0
	U, Garlotta	2.21	17.7
	U, Dorgan	2.03	8.0

As conclusion, when comparing the different molar mass averages it could be seen that the results analyzed with different calibration methods were not similar. With the conventional calibration method, the analyzed molar mass average results were the largest and the farthest from the results of the triple detection method. In the universal calibration, the magnitude of the difference between the results analyzed with universal calibration and triple detection depended on the Mark-Houwink parameters. Therefore, the results of the universal calibration were very much dependent on the used Mark-Houwink parameters. When examining especially the M_ws of PLLA, the results of universal calibration were nearer to the results of the triple detection when the Mark-Houwink parameters were set according to Garlotta[36]. However, when examining the *PD*s of PLLA samples it could be seen that the results of the universal calibration were nearer to the results of the triple detection when the Mark-Houwink parameters were set according to Dorgan *et al*[83]. Yet, the *PD* is determined via molar mass averages and therefore, the molar mass average results are more relevant than the *PD* results when comparing the results obtained with different parameters. There are several different parameters reported in the literature which indicates about the difficulties in the determination of Mark-Houwink parameters.

11.4.2 PLGA AND PLCL SAMPLES

The determined molar mass averages analyzed with the conventional and universal calibration were compared to the results from the triple detection. Both PLGA and PLCL results are gathered in Table 13. In addition, from Figure 30 it can be noticed the difference between the molar masses analyzed with different methods and inherent viscosity of the samples.

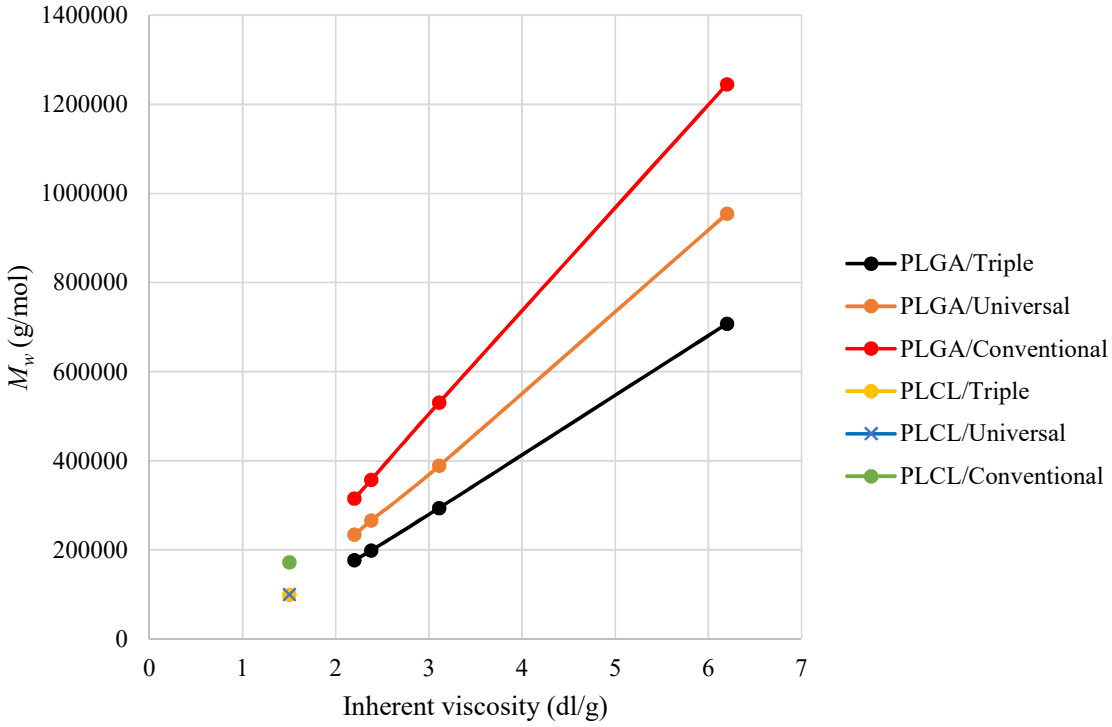


Figure 30. The correlation of inherent viscosity and M_w of the PLGA and PLCL samples analyzed with different methods. The used values are from the Appendix II (Table 2), Appendix V (Table 2), and Appendix VII (Table 2). In the universal calibration, the M-H parameters of Kenley *et al.*[86] (PLGA) and the parameters determined by the triple detection (PLCL) are used.

From the molar mass average results, it could be noticed that with the results of PLGA samples there was a difference of 19.5-34.9 % between the results analyzed with triple detection method and universal calibration (Table 13). The results of different molar mass averages with universal calibration were in every case larger than those from the triple detection method. The observed difference was on its largest in M_ws. If the PLGA triple detection results were compared to the results of conventional calibration, the observed difference was between 48.2 and 80.4 %. Also in this case, the difference was on its largest in the weight average molar masses. The observed difference was notable and larger than the difference between the results of universal calibration and triple detection.

Table 13. Collection of PLGA and PLCL molar mass averages determined with the triple detection (T), conventional calibration (C), and universal calibration I (U) methods with comparison to the triple detection. Term X means C or U. In the universal calibration, different Mark-Houwink parameters are utilized according to Kenley *et al.*[86] (PLGA) and the average values from triple detection (PLCL, Appendix II, Tables 5 and 6). The *M* values used are the average values of the molar mass averages determined in different measurements (see Tables 1-3 in Appendix II and Tables 1-3 in Appendix V, and Tables 1-3 in Appendix VII).

Sample	Analyzing method	M_p	(X-T)/T (%)	M_w	(X-T)/T (%)	M_n	(X-T)/T (%)
PLG22E	T	178774		176821		90431	
	C	294259	64.6	315120	78.2	147000	62.6
	U, Kenley	222506	24.5	234253	32.5	108078	19.5
PLG238P	T	198646		198600		108252	
	C	330175	66.2	356979	79.7	171478	58.4
	U, Kenley	249487	25.6	265847	33.9	135067	24.8
PLG311P	T	291048		294110		156618	
	C	485916	67.0	530662	80.4	256558	63.8
	U, Kenley	361103	24.1	388691	32.2	193101	23.3
PLG62E	T	708353		707732		390574	
	C	1146336	61.8	1245293	76.0	578808	48.2
	U, Kenley	886786	25.2	954654	34.9	467363	19.7
PLC15	T	89570		99610		58936	
	C	145359	62.3	172432	73.1	96798	64.2
	U, triple averages	84106	-6.1	100237	0.6	55213	-6.3

With PLCL samples, the molar mass average results of the triple detection method and universal calibration were relatively close to each other (Table 13). That was how it was assumed because in the universal calibration, the utilized Mark-Houwink parameters were the parameters determined in the triple detection. Thus, the parameters were exactly the same in the universal calibration and the triple detection analyses and therefore, it was assumed that the results would be relatively the same. Generally, the difference was about 6 % (the results of the conventional calibration were smaller than the results of triple detection method) and in case of M_w, the

difference was only 0.6 %. Thus, although the parameters were the same in both analyzing methods, the results were not completely the same. However, the observed differences were quite small. The difference can be explained, for example, by the fact that the integration and baseline regions in the analyses were not completely the same. In the GPC analyses, there are generally differences between the analyzed results according to the analyst because everyone has an own way to do the analysis.

If the PLCL molar mass average results of the triple detection and conventional calibration were compared, the observed difference was much bigger than the difference observed between the results of triple detection and universal calibration. The difference varied between 62.3 and 73.1 %. The difference could be explained by the analysis method. As noted before, in the conventional calibration the Mark-Houwink parameters correspond the standard material (PS) and not the measured material. The difference between the structure of PS and PLCL molecules is quite big and this probably caused the observed differences.

As with the PLLA samples, the triple detection results were probably the most reliable due to the several detectors in the device. The observed differences between the results analyzed with different methods might be a result of the used Mark-Houwink parameters. In the universal calibration, the used parameters for PLGA were from the literature. In this literature reference, there was no reference, for example, about the size of the polymer used in the parameter determination. Thus, the parameters used might be more suitable for different sized polymers than that were used in this thesis. If the parameters were not correct, also the results were not correct. In the case of conventional calibration results, the difference was likely caused by the analysis method. In the conventional calibration, the Mark-Houwink parameters were set according to the standard polymer (in this case PS). As a molecule, PS, PLGA, and PLCL are quite different and thus, not completely comparable.

There was also a difference in the *PD*s of the PLGA and PLCL results analyzed with the conventional calibration, universal calibration, and triple detection. From Table 14 it could be noticed that the results analyzed with the triple detection method were always the smallest. The difference between the PLGA results analyzed with the universal calibration and triple detection varied between 7.2 and 12.4 %, whereas with PLCL sample, the difference was 7.4 %. Correspondingly, when comparing the results analyzed with the triple detection and conventional calibration, the difference in the PLGA results was in the range of 9.4-18.6 %. For PLCL results, the corresponding difference was 5.4 %. Notable was that in the case of PLCL

PD results, the results analyzed with the conventional calibration were nearer to the results analyzed with the triple detection than the results analyzed with the universal calibration. This was surprising when taking into account that in the universal calibration of PLCL, the Mark-Houwink parameters determined in the triple detection were utilized. Because generally the differences were quite small, it was possible that the way of the analysis making had a larger effect to the *PD* results than, for example, in the molar mass average analysis. On the whole, the observed differences were not substantial but some differences between the *PD*s analyzed with different methods could be observed.

Table 14. PLGA and PLCL polydispersities (*PD*s) determined with the triple detection (T), universal calibration (U), and conventional calibration (C) with comparison to the triple detection. Term X means C or U. In the universal calibration, the PLGA samples were analyzed with the Mark-Houwink parameters of Kenley *et al.*[86] and with PLCL, the Mark-Houwink parameters of the triple detection method analysis are used (Appendix II, Tables 5 and 6). The *PD* values used are the average values of the *PD*s determined in different measurements (see Table 4 in Appendix II, Table 4 in Appendix V, and Table 4 in Appendix VII).

Sample	Analyzing method	*PD*	(X-T)/T (%)
PLG22E	T	1.96	
	C	2.15	9.4
	U, Kenley	2.17	10.6
PLG238P	T	1.84	
	C	2.08	13.4
	U, Kenley	1.97	7.2
PLG311P	T	1.88	
	C	2.07	10.2
	U, Kenley	2.01	7.2
PLG62E	T	1.82	
	C	2.15	18.6
	U, Kenley	2.04	12.4
PLC15	T	1.69	
	C	1.78	5.4
	U, triple averages	1.82	7.4

11.5 COMPARISON BETWEEN THE MATERIALS

There were some differences and similarities observed between the PLLA, PLGA, and PLCL samples. When considering the different molar mass averages of PLLA and PLGA measured with GPC, it was noticed that when the inherent viscosity of the material increased, also the molar mass averages increased. This correlation was observed in all the results analyzed with the triple detection, universal calibration I, and conventional calibration methods. In addition, this correlation followed the Mark-Houwink equation (9). When comparing different molar mass averages of the same sample (same material and inherent viscosity), they were in most cases in the order of $M_n < M_p < M_w$ regardless of the material (PLLA, PLGA or PLCL). This order also followed the theory. Also, for all the materials it was observed that the smaller the molar mass of the sample was, the longer the retention time was as it should be according to the operational principle of GPC.

When comparing the measured M_w values and the inherent viscosities of the samples, it could be seen that there was an almost linear correlation between these variables both for the PLLA and PLGA samples. This correlation can be seen in Figure 31. The rounded R^2 values for PLLA and PLGA lines were 0.98 and 1.00, respectively. Thus, the PLGA line was straighter than PLLA line. If the slopes of the linear regression lines were compared, the slope of PLLA line was 113840 $\frac{g^2}{mol \cdot dl}$ and the slope of PLGA line was 133070 $\frac{g^2}{mol \cdot dl}$. The difference of the slopes was large enough to cause the lines to overlap in the low inherent viscosities and in proportion to separate in the higher viscosities.

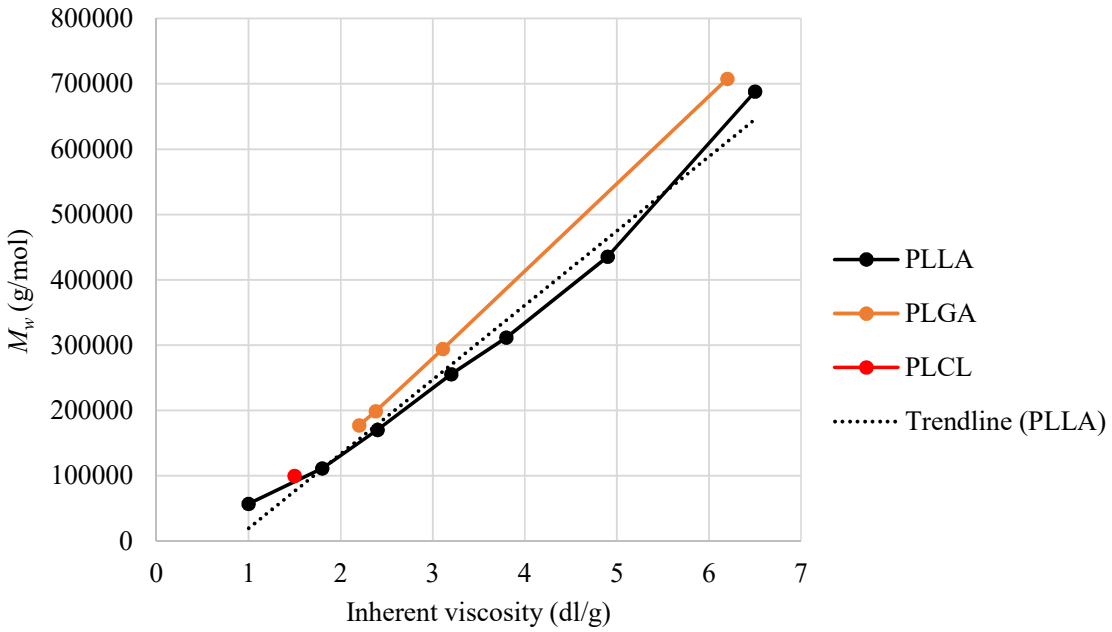


Figure 31. Correlation of inherent viscosity and M_w for the PLLA, PLGA, and PLCL samples. There is also a linear trendline for PLLA. The used M_w values are the average values from Table 2 in Appendix I and Table 2 in Appendix II (analyzed with the triple detection) and the used inherent viscosities are informed by the manufacturers.

It was also possible to compare the materials through the samples with same inherent viscosities. In this comparison, the results obtained with the triple detection were utilized. By this way, the analysis of the materials should be the most comparable. Four different sample pairs were chosen to this examination according to their inherent viscosities: PL10 – PLC15, PL24 – PLG238, PL32 – PLG311, and PL65 – PLG62. The results are gathered in Table 15.

Table 15. Comparison of the different materials (PLLA/PLGA/PLCL). The samples with the inherent viscosity of same magnitude analyzed with the triple detection are compared. The used values are the average values from Tables 1-4 in Appendix I and Tables 1-4 in Appendix II. The used inherent viscosities (IV) are informed by the manufacturers.

Sample	IV	M_p	M_w	M_n	*PD*
PL10	1.0	40836	56693	42253	1.34
PLC15	1.5	89570	99610	58936	1.69
PL24	2.4	132425	170235	103133	1.65
PLG238	2.38	198646	198600	108252	1.84
PL32	3.2	235686	255365	151379	1.69
PLG311	3.11	291048	294110	156618	1.88
PL65	6.5	654597	687988	370112	1.88
PLGA62	6.2	708353	707732	390574	1.82

From Table 15, it could be seen that from the samples with similar inherent viscosities, the PLGA samples always show larger results (M_p, M_w, M_n, and *PD*) than the PLLA samples when the inherent viscosity was between 2.38 and 6.5 dl/g. This could be also concluded from Figure 31. Only exception for this is in the *PD*s of the samples with inherent viscosity of 6.2-6.5 dl/g. In addition, when the inherent viscosity of the sample was 1.0-1.5 dl/g, PLCL obtained a bit larger values than PLLA. Because there was only one PLCL sample, it could not be concluded what kind of the correlation there would be for higher viscosities of PLCL.

Overall according to Figure 31 and Table 15, it seemed that PLLA showed the smallest values of different molar mass averages and *PD*s if it is compared to the PLGA and PLCL. The reason for this could be found out again from the molecular structure of these polymers. As a polymer, PLLA consist completely of lactide monomers, whereas PLGA and PLCL have also glycolides and ε-caprolactones in their structure. The structure of the PLLA and PLGA molecules is quite similar and the observed differences between them are not enormous. In the case of PLCL, the molecules differ a lot from PLLA molecules because of the five-member carbon chain. That is why the observed differences between PLLA and PLCL were larger than the differences between PLLA and PLGA.

The *PD* should not depend much on the inherent viscosity of the polymer. However, there were some indications of this kind of correlations in some measurements (only with the samples of PLLA). Despite of that, generally the *PD* values of the same material analyzed with the same method stayed quite the same. This is because *PD* is defined as a ratio of M_w and M_n. Both of these molar mass averages change relatively the same way when the inherent viscosity of the samples changes so the ratio of them stays quite stable. Therefore, it is reasonable to calculate averages of the *PD*s of different materials by using all of the results from the same material.

If the average value from all the measured results of the same material was calculated, the *PD* of PLLA was 1.63, for PLGA 1.87, and for PLCL 1.69 (the results analyzed with the triple detection method). In proportion for the results analyzed with the universal calibration I method, the corresponding average *PD* of PLLA (Garlotta[36]) was 1.88, for PLLA (Dorgan *et al.*[83]) 1.76, for PLGA 2.05, and for PLCL 1.82. The corresponding results analyzed with the conventional calibration were 1.80 (PLLA), 2.11 (PLGA), and 1.78 (PLCL). Thus, if the materials were arranged according to the *PD*s, the order was PLLA < PLCL < PLGA (results analyzed with the triple detection method), PLLA(Dorgan *et al.*[83]) < PLCL < PLLA(Garlotta[36]) < PLGA (results analyzed with the conventional calibration), and PLLA < PLCL < PLGA (results analyzed with the conventional calibration). Consequently, the order of the *PD*s of the materials stayed quite stable (except for the PLLA results analyzed with the conventional calibration with Garlotta's parameters) and did not considerably change because of the analyzing method.

When analyzing the material specific *dn/dc* values of these materials it could be observed that PLLA has the lowest *dn/dc* value (approximately 0.047 ml/g according to Table 7 in Appendix IV). The corresponding values for PLGA and PLCL were 0.048 ml/g and 0.052 ml/g, respectively, (Table 7 in Appendix II). These values are also presented in Table 16 with comparison to the literature values. The *dn/dc* values of PLLA and PLGA were quite near to each other but PLCL obtained clearly a larger *dn/dc* value. This might be a result from the fact that the molecular structures of PLLA and PLGA are quite similar but the structure of PLCL is a bit different. The molecular structure of PLCL differs from the structures of PLLA and PLGA due to the 5-carbon chain in the middle of the polymer which does not exist in the PLLA and PLGA molecules. However, the differences in the structures are not significantly large and thus, in larger perspective, all of these values were in the same order of magnitude.

Table 16. Average values of α, K, and dn/dc for PLLA, PLGA, and PLCL. The results are analyzed by the triple detection and averages are calculated using Tables 5-7 in Appendix I and Tables 5-7 in Appendix II. There are no literature values for PLCL.

Variable	Average of all the measurements	Literature value
α(PLLA)	0.91	0.65[36], 0.736[83]
K(PLLA)	$47.71 \cdot 10^{-5}$ dl/g	$100 \cdot 10^{-5}$ dl/g[36], $17.4 \cdot 10^{-5}$ dl/g[83]
dn/dc(PLLA)	0.047 ml/g	0.042 ml/g[91], 0.0558 ml/g[90]
α(PLGA)	0.81	0.761[86]
K(PLGA)	$13.77 \cdot 10^{-5}$ dl/g	$10.7 \cdot 10^{-5}$ dl/g[86]
dn/dc(PLGA)	0.048 ml/g	0.05 ml/g[89]
α(PLCL)	0.68	-
K(PLCL)	$44.84 \cdot 10^{-5}$ dl/g	-
dn/dc(PLCL)	0.052 ml/g	-

If the Mark-Houwink parameters of different materials measured with the triple detection method were compared by using the averages of all the measured data of the same material, the α parameters were in order α(PLCL) < α(PLGA) < α(PLLA). Respectively, the K parameters were in order K(PLGA) < K(PLCL) ≈ K(PLLA). Therefore, in both of these orders the parameter of PLLA was the largest. These average values and corresponding literature values are collected in Table 16.

However, it should be noted that a clear correlation between α parameters and inherent viscosities of the samples was observed in the measurements of PLLA samples (Table 5, Appendix I). This correlation was not observed with PLGA and PLCL samples which might be a results from the fact that there were fewer measuring data on PLGA and PLCL materials than on PLLA. In that case, the possible correlation could not be observed. Thus, the way to calculate only one average value for these parameters according to the material and use it in the comparison is a bit questionable. The fact that there is possibly some correlation between α values and inherent viscosities can be also concluded on the basis of the Mark-Houwink equation (equation 9, $[\eta] = K \cdot M^{\alpha}$). Clear correlation between inherent viscosities and K parameters was not observed in the measurements. Only possible correlation for all the materials observed between inherent viscosity and K was that with samples of high inherent viscosity the K was quite small.

INHERENT VISCOSITY PART

12 EQUIPMENT

The inherent viscosity measurements were carried out with an Anton Paar -microviscometer (Lovis 2000 M/ME). There was also an automatic sample changer in the equipment. The ball used in the measurements was steel (ø 1.5 mm, ball density 7.66 g/cm^3). The measuring conditions were set to respond the conditions of the Ubbelohde-viscometer: the measuring angle was set to 30°, the measuring distance was 100.12 mm, and the temperature was 25 °C. The temperature stabilization time was adjusted to 10 s. From the capillaries of Arctic Biomaterials Oy, the capillary D (serial number 21617423, ø 1.59 mm) was used.

The weighing of the samples was done with Mettler Toledo XP205 –scales. During the sample preparation, the dosing feeder (Brand Dispensette organic) and sample shaker (Stuart orbital shaker SSL1, rate 80 rpm) were utilized. The samples were filtered with a syringe (10 ml, Henke Sass Wolf, Norm-Ject luer lock, filter Agilent Captiva PTFE 0.2 µm) to the test tubes (glass, 12 mm), which were closed with plastic caps (Uni-flex safety caps, 16 mm, Anton Paar). The solvent used to dissolve the samples and also used in the microviscometer for rinsing the capillaries was chloroform (Honeywell Chromasolv for HPLC, amylene stabilized). Also ethanol (Etax A, manufacturer Altia Oyj) was used as secondary solvent in special cleaning.

13 SAMPLES

The samples were the same as used in the GPC measurements (see section 10) and they included PLLA, PLGA, and PLCL samples with different inherent viscosities. The PLLA samples (Purasorb PL, Poly(L-lactide)) were manufactured by Corbion Purac and the samples had the inherent viscosities of 1.0, 1.8, 2.4, 3.2, 3.8, 4.9, and 6.5 dl/g. The PLGA samples were manufactured by Evonik and Corbion Purac and the inherent viscosities of these samples were 2.2, 2.38, 3.11, and 6.2 dl/g. There was only one PLCL sample with inherent viscosity of 1.5 dl/g and it was produced by Evonik. These values were determined by using the Ubbelohde method.

13.1 PREPARATION

At first, the samples were taken out from the freezer (-60 °C) and were let to settle at room temperature (22 °C). After that, the samples were weighed in the 20 ml measuring bottles so that there was 19.6-20.4 mg of the sample in the measuring bottle. First the samples were measured in the plastic weighing boat and then transferred to the measuring bottles. The weighed amounts of the samples in the weighing boats were written down for later concentration calculation (about 0.1 g/dl).

After the samples were weighed, the measuring bottles were filled in half with chloroform with the help of dosing feeder. The samples were let to dissolve overnight in a shaker. On the next day the measuring bottles were filled to the mark with chloroform using the dosing feeder and a glass Pasteur pipette. The measuring bottles were shaken by hand and the samples were filtered to the 12 ml glass test tubes which were closed with caps. The sample solution was poured straight from the measuring bottle to the syringe attached with filter and then pressed to the test tube. The test tubes were not filled full and they were let about 10 mm short. After that, the samples were ready for measuring.

13.2 MEASUREMENTS

Before the measurements, the capillary was assembled into the device and the microviscometer was let to warm up at least 20 minutes. When the microviscometer was warm, first the pure chloroform was measured (chloroform check). By this way, the device got the information of the solvent which was used to dissolve the PLLA samples. The used density for chloroform was 1.492 g/cm^3. During the chloroform check, the device determined the dynamic viscosity of the chloroform which was written down. After the chloroform check, the rolling time of the ball in pure chloroform was measured in the same way as a normal sample measurement and the time was written down for later measurements.

After the starting procedure, it was time for sample measurements. In the measurements, the previously measured ball's rolling time in pure chloroform was utilized and also the concentrations of the samples (g/dl, calculated from the weighing results) were entered. The samples were set in known order in the sample carousel and the run was started. After the measurements, the microviscometer reported if the measurements were valid (variation between two parallel rolling times of the same sample was small enough) and the results could

be downloaded from the device. After that, the capillary was unassembled and washed with chloroform with a small brush. The capillary was dried with compressed air and set back to the capillary case.

14 RESULTS (INHERENT VISCOSITY) AND DISCUSSION

14.1 PLLA SAMPLES

There were seven different PLLA samples which differed with their inherent viscosity. The samples were the same as used in the GPC measurements (see section 10). The viscosities of the samples were 1.0, 1.8, 2.4, 3.2, 3.8, 4.9, and 6.5 dl/g according to the manufacturers. Four separate measurements were carried out for all of these samples on 4.5.2018, 17.5.2018, 27.6.2018, and 28.6.2018. The results are gathered in Table 1 in Appendix VIII. Two parallel samples (separately weighed) were prepared from each material excluding the measurement done on 4.5.2018. The variation between the measurements was small and the RSD was between 1.1 and 3.6 %.

If the inherent viscosity values of the PLLA samples reported by Corbion Purac were compared with the measured results, the inherent viscosities were quite close to each other. The informed and measured inherent viscosities (averages) are gathered in Table 17. From Table 17 it can be seen that the biggest difference between the informed and measured inherent viscosities was in the sample of PL49 with the difference on 0.2 dl/g. Of all the samples, except for the PL10 and PL65, the measured values for inherent viscosity were lower than informed.

Table 17. Informed and measured inherent viscosities of the PLLA samples. The used measured inherent viscosities are the averages of several measurements (see Table 1 in Appendix VIII). The relative standard deviations (RSD) of the measurements are included. In addition, the allowed ranges of inherent viscosities informed by the manufacturer are presented.

Sample	Inherent viscosity informed by the manufacturer (dl/g)	Measured inherent viscosity (dl/g)	RSD of the measurements (%)	Allowed range of inherent viscosity (dl/g)
PL10	1.0	1.0	1.8	0.9-1.2[108]
PL18	1.8	1.7	1.1	1.5-2.0[109]
PL24	2.4	2.3	1.9	2.0-2.7[110]
PL32	3.2	3.1	3.6	2.7-3.6[111]
PL38	3.8	3.7	1.5	3.2-4.3[112]
PL49	4.9	4.7	3.3	4.3-5.5[113]
PL65	6.5	6.5	1.6	5.5-7.5[114]

In the manufacturer's websites, there was a certain range informed in which the product's inherent viscosity should be (Table 17). If these ranges were compared to the measured results, all the results were in the informed region. When looking for the size of the range it could be observed that the region was smaller with the samples of low inherent viscosity and quite big with the samples of high inherent viscosity. This might indicate that the samples with larger inherent viscosity are more difficult to manufacture with a certain inherent viscosity.

14.2 PLGA AND PLCL SAMPLES

There were four PLGA samples with different inherent viscosities and one PLCL sample which were measured with microviscometer. The samples were the same as those used in the GPC measurements (see section 10). The inherent viscosities of the PLGA samples were 2.2, 2.38, 3.11, and 6.2 dl/g according to the manufacturers. The inherent viscosity of the PLCL sample was informed to be 1.5 dl/g. Four different measurements were performed on 27.6.2018, 28.6.2018, 3.7.2018, and 4.7.2018 and the results are collected in Table 2 in Appendix VIII. There were two separately weighed parallel samples for every material. The variation between the measurements was small and the RSD for PLGA samples was between 0.8 and 1.4 % and for PLCL samples 3.9 %.

From Table 2 in Appendix VIII and from Table 18 it can be noticed that the measured values for inherent viscosities of the samples and by that way the averages of different sample types were in every case smaller than the values informed in the certificates by the manufacturer. Only exception from this was the sample PLC15. The biggest difference between the informed and measured viscosities was in the sample PLG62 in which the difference was 0.5 dl/g. With the other samples, the measured and informed inherent viscosities were quite near to each other. Also in case of PLGA and PLCL samples, there was a certain range informed in which the product's inherent viscosity should be in the manufacturer's websites. If these ranges were compared to the measured results, all the results were in these limits.

Table 18. Informed and measured inherent viscosities of the PLGA and PLCL samples. The used measured inherent viscosities are the averages of several measurements (see Table 2 in Appendix VIII). The relative standard deviations (RSD) of the measurements are included. In addition, the allowed ranges of inherent viscosities informed by the manufacturer are presented.

Sample	Inherent viscosity informed by the manufacturer (dl/g)	Measured inherent viscosity (dl/g)	RSD of the measurements (%)	Allowed range of inherent viscosity (dl/g)
PLG22	2.2	2.1	0.8	1.7-2.6[115]
PLG238	2.38	2.2	1.0	2.0-2.5[116]
PLG311	3.11	3.0	1.0	2.7-3.5[117]
PLG62	6.2	5.7	1.4	5.0-7.0[115]
PLC15	1.5	1.5	3.9	1.3-1.8[115]

14.3 DISCUSSION

When considering the inherent viscosity results measured with microviscometer, it could be noticed that with every sample type (PLLA, PLGA, and PLCL) the measured results were quite near to the inherent viscosities informed by the manufacturers. The values informed by the manufacturers were measured with the Ubbelohde method. Generally, the measured values were smaller than informed. The smaller measured inherent viscosities could be explained, for example, with the naturally occurring polymer degradation. The manufacturing dates of the samples are gathered in Table 19. If the manufacturing dates of the materials were observed it could be seen that the sample PLG62 was notably older than the other samples. In addition, the

biggest difference was observed in the very same sample PLG62 where the measured inherent viscosity was 0.5 dl/g smaller than the informed value. This confirmed the idea that the drop in the inherent viscosities of the samples may be caused by the degradation of the polymers over the time. Also, although the samples were stored in the freezer, the degradation may have been accelerated because the bags were opened and the material was not in the original bags. However, all the measured results were still in the range of allowed variation announced by manufacturers so it could be assumed that the samples were still usable and the measurements were successful.

Table 19. Manufacturing dates of the measured sample materials. The degradation timeframe is informed by the manufacturer. The precise manufacturing date of PLLA samples was not known.

Sample material	Manufacturing date	Degradation timeframe
PLLA -samples	2017	5 years (-15 °C)[108–114]
PLG22	30.8.2015	1-2 years[118]
PLG238	23.12.2014	5 years (-15 °C)[116]
PLG311	13.5.2014	5 years (-15 °C)[117]
PLG62	1.11.2013	1-2 years[118]
PLC15	2.1.2017	Not measured[118]

In addition, the biggest differences between the informed and measured inherent viscosities were observed with the samples of the highest inherent viscosities. When examining the allowed ranges of variation of different sample types provided by the manufacturers there was the biggest ranges for the samples with higher inherent viscosities. This might denote that it was difficult for the manufacturers to produce samples with high inherent viscosity accurately and the inherent viscosity may change over the time.

If the correlation of M_w and inherent viscosity of PLLA and PLGA samples was estimated again as in the section 11.4 but now with the measured inherent viscosities, a new figure can be drawn (Figure 32).

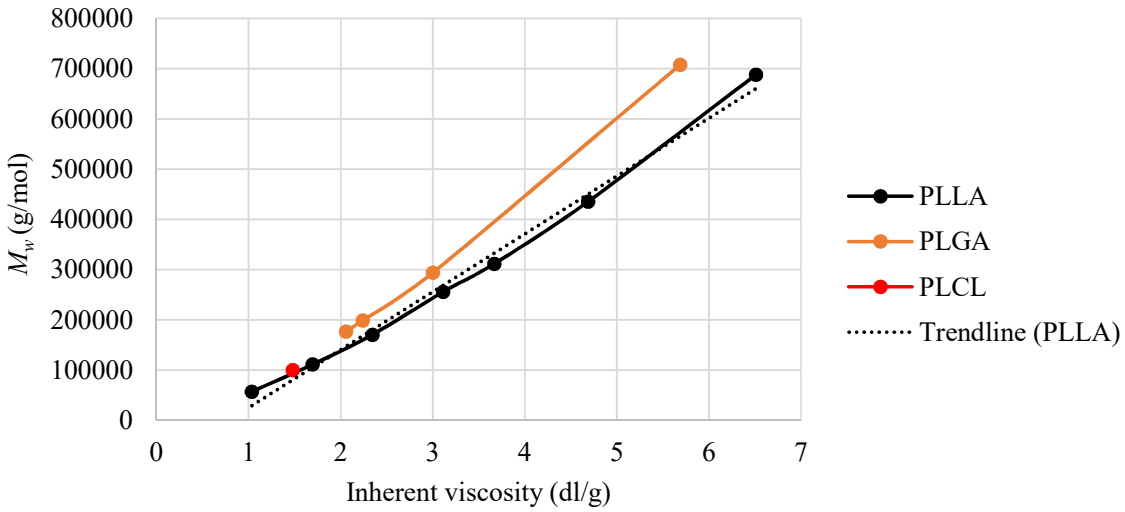


Figure 32. Correlation of inherent viscosity and M_w for measured PLLA, PLGA, and PLCL samples. There is also a linear trendline for PLLA. The used M_w values are the average values from Table 2 in Appendix I and Table 2 in Appendix II (analyzed with the triple detection) and the used inherent viscosities were the average values from Tables 1 and 2 in Appendix VIII.

Figure 32 can be compared to Figure 31. The rounded R^2 value for PLLA line in Figure 32 was 0.99 and for PLGA it was 1.00. In Figure 31, the corresponding values for PLLA and PLGA curves were 0.98 and 1.00, respectively. In Figure 31 the PLGA curve was a bit nearer to be linear than in Figure 32 and in the case of PLLA, the corresponding order was the other way round. In addition, it could be noticed the PLGA curve was nearer to be linear than the PLLA curve in both figures. This might be due to the fact that there were more measuring points in the PLLA curve than in the PLGA curve. Visually, the difference between the figures was not large and this could have been also assumed because the values of the measured inherent viscosities were so close to the manufacturers' values. For example, the order of the lines did not change in the figures.

However, when observing the linear regression lines of Figure 32, the slope of the PLLA was 115310 $\frac{g^2}{mol \cdot dl}$. Respectively, the slope of the PLGA was 147440 $\frac{g^2}{mol \cdot dl}$. The corresponding values for the lines formed with the inherent viscosities informed by the manufacturers (Figure 31) were for PLLA 113840 $\frac{g^2}{mol \cdot dl}$ and for PLGA 133070 $\frac{g^2}{mol \cdot dl}$. When comparing the slopes, it could be seen that in the case of PLLA, the slope was almost the same in these figures. In the case of PLGA, the slope was larger in Figure 32. However, the differences observed were not significant.

15 CONCLUSIONS OF THE EXPERIMENTAL PART

In the experimental part of this thesis, three different types of lactide-based polymers with different inherent viscosities (PLLA (7 samples), PLGA (4 samples), and PLCL (1 sample)) were measured by GPC. The inherent viscosities of the samples were also measured with the help of microviscometer and in these measurements, it was found out that the measured inherent viscosities were a bit smaller than informed by the manufacturers. This could be explained, for example, with the natural degradation of the polymer chains over the time. However, the measured values were in the range informed by the manufacturers so there had not been too much degradation.

With GPC, the same results were analyzed with three different methods; the triple detection, universal calibration I, and conventional calibration to see if there was a difference in the results. In general, the devices with the conventional calibration method have been the most utilized analysis method in GPC so it is important to know about the possible differences in the results which are followed by using different analyzing methods. In addition to find out the possible differences caused by different analysis methods, it was studied how the different Mark-Houwink parameters utilized in the universal calibration I method affect the results.

In the results, the focus was on the different molar mass averages (M_p, M_w, and M_n) and *PD*s. Also when utilizing the triple detection method, the Mark-Houwink parameters (α and K) and *dn/dc* values of the samples were found out and compared to the literature values. In addition, the measured *PD*s were compared to the literature values. All the results were relatively comparable to the literature values (if the literature values were found). Also, the comparison between the materials was performed and material-specific differences, for example, in the *dn/dc* values were found.

When comparing the results analyzed with different methods, there were notable differences observed. Generally, the results analyzed with the universal and conventional calibration methods were compared to the results analyzed with the triple detection system. This was done because the results analyzed with triple detection system were assumed to be the most realistic due to the analysis method: there are many detectors in use and no need for literature values. In the universal calibration, the difference between the molar mass average results analyzed with universal calibration and triple detection methods in case of PLLA was about 20 % (results analyzed with parameters of Garlotta[36]) and 20-30 % (results analyzed with parameters of

Dorgan *et al.*[83]). In the conventional calibration, the corresponding observed difference between the molar mass average results was large, 80-100 %. The order of magnitude of the molar mass averages analyzed with different methods was M(Universal, Garlotta[36]) < M(Triple) < M(Universal, Dorgan *et al.*[83]) < M(Conventional).

For PLGA, the differences between the molar mass averages analyzed with the universal calibration and triple detection methods were 20-30 %. Correspondingly, the difference in the results analyzed with the conventional calibration and triple detection methods was 60-80 % and thus, significantly large. These percentages were similar to the percentages observed with in the case of PLLA. The order of magnitude in the molar mass average results for PLGA was M(Triple) < M(Universal, Kenley *et al*.[86]) < M(Conventional).

For PLCL, the observed difference between the results analyzed with the triple detection and the universal calibration methods was about 6 % in the case of M_n and M_p and only 0.6 % in the case of M_w. Thus, the difference in the case of PLCL was quite small (under 10 %) as expected because in the conventional calibration of PLCL the Mark-Houwink parameters determined from the triple detection method were utilized. From these results it could be seen that even when the same parameters were utilized in the different analyses, the results were not exactly the same. Possible reasons for this were, for example, that the utilized integration and baseline regions were not absolutely identical in the analyses. Thus, the analysis made by hand could be seen in the results. When comparing the results analyzed with the conventional calibration and triple detection methods it could be seen that the difference observed was 60-70 % which was observed also with the PLLA and PLGA samples. Therefore, despite of the material, the molar mass average results analyzed with conventional calibration were notably larger than the results analyzed with triple detection.

When the *PD*s of the samples analyzed with different analyzing methods were compared, for all of the materials the *PD* analyzed with the triple detection method was smaller than the *PD* analyzed with the universal or conventional calibration methods. For PLLA, the differences between *PD*s analyzed with universal calibration and triple detection were 10-20 % (Garlotta[36]) and about 10 % (Dorgan *et al.*[83]), and respectively for PLGA, the difference was about 10 %. For PLCL, the difference was under 10 %. When comparing the differences between the *PD*s analyzed with the conventional calibration and triple detection methods, for the PLLA samples the difference was 10-20 %, for PLGA samples about 10 %, and for PLCL sample under 10 %. Therefore, the analyzing method did not have a great effect on *PD*. *PD* is defined as a ratio of

M_w and M_n and because both of these variables change similarly depending on the analysis method, the ratio (i.e. *PD*) stays quite the same.

When comparing the PLLA results analyzed twice with the universal calibration method but with different Mark-Houwink parameters, notable differences could be observed. For example, if the molar mass average results of PLLA analyzed with different parameters (according to Garlotta[36] and Dorgan *et al.*[83]) were compared, the difference was significant (about 50 %). In the case of *PD*, the corresponding difference was under 10 %. Thus, the results analyzed with the universal calibration I method greatly depended on the Mark-Houwink parameters. In molar mass averages, the results analyzed with the universal calibration method with parameters of Garlotta[36] were closer to the results from the triple detection method than the results calculated with parameters of Dorgan *et al*[83]. At the same time, the *PD*s of PLLA from the universal calibration with parameters of Dorgan *et al.* were closer to the results analyzed with the triple detection method (difference about 10 % or under) than the results obtained with the universal calibration with the parameters of Garlotta[36] (difference 10-20 %). Thus, it was not clear which parameters were the best in the universal calibration so that the results would be similar to the results of triple detection method.

If the measured Mark-Houwink parameters were observed, it could be noticed that in some cases there was some correlation between the parameters and inherent viscosities (and thus with the molar masses) of the samples. Generally in the literature, there is only one Mark-Houwink parameter pair informed per material but according to the measurements made in this thesis, it seemed that the parameters depended not only on the material but also on the size of the polymer. Therefore, using the same Mark-Houwink parameters for every molar mass polymers may falsify the results analyzed with the universal calibration I method. This observation is important when considering different tests where the effect of hydrolysis to the polymers over the time is studied. During this type of hydrolysis series, the size of the polymer significantly changes and thus, the parameters used in the analysis should be also changed as the measurements proceed. This problem does not have to be taken into account if the results are analyzed with the triple detection method.

Altogether, it seemed that the Mark-Houwink parameters have a great impact to the GPC results analyzed with the universal calibration I method (especially, in case of different molar mass averages). In addition, there were notable differences observed in the GPC results analyzed with the triple detection, universal calibration, and conventional calibration methods. This is

why these results are not necessarily comparable with each other. It is noteworthy, since all these analysis methods are used in the scientific world. The results analyzed with the triple detection method can be considered to be the most realistic of the different analyzing methods due to the several detectors. If the results of the universal calibration are wanted to be quite similar to the results of the triple detection method, it is not unambiguous which Mark-Houwink parameters should be utilized. In the conventional calibration, more similar results as in the triple detection could be obtained only if the used standards in the calibration would be more equivalent to the measured polymers than what, for example, PSs are for polylactides. However, it is not easy to find suitable standards separately for every polymer.

There were several things which could have caused some error to the results. For example, possible small weighing and volume errors in the sample preparation could have a slight effect on the results. In addition, the way to do the GPC analysis improved during the period of measurements. Because analysis style developed during the project, there might be some differences in the results when comparing the analysis made, for example, in February and June. Also, if the samples were not at room temperature when the plastic bags from the freezer were opened, they could have absorbed some moisture. This could have an influence on weighing so that the weighed amounts of polymer may be a bit lower than they should be. For example, the measured inherent viscosities were smaller than the values informed by the manufacturers which could be explained with the possible humidity in the samples. However, the possible humidity was not likely the only explanation to the differences in the results but the possible error consisted of several different reasons.

On the whole, the measured results did not vary substantially and they went along with different theories including the Mark-Houwink equation and the operational principle of GPC. From this point of view, it seemed that the preparation and measuring of the samples succeeded quite well. In addition, it seemed that the method was quite repeatable. The GPC results analyzed with different methods would have been even more comparable if the exactly the same integration and baseline areas would have been used in the analysis for corresponding samples. Also, the possible moisture problem in the samples could have been avoided if the samples from the freezer would have stayed on the table more than two hours. However, in case of the most of the samples, the standing time was longer than two hours so it could be assumed that most of the samples were confidently at room temperature when the bags were opened.

In further studies, it could be tried to determine the Mark-Houwink parameters with the triple detection system for different polymers so that the results analyzed with the universal calibration I method with those parameters would be equivalent to the results analyzed with the triple detection method. Especially, the parameters for PLCL would be needed because there are no parameters for PLCL in the literature. It would be also profitable to investigate how the inherent viscosities of the samples affect the *PD*s and the Mark-Houwink parameters of samples. Is it a good way to determine only one parameter for the material or should there be parameters separately for the samples with high and low inherent viscosities? For this research, numerous new measurements would be required with samples of several different inherent viscosities.

Also, the way to do the GPC analysis and its effects on the results could be studied more elaborately. For instance, in the triple detection method, it could be figured out how exactly the choosing of the area where red line was tried to answer the results (log M_w) affect the Mark-Houwink parameters. For example, in the measurements made in this thesis it seemed that the chosen area had a notable effect on α values so that when the area was narrow, the values of α increased. Additionally, it could be studied how the selection of the integration area affects the *PD* of the sample. Does the *PD* grow if there is a larger area selected as integration region and through that more data to calculate the *PD*? In addition, it could be studied that if the same data have been analyzed both with the universal calibration and the triple detection method but with exactly the same integration and baseline regions, is there still a notable difference in the analyzing results or does the difference decrease. Also for these studies, several measurements would be needed.

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APPENDIXES

APPENDIX I. THE GPC RESULTS OF PLLA ANALYZED WITH THE TRIPLE DETECTION METHOD (four pages)

APPENDIX II. THE GPC RESULTS OF PLGA AND PLCL ANALYZED WITH THE TRIPLE DETECTION METHOD (four pages)

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APPENDIX I. THE GPC RESULTS OF PLLA ANALYZED WITH THE TRIPLE DETECTION METHOD (Tables 1-7)

Table 1. The M_p averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the triple detection method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-14.2.18	40097	72203	150628	220493	268592	325335	584593
2-14.2.18	38774	71392	153733	224600	254429	354163	607830
1-19.4.2018	40753	75793	114780	231175	262745	343377	654341
2-19.4.2018	40777	73941	116275	228435	273671	342202	645480
1-22.5.2018	42927	75292	121445	242424	304582	426439	679421
2-22.5.2018	41015	73771	121062	245728	285563	361758	693551
1-23.5.2018	*	76029	160494	249356	286579	382475	683356
2-23.5.2018	41509	75241	120980	243276	287435	430587	688201
Average	40836	74208	132425	235686	277950	370792	654597
σ	1175	1592	17770	10108	15091	36745	37518
RSD (%)	2.9	2.1	13.4	4.3	5.4	9.9	5.7

Table 2. The M_w averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the triple detection method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-14.2.18	56600	108649	162815	236950	293559	403952	642722
2-14.2.18	54099	107542	163112	244869	293896	410439	635673
1-19.4.2018	56931	113431	168042	251659	301105	412844	676438
2-19.4.2018	57171	111558	167543	249702	310797	420312	651295
1-22.5.2018	57854	111335	173049	264209	336118	460631	718245
2-22.5.2018	56271	111170	178184	264008	317367	457024	726140
1-23.5.2018	*	113225	173407	266847	320031	447225	736558
2-23.5.2018	57925	111858	175728	264675	319079	470134	716834
Average	56693	111096	170235	255365	311494	435320	687988
σ	1199	1919	5352	10407	13743	24492	38451
RSD (%)	2.1	1.7	3.1	4.1	4.4	5.6	5.6

Table 3. The M_n averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the triple detection method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-14.2.18	41977	68491	97110	135001	172436	260811	359604
2-14.2.18	40125	69390	96178	142529	181284	261407	336749
1-19.4.2018	41956	72529	105040	152511	186585	242863	382001
1-19.4.2018	42450	67318	100699	150051	192779	257666	386130
1-22.5.2018	44255	68156	103495	162137	209424	294091	434543
2-22.5.2018	43005	67757	113344	158919	200978	291113	360314
1-23.5.2018	*	68328	99356	158580	190964	277566	305111
2-23.5.2018	42001	68349	109845	151307	190833	334569	396441
Average	42253	68790	103133	151379	190660	277511	370112
σ	1157	1520	5678	8471	10605	27037	36720
RSD (%)	2.7	2.2	5.5	5.6	5.6	9.7	9.9

Table 4. *PD* values for PLLA samples with different inherent viscosities analyzed with the triple detection method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages of the results have been calculated from the unrounded values.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-14.2.18	1.35	1.59	1.68	1.76	1.70	1.55	1.79
2-14.2.18	1.35	1.55	1.70	1.72	1.62	1.57	1.89
1-19.4.2018	1.36	1.56	1.60	1.65	1.61	1.70	1.77
2-19.4.2018	1.35	1.66	1.66	1.66	1.61	1.63	1.69
1-22.5.2018	1.31	1.63	1.67	1.63	1.61	1.57	1.65
2-22.5.2018	1.31	1.64	1.57	1.66	1.58	1.57	2.02
1-23.5.2018	*	1.66	1.75	1.68	1.67	1.61	2.41
2-23.5.2018	1.38	1.64	1.60	1.75	1.67	1.41	1.81
Average	1.34	1.62	1.65	1.69	1.64	1.58	1.88
σ	0.02	0.04	0.05	0.04	0.04	0.08	0.23
RSD (%)	1.8	2.5	3.3	2.6	2.4	5.0	12.2

Table 5. Mark-Houwink α parameters for the PLLA-samples analyzed with the triple detection method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-14.2.18	0.62	0.60	0.83	0.95	1.17	1.38	1.26
2-14.2.18	0.55	0.63	0.82	1.12	1.24	1.53	1.41
1-19.4.2018	0.55	0.53	0.78	0.94	1.05	1.27	1.08
2-19.4.2018	0.56	0.56	0.70	0.60	0.93	0.94	1.62
1-22.5.2018	0.65	0.67	0.81	0.92	0.95	1.09	1.27
2-22.5.2018	0.61	0.70	0.78	0.85	1.09	1.11	1.13
1-23.5.2018	*	0.72	0.77	0.91	0.88	1.08	1.04
2-23.5.2018	0.63	0.70	0.74	0.83	0.96	1.03	1.22
Average	0.60	0.64	0.78	0.89	1.03	1.18	1.25
σ	0.04	0.07	0.04	0.14	0.12	0.18	0.18
RSD (%)	6.5	10.3	5.2	15.4	11.4	15.7	14.1

Table 6. Mark-Houwink K parameters (10^{-5} dl/g) for the PLLA-samples analyzed with the triple detection method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-14.2.18	106.91	154.15	11.82	2.85	0.23	0.02	0.08
2-14.2.18	245.14	108.25	13.67	0.40	0.10	0.00	0.01
1-19.4.2018	222.01	360.14	22.25	3.80	1.21	0.08	0.74
2-19.4.2018	203.37	256.32	56.69	273.39	4.66	4.69	0.00
1-22.5.2018	62.52	57.81	12.12	3.49	2.50	0.47	0.04
2-22.5.2018	106.89	40.43	16.68	8.71	0.50	0.34	0.27
1-23.5.2018	*	34.56	20.82	3.93	6.53	0.53	1.03
2-23.5.2018	82.18	42.81	27.29	10.82	2.36	1.06	0.09
Average	137.61	131.81	22.67	38.42	2.26	0.90	0.28
σ	69.86	111.75	13.82	88.86	2.15	1.47	0.36
RSD (%)	50.8	84.8	61.0	231.3	95.2	163.5	128.8

Table 7. The *dn/dc* values (ml/g) for PLLA-samples analyzed with the triple detection method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-14.2.18	0.048	0.048	0.049	0.049	0.049	0.048	0.049
2-14.2.18	0.048	0.048	0.048	0.048	0.048	0.049	0.049
1-19.4.2018	0.047	0.047	0.047	0.047	0.047	0.048	0.047
2-19.4.2018	0.047	0.048	0.047	0.047	0.047	0.047	0.047
1-22.5.2018	0.047	0.047	0.047	0.047	0.046	0.046	0.046
2-22.5.2018	0.046	0.047	0.046	0.047	0.046	0.046	0.046
1-23.5.2018	*	0.047	0.047	0.046	0.046	0.046	0.046
2-23.5.2018	0.047	0.047	0.046	0.047	0.047	0.046	0.046
Average	0.047	0.047	0.047	0.047	0.047	0.047	0.047
σ	0.0006	0.0005	0.0009	0.0008	0.0010	0.0011	0.0012
RSD (%)	1.4	1.0	2.0	1.8	2.1	2.4	2.6

APPENDIX II. THE GPC RESULTS OF PLGA AND PLCL ANALYZED WITH THE TRIPLE DETECTION METHOD (Tables 1-7)

Table 1. The M_p averages (g/mol) for PLGA and PLCL samples with different inherent viscosities analyzed with the triple detection method. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	186072	197013	294665	710137	91948
2-14.6.18	183269	201495	295203	720001	89867
1-19.6.2018	177249	197523	296383	704090	88165
2-19.6.2018	173650	201895	287983	712847	88923
1-20.6.2018	179636	202905	287421	714237	88626
2-20.6.2018	172768	191043	284634	688803	89893
Average	178774	198646	291048	708353	89570
σ	4811	4053	4518	9950	1234
RSD (%)	2.7	2.0	1.6	1.4	1.4

Table 2. The M_w averages (g/mol) for PLGA and PLCL samples with different inherent viscosities analyzed with the triple detection method. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	181037	200617	297123	712078	101614
2-14.6.18	180046	200797	292899	714702	99234
1-19.6.2018	174038	201000	298915	710056	99652
2-19.6.2018	173789	200715	294170	711825	99297
1-20.6.2018	178423	200064	292494	710099	97597
2-20.6.2018	173593	188405	289057	687633	100267
Average	176821	198600	294110	707732	99610
σ	3112	4568	3209	9121	1207
RSD (%)	1.8	2.3	1.1	1.3	1.2

Table 3. The M_n averages (g/mol) for PLGA and PLCL samples with different inherent viscosities analyzed with the triple detection method. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	96809	113095	165619	400863	61259
2-14.6.18	97368	112716	157750	410082	58409
1-19.6.2018	84077	107017	158144	365680	57383
2-19.6.2018	81790	109033	155943	405087	57327
1-20.6.2018	94958	111232	152259	397492	59395
2-20.6.2018	87585	96416	149991	364240	59845
Average	90431	108252	156618	390574	58936
σ	6224	5693	4967	18521	1397
RSD (%)	6.9	5.3	3.2	4.7	2.4

Table 4. *PD* values for PLGA and PLCL samples with different inherent viscosities analyzed with the triple detection method. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	1.87	1.77	1.79	1.78	1.66
2-14.6.18	1.85	1.78	1.86	1.74	1.70
1-19.6.2018	2.07	1.88	1.89	1.94	1.74
2-19.6.2018	2.13	1.84	1.89	1.76	1.73
1-20.6.2018	1.88	1.80	1.92	1.79	1.64
2-20.6.2018	1.98	1.95	1.93	1.89	1.68
Average	1.96	1.84	1.88	1.82	1.69
σ	0.11	0.06	0.04	0.07	0.04
RSD (%)	5.4	3.4	2.4	4.0	2.1

Table 5. Mark-Houwink α parameters for the PLGA and PLCL samples analyzed with the triple detection method. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	0.77	0.77	0.83	0.91	0.70
2-14.6.18	0.78	0.78	0.80	1.02	0.69
1-19.6.2018	0.77	0.74	0.78	0.77	0.66
2-19.6.2018	0.75	0.72	0.77	0.85	0.66
1-20.6.2018	0.77	0.77	0.79	0.91	0.68
2-20.6.2018	0.76	0.75	0.81	0.87	0.69
Average	0.77	0.76	0.80	0.89	0.68
σ	0.01	0.02	0.02	0.08	0.02
RSD (%)	1.2	2.7	2.5	8.5	2.2

Table 6. Mark-Houwink K parameters (10^{-5} dl/g) for the PLGA and PLCL samples analyzed with the triple detection method. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	15.87	16.24	7.97	3.20	36.68
2-14.6.18	12.87	14.13	12.18	0.85	40.76
1-19.6.2018	15.78	21.01	14.53	21.34	57.39
2-19.6.2018	19.30	28.31	18.60	7.27	53.60
1-20.6.2018	14.69	15.74	13.00	3.50	41.29
2-20.6.2018	16.08	20.66	11.22	6.11	39.32
Average	15.77	19.35	12.92	7.05	44.84
σ	1.92	4.74	3.24	6.72	7.75
RSD (%)	12.2	24.5	25.0	95.4	17.3

Table 7. The *dn/dc* values (ml/g) for PLGA and PLCL samples analyzed with the triple detection method. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	0.048	0.047	0.047	0.047	0.052
2-14.6.18	0.048	0.047	0.048	0.047	0.052
1-19.6.2018	0.049	0.047	0.047	0.048	0.052
2-19.6.2018	0.049	0.048	0.048	0.048	0.052
1-20.6.2018	0.048	0.047	0.048	0.048	0.053
2-20.6.2018	0.048	0.049	0.048	0.049	0.052
Average	0.048	0.048	0.048	0.048	0.052
σ	0.0005	0.0008	0.0005	0.0007	0.0004
RSD (%)	1.0	1.6	1.0	1.4	0.7

APPENDIX III. THE GPC RESULTS OF PLLA ANALYZED WITH THE UNIVERSAL CALIBRATION I METHOD USING THE PARAMETERS ACCORDING TO GARLOTTA (Tables 1-4)

Table 1. The M_p averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters are $\alpha = 0.65$ and $K = 1.00 \cdot 10^{-3}$ dl/g according to Garlotta[36]. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	31250	60986	96391	113740	237133	307922	569919
2-19.4.2018	31250	59860	96391	204289	242285	301207	569919
1-22.5.2018	30668	58450	95840	208395	258800	370332	562782
2-22.5.2018	30668	59543	93986	212906	242377	289006	575941
1-23.5.2018	*	61971	139198	214004	242837	321161	575941
2-23.5.2018	32378	60833	97646	209576	248047	328251	537446
Average	31243	60274	103242	193818	245247	319647	565325
σ	624	1135	16117	35951	6833	26018	13237
RSD (%)	2.0	1.9	15.6	18.5	2.8	8.1	2.3

Table 2. The M_w averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters are $\alpha = 0.65$ and $K = 1.00 \cdot 10^{-3}$ dl/g according to Garlotta[36]. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	45945	101748	160656	132598	301613	422655	652847
2-19.4.2018	46120	102569	158978	242790	305838	417616	636438
1-22.5.2018	45411	98664	157979	254998	322736	452417	654997
2-22.5.2018	45746	100757	160768	251847	301946	423691	661071
1-23.5.2018	*	102968	163235	249761	295849	417474	636647
2-23.5.2018	49544	101764	161305	249822	300380	417355	625741
Average	46553	101412	160487	230303	304727	425201	644624
σ	1514	1413	1678	43848	8570	12438	12470
RSD (%)	3.3	1.4	1.0	19.0	2.8	2.9	1.9

Table 3. The M_n averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters are $\alpha = 0.65$ and $K = 1.00 \cdot 10^{-3}$ dl/g according to Garlotta[36]. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	32425	55698	81420	70858	150837	217357	301165
2-19.4.2018	31940	54495	82731	117549	152551	213860	279478
1-22.5.2018	31722	55253	80227	130926	163723	238157	299757
2-22.5.2018	31848	56839	85511	127028	157159	229481	295680
1-23.5.2018	*	55435	86281	131258	158295	229895	300221
2-23.5.2018	32864	57151	87667	128404	159892	224303	274768
Average	32160	55812	83973	117671	157076	225509	291845
σ	425	917	2690	21426	4340	8151	10637
RSD (%)	1.3	1.6	3.2	18.2	2.8	3.6	3.6

Table 4. *PD* values for PLLA samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters are $\alpha = 0.65$ and $K = 1.00 \cdot 10^{-3}$ dl/g according to Garlotta[36]. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	1.42	1.83	1.97	1.87	2.00	1.95	2.17
2-19.4.2018	1.44	1.88	1.92	2.07	2.01	1.95	2.28
1-22.5.2018	1.43	1.79	1.97	1.95	1.97	1.90	2.19
2-22.5.2018	1.44	1.77	1.88	1.98	1.92	1.85	2.24
1-23.5.2018	*	1.86	1.89	1.90	1.87	1.82	2.12
2-23.5.2018	1.51	1.78	1.84	1.95	1.88	1.86	2.28
Average	1.45	1.82	1.91	1.95	1.94	1.89	2.21
σ	0.03	0.04	0.05	0.06	0.05	0.05	0.06
RSD (%)	2.2	2.3	2.5	3.2	2.8	2.7	2.6

APPENDIX IV. THE GPC RESULTS OF PLLA ANALYZED WITH THE UNIVERSAL CALIBRATION I METHOD USING THE PARAMETERS ACCORDING TO DORGAN *ET AL.* (Tables 1-4)

Table 1. The M_p averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters are $\alpha = 0.736$ and $K = 1.74 \cdot 10^{-4}$ dl/g according to Dorgan *et al.*[83] The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	51245	96749	149486	311447	351716	450837	809369
2-19.4.2018	51245	95051	149486	305247	358974	441487	809369
1-22.5.2018	50338	92920	148675	311075	382192	537279	799733
2-22.5.2018	50338	94572	145940	317472	359103	424473	817495
1-23.5.2018	*	98234	211979	319029	359752	469241	817495
2-23.5.2018	53001	96518	151336	312751	367083	479082	765474
Average	51233	95674	159484	312837	363137	467067	803156
σ	972	1713	23531	4518	9610	36080	17889
RSD (%)	1.9	1.8	14.8	1.4	2.6	7.7	2.2

Table 2. The M_w averages (g/mol) for PLLA samples with different inherent viscosities analyzed with universal calibration I method. The used Mark-Houwink parameters are $\alpha = 0.736$ and $K = 1.74 \cdot 10^{-4}$ dl/g according to Dorgan *et al.*[83] The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	73342	155494	239994	363428	435501	601528	908234
2-19.4.2018	73556	155905	237486	356297	441445	595579	886455
1-22.5.2018	72419	150958	236006	371257	464149	642362	913211
2-22.5.2018	73337	154448	240725	368281	436636	602153	917871
1-23.5.2018	*	157198	242937	365078	429127	594417	887213
2-23.5.2018	78440	155579	240936	364571	435416	593659	872970
Average	74219	154930	239681	364819	440379	604950	897659
σ	2147	1951	2298	4621	11219	17056	16363
RSD (%)	2.9	1.3	1.0	1.3	2.5	2.8	1.8

Table 3. The M_n averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters were $\alpha = 0.736$ and $K = 1.74 \cdot 10^{-4}$ dl/g according to Dorgan *et al.*[83] The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	53435	90149	135477	196348	237637	331492	452481
2-19.4.2018	53113	88612	132445	194180	235714	329314	425917
1-22.5.2018	52565	90093	128607	202415	248497	367084	461127
2-22.5.2018	53732	94187	139896	202016	241875	342404	434463
1-23.5.2018	*	89580	132991	203754	246271	342482	456940
2-23.5.2018	54204	93243	143325	195954	249941	333189	425848
Average	53410	90977	135457	199111	243323	340994	442796
σ	554	2019	4894	3715	5348	12731	14557
RSD (%)	1.0	2.2	3.6	1.9	2.2	3.7	3.3

Table 4. *PD* values for PLLA samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters are $\alpha = 0.736$ and $K = 1.74 \cdot 10^{-4}$ dl/g according to Dorgan *et al.*[83] The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	1.37	1.73	1.77	1.85	1.83	1.82	2.01
2-19.4.2018	1.39	1.76	1.79	1.84	1.87	1.81	2.08
1-22.5.2018	1.38	1.68	1.84	1.83	1.87	1.75	1.98
2-22.5.2018	1.37	1.64	1.72	1.82	1.81	1.76	2.11
1-23.5.2018	*	1.76	1.83	1.79	1.74	1.74	1.94
2-23.5.2018	1.45	1.67	1.68	1.86	1.74	1.78	2.05
Average	1.39	1.70	1.77	1.83	1.81	1.78	2.03
σ	0.03	0.05	0.06	0.02	0.05	0.03	0.06
RSD (%)	1.9	2.6	3.1	1.2	3.0	1.7	2.9

APPENDIX V. THE GPC RESULTS OF PLGA AND PLCL ANALYZED WITH THE UNIVERSAL CALIBRATION I METHOD USING THE PARAMETERS ACCORDING TO KENLEY *ET AL.* (Tables 1-4)

Table 1. The M_p averages (g/mol) for PLGA and PLCL samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters for PLGA are $\alpha = 0.761$, $K = 1.07 \cdot 10^{-4}$ dl/g and for PLCL $\alpha = 0.68$, $K = 44.84 \cdot 10^{-5}$ dl/g. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	228852	242362	359020	996361	83832
2-14.6.18	220314	247062	366328	1019109	83832
1-19.6.2018	228437	246604	365345	830914	83556
2-19.6.2018	219917	256287	358082	830914	85113
1-20.6.2018	220823	252303	358922	830749	84150
2-20.6.2018	216694	252303	358922	812671	84150
Average	222506	249487	361103	886786	84106
σ	4538	4602	3373	86016	495
RSD (%)	2.0	1.8	0.9	9.7	0.6

Table 2. The M_w averages (g/mol) for PLGA and PLCL samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters for PLGL are $\alpha = 0.761$, $K = 1.07 \cdot 10^{-4}$ dl/g and for PLCL $\alpha = 0.68$, $K = 44.84 \cdot 10^{-5}$ dl/g. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	236559	265293	389704	1088635	99618
2-14.6.18	232261	265976	389271	1101149	99535
1-19.6.2018	238381	265095	389642	891016	100911
2-19.6.2018	233332	268086	386431	888658	100858
1-20.6.2018	233542	264707	388129	885499	100155
2-20.6.2018	231441	265927	388970	872967	100345
Average	234253	265847	388691	954654	100237
σ	2435	1096	1138	99392	538
RSD (%)	1.0	0.4	0.3	10.4	0.5

Table 3. The M_n averages (g/mol) for PLGA and PLCL samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters for PLGA are $\alpha = 0.761$, $K = 1.07 \cdot 10^{-4}$ dl/g and for PLCL $\alpha = 0.68$, $K = 44.84 \cdot 10^{-5}$ dl/g. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	105467	135431	195634	520514	55227
2-14.6.18	112371	139190	191149	517673	54287
1-19.6.2018	105204	133127	198030	449599	56983
2-19.6.2018	102675	139422	195891	454644	55992
1-20.6.2018	111360	136466	189050	445535	53873
2-20.6.2018	111390	126763	188849	416211	54916
Average	108078	135067	193101	467363	55213
σ	3751	4297	3578	38571	1039
RSD (%)	3.5	3.2	1.9	8.3	1.9

Table 4. The *PD* values for PLGA and PLCL samples with different inherent viscosities analyzed with the universal calibration I method. The used Mark-Houwink parameters for PLGA are $\alpha = 0.761$, $K = 1.07 \cdot 10^{-4}$ dl/g and for PLCL $\alpha = 0.68$, $K = 44.84 \cdot 10^{-5}$ dl/g. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	2.24	1.96	1.99	2.09	1.80
2-14.6.18	2.07	1.91	2.04	2.13	1.83
1-19.6.2018	2.27	1.99	1.97	1.98	1.77
2-19.6.2018	2.27	1.92	1.97	1.96	1.80
1-20.6.2018	2.10	1.94	2.05	1.99	1.86
2-20.6.2018	2.08	2.10	2.06	2.10	1.83
Average	2.17	1.97	2.01	2.04	1.82
σ	0.09	0.06	0.04	0.07	0.03
RSD (%)	4.2	3.2	1.9	3.3	1.5

APPENDIX VI. THE GPC RESULTS OF PLLA ANALYZED WITH THE CONVENTIONAL CALIBRATION METHOD (Tables 1-4)

Table 1. The M_p averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the conventional calibration method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	72964	139620	217724	460721	521632	672164	1221754
2-19.4.2018	72964	137118	217724	451358	532628	657932	1221754
1-22.5.2018	71645	133980	216516	460159	567830	804024	1206902
2-22.5.2018	71645	136412	212450	469825	532823	632050	1234282
1-23.5.2018	*	141808	311035	472177	533806	700196	1234282
2-23.5.2018	75519	139280	220475	472177	544916	715195	1154131
Average	72947	138036	232654	464403	538939	696927	1212184
σ	1415	2524	35134	7660	14569	55011	27562
RSD (%)	1.9	1.8	15.1	1.6	2.7	7.9	2.3

Table 2. The M_w averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the conventional calibration method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	105459	227980	355586	541820	651504	905081	1384131
2-19.4.2018	105824	229206	352172	532493	663009	895570	1352849
1-22.5.2018	103907	221842	350829	552897	696213	967850	1388472
2-22.5.2018	104229	225877	355529	546292	655301	908870	1404320
1-23.5.2018	*	231415	361472	543984	640109	897442	1347963
2-23.5.2018	111959	227708	357300	544768	650576	896377	1324769
Average	106276	227338	355481	543709	659452	911865	1367084
σ	2932	2972	3459	6078	17783	25504	27366
RSD (%)	2.8	1.3	1.0	1.1	2.7	2.8	2.0

Table 3. The M_n averages (g/mol) for PLLA samples with different inherent viscosities analyzed with the conventional calibration method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	76122	128789	198861	279998	335661	467940	680810
2-19.4.2018	75965	126221	197549	289468	352562	470591	655752
1-22.5.2018	74986	128188	199243	285063	366600	532212	676174
2-22.5.2018	74892	133519	200602	276413	355090	500175	696764
1-23.5.2018	*	132695	201322	287361	344410	515659	670913
2-23.5.2018	77848	134219	208016	296458	355363	499810	628892
Average	75963	130605	200932	285794	351614	497731	668218
σ	1066	3008	3392	6478	9644	22894	21393
RSD (%)	1.4	2.3	1.7	2.3	2.7	4.6	3.2

Table 4. *PD* values for PLLA samples with different inherent viscosities analyzed with the conventional calibration method. The symbol * means that there is no result. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-19.4.2018	1.39	1.77	1.79	1.94	1.94	1.93	2.03
2-19.4.2018	1.39	1.82	1.78	1.84	1.88	1.90	2.06
1-22.5.2018	1.39	1.73	1.76	1.94	1.90	1.82	2.05
2-22.5.2018	1.39	1.69	1.77	1.98	1.85	1.82	2.02
1-23.5.2018	*	1.74	1.80	1.89	1.86	1.74	2.01
2-23.5.2018	1.44	1.70	1.72	1.84	1.83	1.79	2.11
Average	1.40	1.74	1.77	1.90	1.88	1.83	2.05
σ	0.02	0.04	0.03	0.05	0.04	0.07	0.03
RSD (%)	1.4	2.4	1.4	2.7	2.0	3.6	1.6

APPENDIX VII. THE GPC RESULTS OF PLGA AND PLCL ANALYZED WITH THE CONVENTIONAL CALIBRATION METHOD (Tables 1-4)

Table 1. The M_p averages (g/mol) for PLGA and PLCL samples with different inherent viscosities analyzed with the conventional calibration method. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	302954	321499	483013	1134882	144892
2-14.6.18	291254	327961	493200	1161348	144892
1-19.6.2018	302385	327331	491830	1152053	144421
2-19.6.2018	290710	333919	481704	1152053	147079
1-20.6.2018	291950	335169	482875	1151816	145435
2-20.6.2018	286298	335169	482875	1125861	145435
Average	294259	330175	485916	1146336	145359
σ	6218	5036	4703	12050	845
RSD (%)	2.1	1.5	1.0	1.1	1.6

Table 2. The M_w averages (g/mol) for PLGA and PLCL samples with different inherent viscosities analyzed with the conventional calibration method. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	317787	356571	534020	1248599	171685
2-14.6.18	311816	356584	530592	1264134	171545
1-19.6.2018	321076	356312	532036	1246373	172390
2-19.6.2018	315772	359407	527390	1246437	173523
1-20.6.2018	312054	355298	530120	1242321	172629
2-20.6.2018	312215	357703	529815	1223895	172818
Average	315120	356979	530662	1245293	172432
σ	3459	1292	2037	11798	674
RSD (%)	1.1	0.4	0.4	0.9	0.4

Table 3. The M_n averages (g/mol) for PLGA and PLCL samples with different inherent viscosities analyzed the with conventional calibration method. Standard deviation (σ) and relative standard deviation (RSD) are also included.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	143571	173173	269044	588493	97966
2-14.6.18	147883	177312	247594	577614	98636
1-19.6.2018	144205	171291	261319	569465	96731
2-19.6.2018	151883	172610	260288	604770	98158
1-20.6.2018	140667	173742	255503	586034	93664
2-20.6.2018	153792	160741	245598	546473	95633
Average	147000	171478	256558	578808	96798
σ	4662	5142	8104	18048	1721
RSD (%)	3.2	3.0	3.2	3.1	1.8

Table 4. *PD* values for PLGA and PLCL samples with different inherent viscosities analyzed with the conventional calibration method. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-14.6.18	2.21	2.06	1.99	2.12	1.75
2-14.6.18	2.11	2.01	2.14	2.19	1.74
1-19.6.2018	2.23	2.08	2.04	2.190	1.78
2-19.6.2018	2.08	2.08	2.03	2.06	1.77
1-20.6.2018	2.22	2.05	2.08	2.12	1.84
2-20.6.2018	2.03	2.23	2.16	2.24	1.81
Average	2.15	2.08	2.07	2.15	1.78
σ	0.08	0.07	0.06	0.06	0.03
RSD (%)	3.6	3.2	3.0	2.7	2.0

APPENDIX VIII. THE INHERENT VISCOSITY RESULTS OF PLLA, PLGA, AND PLCL (Tables 1 and 2)

Table 1. Inherent viscosities for the PLLA-samples measured with an Anton Paar microviscometer (dl/g). On 4.5.2018 there were no parallel samples. Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded values and calculated from the unrounded values.

Run number - date	PL10	PL18	PL24	PL32	PL38	PL49	PL65
1-4.5.2018	1.0	1.7	2.2	3.1	3.6	4.3	6.5
2-4.5.2018	-	-	-	-	-	-	-
1-17.5.2018	1.0	1.7	2.3	3.1	3.7	4.8	6.4
2-17.5.2018	1.0	1.7	2.4	3.0	3.7	4.7	6.4
1-27.6.2018	1.0	1.7	2.3	3.3	3.7	4.8	6.6
2-27.6.2018	1.1	1.7	2.4	3.2	3.8	4.7	6.7
1-28.6.2018	1.0	1.7	2.4	3.0	3.6	4.7	6.5
2-28.6.2018	1.0	1.7	2.4	3.2	3.6	4.8	6.4
Average	1.0	1.7	2.3	3.1	3.7	4.7	6.5
σ	0.02	0.02	0.05	0.11	0.05	0.15	0.11
RSD (%)	1.8	1.1	1.9	3.6	1.5	3.3	1.6

Table 2. Inherent viscosities for the PLGA and PLCL samples measured with an Anton Paar microviscometer (dl/g). Standard deviation (σ) and relative standard deviation (RSD) are also included. The averages are rounded and calculated from the unrounded values.

Run number - date	PLG22	PLG238	PLG311	PLG62	PLC15
1-27.6.2018	2.0	2.2	3.0	5.5	1.4
2-27.6.2018	2.1	2.2	3.0	5.7	1.4
1-28.6.2018	2.0	2.2	3.0	5.7	1.4
2-28.6.2018	2.1	2.2	3.0	5.7	1.4
1-3.7.2018	2.1	2.2	3.0	5.7	1.5
2-3.7.2018	2.0	2.3	3.1	5.7	1.5
1-4.7.2018	2.0	2.3	3.0	5.7	1.6
2-4.7.2018	2.1	2.3	3.0	5.8	1.6
Average	2.1	2.2	3.0	5.7	1.5
σ	0.02	0.02	0.03	0.08	0.06
RSD (%)	0.8	1.0	1.0	1.4	3.9