TAMPEREEN TEKNILLINEN YLIOPISTO
TAMPERE UNIVERSITY OF TECHNOLOGY

TUIJA ALANKO

SYNTHESIS OF BIODEGRADABLE AND BIOCOMPATIBLE POLYMERS FOR DRUG DELIVERY APPLICATIONS

Master of Science Thesis

Supervisors and examiners:
Prof. Minna Kellomäki and Docent Terttu Hukka
Supervisor:
Dr. Musammir Khan
Examiners and topic approved by the Faculty Council of the Faculty of Natural Sciences
on 8th of June 2016

ABSTRACT

TUIJA ALANKO: Synthesis of Biodegradable and Biocompatible Polymers for Drug Delivery Applications
Tampere University of Technology
Master of Science Thesis, 83 pages, 10 Appendix pages
August 2016
Master's Degree Programme in Teacher Training
Major: Chemistry
Examiners: Prof. Minna Kellomäki, Docent Terttu Hukka

Keywords: Ring Opening Polymerization, Random Copolymer, Block Polymer, Biodegradable, Biocompatible, Organic Catalyst, Drug Entrapment, Simulation of Reaction Kinetics, Computer Controlled Monomer Feed

Many polymers, like PLA and PCL, are known to be biodegradable, but the long degradation time have limited their use for drug delivery applications. PLGA 50:50 has been proved to be the most efficiently biodegradable polymer. For nanoparticles and drug encapsulation PLGA alone has not been very successful due to solubility issue in aqueous environment. Different water soluble blocks are thus added to improve its aqueous solubility and at the same time, the end groups have been modified to facilitate drug entrapment.

In this study PEG-b-PLGA-LL was synthesized with organic catalyst at ambient conditions, because completely biocompatible material is not available commercially. Since monomers have very different rate constants, glycolide feed to the reaction mixture needs to be restricted. Reaction kinetics was solved manually and based on results, 3–4 different target feed rates were used during the reaction. In the best cases manual feed yielded to either PLGA block length only 75 % of the target length, with 65:35 ratio or to 52:48 ratio and only one third of the target length.

Solvent content of the reaction mixture varies throughout the process, not only concentrations. That makes the reaction kinetics more complex and manual computing is hard and requires approximate methods. Opportunity to employ a syringe pump in the feed protocol improved calculated feed rate fulfilment in practice significantly. Thus simulation model of the reaction kinetics was employed in the reaction kinetics calculations, which gave directly the feed rate demand needed from the pump as output.

Reaction kinetics simulation was found very effective tool. Improvements were obtained rapidly and already second experiment yielded to almost target product. Final product successfulness remained a little bit of a mystery since the 56:44 was the most glycolide rich fraction that was soluble in any solvent at hand, thus over 20 % of the product could not be characterized. Lysine addition to the polymer failed partly, because of the solubility issue, among other things.

TIIVISTELMÄ

TUIJA ALANKO: Biohajoavien ja bioyhteensopivien polymeerien syntetisointi lääkkeenkuljetussovelluksiin
Tampereen teknillinen yliopisto
Diplomityö, 83 sivua, 10 liitesivua
Elokuu 2016
Matemaattisten aineiden DI-opettajankoulutus
Pääaine: Kemia
Tarkastajat: professori Minna Kellomäki ja dosentti Terttu Hukka

Avainsanat: Renkaanavautumispolymeraatio, Satunnaiskopolymeeri, Lohkopolymeeri, Biohajoava, Bioyhteensopiva, Lääkkeen sitoutuminen, Reaktiokinetiikan simulointi, Tietokoneohjattu monomeerin syöttö

Monet polymeerit tiedetään biohajoaviksi, mutta lääkkeenkuljetussovelluksisssa PLGA 50:50 suhteella on osoitettu kaikkein tehokkaimmin biohajoavaksi polymeeriksi. Nanohiukkasiin ja lääkkeen sitomiseen PLGA ei kuitenkaan ole tehokas sinällään, vaan tarvitsee vesiliukoisen lohkon joko toiseen tai molempiin päihin. Lääkkeen sitoutumista parannetaan funktionalisoivilla pääteryhmillä.

Tässä työssä syntetisoitiin PEG-b-PLGA-LL polymeeriä orgaanisella katalyytillä ympäristön lämpötilassa ja paineessa, koska kaupallisesti täysin bioyhteensopivaa materiaalia ei ole saatavana. Laktidi- ja glykolidimonomeerit ovat reaktionopeusvakioiltaan oleellisesti erilaiset, joten glykolidin syöttöä reaktioon pitää rajoittaa. Reaktiokinetiikka ratkaistiin käsin ja tulosten avulla valittiin 3–4 eri tavoitenopeutta glykolidin syötölle reaktion eri vaiheissa. Glykolidin syöttö reaktioon manuaalisesti johti parhaimmillaan pituudeltaan n. 75 % tavoitteesta PLGA-lohkoon, jolloin suhteeksi tuli vain 65:35. 52:48 suhteeseen päästiin, mutta tällöin PLGA-lohkon pituus jäi vain noin kolmannekseen tavoitteesta.

Reaktioseoksen liuotinkoostumus vaihtelee konsentraatioiden ohella läpi koko prosessin tehden reaktiokinetiikasta monimutkaisemman, hankalasti käsin ratkaistavan ja likimääräisiä menetelmiä vaativan. Lääkeruiskupumpun käyttömahdollisuus paransi oleellisesti lasketun syöttönopeuden toteutumisen tarkkuutta, joten reaktiokinetiikan laskemisessa otettiin käyttöön yksinkertainen polymeroitumisprosessin kinetiikan simulointimalli, josta ulostulona saatiin lääkeruiskupumpulta tarvittava syöttönopeus.

Reaktiokinetiikan simulointi todettiin erittäin tehokkaaksi työkaluksi. Prosessia onnistuttiin parantamaan nopeasti ja jo toinen koe tuotti lähes tavoiteltua tuotetta. Lopullisen tuotteen onnistuneisuus jäi osittain tuntemattomaksi, sillä 56:44 oli glykolidirikkain fraktio, joka vielä liukeni mihinkään käytettävissä olleista liuottimista, joten yli 20 % tuotteesta jäi karakterisoimatta. Myös L-lysiinin lisääminen polymeeriin epäonnistui osittain mm. liukoisuusongelman vuoksi.

PREFACE

Cooperation between Chemistry and Biomaterials made this great opportunity to accomplish Chemistry Master thesis in the surroundings of Biomaterials, with support of the excellence of Chemistry crew, possible. Before taking this challenge I was unaware of many of the biomedical applications of polymeric chemistry.

First of all, I want to thank Professor Minna Kellomäki for offering me the facilities and making this study financially possible. I am also grateful to Docent Terttu Hukka for arranging this possibility, as well as giving me valuable advices throughout this process.

Alexandre Efimov's advices and help with NMR, in interpretation and in organic chemistry was priceless, he was always helpful and ready to offer his time. Nuno Rafael Candeias was my lifeline when I was struggling in the middle of contradictory information. Thanks to Anne-Maarit Tikkanen's helpfulness I had glass ware and other equipment I needed in my use.

Suvi Heinämäki has been extremely helpful in whatever was needed and her flexibility was sometimes redemption. With the help of Laura Honkamäki I got access to the syringe pump, but without Jari Väliaho's and Joose Kreutzer's help I would not have got even a chance to succeed with the very limited time left at that point. I am also grateful to all the Biomaterial and tissue engineering lab crew for their help and our moments during this period. Musammir Khan I want to thank his helpfulness, all the help offered in implementations and all the help offered based on his expertise in medical care applications, needs and requirements.

I also want to thank my daughter Paula and my husband Risto for help, support and understanding towards my overloaded days and weeks.

In Lempäälä, Finland, on 25 July 2016

Tuija Alanko

CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

^{13}C	Carbon 13 isotope
^{1}H	Hydrogen 1 isotope, Proton
BOC	*tert*-Butyloxycarbonyl
CBZ	Carbobenzyloxy
DBF	Dibentzofulvene
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DMAP	*N,N*-Dimethylpyridin-4-amine
DMF	Dimethylforamide, *N,N*-Dimethylformamide
DSC	Differential Scanning Calorimetry
dx/dt	First time derivative of x
Et_2O	Diethyl ether
Et_3N	Triethylamine
EtAc	Ethyl acetate
eq.	equivalent
Fmoc	Fluorenylmethyloxycarbonyl Chloride
Fmoc-LL	Fmoc-Lys(Fmoc)-OH, *N,N*-di-Fmoc-L-lysine
GA	Glycolide monomer, 1,4-dioxane-2,5-dione
iPrOH	2-Propanol
LA	Lactide monomer, (3S)-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione
LL	L-lysine
MeOH	Methanol
mPEG-OH	Poly(ethylene glycol) methyl ether
MTBD	7-Methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene
ND	Not Defined
NMR	Nuclear Magnetic Resonance
NP	Nanoparticle
PDI	Polydispersity index
PDLA	Poly(D-lactide)
PDLLA	Poly(D,L-lactide)
PEG	Polyethylglycol
PGA	Polyglycolide
PhCOOH	Benzoic Acid
PID	Proportional Integral Derivative
PLA	Polylactide
PLGA	Poly(lactide-co-glycolide)
PLL	Poly(L-lysine)
PLLA	Poly(L-lactide)
PS	Polystyrene
PVP	Polyvinyl-pyrrolidone
R	Group, main part of the molecule, inactive in the reaction
RI	Refractive Index
ROP	Ring Opening Polymerization
rt, RT	Room Temperature
TBD	1,5,7-Triazabicyclo[4.4.0]dec-5-ene
THF	Tetrahydrofuran
TMS	Tetramethylsilane

l	dm^3 in SI units
ml	cm^3 in SI units
µl	mm^3 in SI units
[x]	concentration of x
$[x]_t$	concentration of x at time t
b	block
c	concentration
d	doublet, duct length
F	force
f	frequency
g	gravity
h	hour, height
i	index, insoluble
k_G, k_2, *k2*	glycolide rate constant
ki'	evaporation correction constant for state i
k_L, k_1, *k1*	lactide rate constant
l	litre, duct length, nucleon spin
M	molar mass
m	mass (weight), multiplet, angular momentum quantum number
min	minute
M_n	number average molar mass
M_w	mass (weight) average molar mass
n	the amount of a substance
Ø, iD	inner diameter
p	pressure
ppm	parts per million
q	quartet
Q	volume flow rate
r	radius
ran	random
s	second, singlet, soluble
sep	septet
ss	slightly soluble
t	time, triplet
T_g	glass transition temperature
T_m	melting temperature
u	kinematic viscosity
v	velocity
V	volume
V_0	initial volume
V_i	volume at state i
vs	very soluble
w-%	mass (weight) percentage
x	variable
y	variable
z	variable
γ	surface tension
δ	chemical shift in ppm
η	dynamic viscosity

1. INTRODUCTION

The aim of this study was to develop synthesis of poly(ethylene glycol)-*block*-poly(L-lactide-*ran*-glycolide)-L-lysine (PEG-PLGA-LL) for biodegradable, biocompatible nanoparticle material. Target nanoparticle (NP) size is less than 200 nm in diameter with short degradation time and effective drug encapsulation. Biocompatibility is required, thus only organic catalysts can be considered. Target polymer has equal amounts of L-lactide (LA) and glycolide (GA) and molar mass is 9–10 kg/mol (less than 15 kg/mol) in order to avoid solubility issues and ensure fast degradation [1][2][3]. For now, all commercially available 50:50 PLGAs are synthesized with metal catalysts, thus not completely biocompatible.

GA and LA are known to have even several orders different polymerization rate constants [4][5][6], which means in practice that in order to synthesize 50:50 PLGA random copolymer block, glycolide feed into the reaction needs to be controlled. Ring-opening polymerization (ROP) was chosen over condensation polymerization because in condensation reaction water needs to be removed from the reaction, which requires elevated temperature [7]. In melt polymerization 180 °C temperature is needed and only 10 kg/mol would be expected [5][7]. Presence of water would also limit the choice of catalyst and ROP favours lower temperatures since both monomers' ring openings are exothermic [8][9]. Degree of LA conversion have also been found to decrease with increasing temperature [8].

For example, 11 choices of metal-free catalysts are presented by Suriano et al. [10]. Phosphines [11], *N,N*-Dimethylpyridin-4-amine (DMAP) (pure and as different salts) [12], 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU), 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD) and 7-Methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTBD) [4][13][14][15] were compared. Based on polymerization conditions, reaction times, products molar masses and PDIs, DBU was used as catalyst throughout this study even though it has been reported very sensitive to carbon dioxide and water [16]. Process was thus set to ROP in solution, since DBU flash point is 116 °C [17].

PEG is the most popular hydrophilic block used as a macroinitiator during PLGA synthesis and the choice is most commonly made between 2 kDa and 5 kDa PEG, for example [3][4][18]. Early precipitation was a problem and since 5 kDa PEG solubility in tetrahydrofuran (THF) was found very limited, all the reported polymerizations were carried out with PEG 2 kDa. Because the fast biodegradation takes place via ester hydrolysis, PEG does not take part in the degradation process and thus the shorter block is better choice to help only in a good aqueous dispersion of the final copolymer. With

hydroxyl group in one end, PEG initiates the polymerization via nucleophilic attack to monomer's carbonyl carbon by hydroxyl group's oxygen's free sp^2 electron pair [19, p. 262, 304].

In recent studies many different solutions to increase the PLGA NP drug loading and drug release has been presented, like PVP conjugation to NP surface [20], new NP preparation technique [21], adding calcium phosphate to the inner water phase [22], PEG (earlier) [23] and connecting lactose acid to both PLGA ends or lactose acid to one end and poly(L-lysine) (PLL) to another end [24]. With the PLL end group burst release decreased and release rate remained almost constant over the first 24 h.

Du et. al synthesized PEG-PLGA-PLL with dibentzofulvene (DBF) and carbobenzyloxy (CBZ) protecting groups and used HBr/Acetic acid for deprotection of CBZ from PLL [25]. Three years later another group, sharing three common members with the Du et. al, used almost exactly same procedure, only deprotection time was decreased from 24 h to 1 h [26]. For this study use of *N*,*N*-di-Fmoc-L-lysine (Fmoc-Lys(Fmoc)-OH, Fmoc-LL) was pre-determined, but no reports of Fmoc protected L-lysine (LL) addition to PLGA, including deprotection, was found.

PLGA 50:50 is apparently seldom synthesized with organic catalyst and in solution, since only one article [4] was found. More lactide rich, for example 75:25 and 67:33 [18] are commonly found. Thus the choice of solvents was made accordingly [4].

This thesis consists of literature review, theoretical approach and experimental part. Theoretical part starts with a short introduction of the history and continues through the polymerization theory and results found with different implementations to the reaction mechanism design, GA feed method studies and reaction kinetics formulation. It is shown in the experimental part how the equations of reaction kinetics are formulated to Simulink simulation model used in polymerization studies. Simulink is a block-diagram based, graphical user interface equipped environment in Matlab, aimed for modelling and simulation of dynamic systems. Finally, experimental methods are documented, before actual experimental part.

In the experimental part most significant syntheses are presented in detail, as well as simulation model refinements. All the synthesis and individuals steps in this study are presented in the order that best describes how the results from previous experiments was used for reasoning of the changes made in the following experiment.

Main results are gathered together in discussion, where they are compared, also with results found in the literature and discussed. Proposals for further work and summary of this study are gathered to summary and conclusions.

2. LITERATURE REVIEW

Biodegradable materials are natural or synthetic in origin, which on degradation in vitro or in vivo, either enzymatically or non-enzymatically, produce biocompatible, toxicologically safe by-products, that are eliminated from the living system by the normal metabolic pathways [1]. Biocompatible means "*compatible with living cells, tissues, organs, or systems, and posing no risk of injury, toxicity, or rejection by the immune system [27]*".

2.1 PLA and PLGA

Polylactic acid, or polylactide (PLA), is a biodegradable, thermoplastic, aliphatic polyester, usually produced from corn starch or sugarcane. It was discovered already in 1920s, but commercially manufactured first in 1989. In 2010, PLA had the second highest consumption volume of any bioplastic in the world. PLA is used as medical implants in the form of anchors, screws, plates, pins, rods, and as a mesh. It degrades slowly in living tissue, producing lactic acid. PLA has many non-medical uses, as compostable material for packaging and disposable products and as feed material in 3D-printing. [28]

Polyglycolic acid, or polyglycolide (PGA), was first discovered in 1954. Its first medical application was PGA suture thread, first used in 1962. It is used as suture, support plates, pins and non-wowen mesh. It is quickly degraded in human tissue, but this property can be adjusted with PLA as copolymer. [7]

PLGA is a copolymer of two different monomers, glycolic acid and lactic acid. The time required for degradation and crystalline structure of PLGA is related to the monomers ratio used: the higher the content of glycolide units, the lower the time required for degradation as compared to predominantly lactide materials. The degradation by hydrolysis in living tissue produces the original monomers that are normally present in human body, but creating acidic environment. The ability to tailor the degradation makes PLGA suitable for implants, sutures, prosthetic devices, and also drug carrier as nanoparticles. [1]

PLGA pros are biocompatibility, biodegradability, highly tuneable mechanical properties and it is well suited for controlled drug delivery. PLA can be made highly crystalline poly(L-lactide) (PLLA) or amorphous poly(D,L-lactide) (PDLLA) while PGA is highly crystalline. Methyl groups in PLA causes hydrophobicity of PLGA. "*Mechanical strength, swelling behaviour, capacity to undergo hydrolysis and subse-*

quently biodegradation rate of the polymer are directly influenced by the degree of crystallinity of the PLGA" [1]. They also proposed that drug presence may affect the degradation mechanism. PLGA with 50:50 ratio LA and GA degrades fastest. In PLGA glass transition temperature (T_g) decreases when the relative amount of GA decreases and increases when relative amount of LA increases, as well as with the polymer length [1]. However, a Japanese group has even proposed that PLLA would degrade faster because it has highly crystalline regions, because they detected degradation specifically on those regions [29].

In general, highly crystalline PLLA has been found to degrade slower than amorphous stereoisomer PDLLA, like found in review published by Gentile et. al [2]. Degradation time of 12-18 weeks was reported for Poly(L-lactide), 11-15 weeks for poly(D,L-lactide) and 1-2 weeks for poly(D,L-lactide-co-glycolide) [2]. Reported times are in relation to each other and in good agreement with information found from Sigma Aldrich on-line catalog: over 24 months for PLLA, 12-16 months for PDLLA and 6 months for PDLA [30] as well as with degradation times presented by Huh et. al [31]: 2-3 months for PGA, more than 2 years for PLLA, 12-16 months for PDLLA and 1-6 months for PLGA.

PLGA's only remarkable disadvantage is the acidic degradation products, which may accumulate on the implantation site [1]. Another remarkable disadvantage is, with commercial PLGA, related with the stannous octoate "*The amount of residual tin found in commercial polylactides can be as high as 530 ppm, which is slowly incorporated into the bloodstream of the patient [32]*" [33] and even with other metal catalysts "*Zirconium compounds are reported to be 10–20 times less toxic than tin compounds and are allowed to be used in cosmetics and drugs. However, this does not take into account the toxicity of the ligand, which will need to be fully addressed for any industrially viable system [32]*" [34].

Since PLGA has not shown very efficient drug loading [20][21][22] properties and it is also insoluble in water, simple PLGA is not favourable in drug carrier application [21], thus some modifications are essential. Thus PLGAs are used typically with water soluble polymers like PEG as AB diblockcopolymers or ABA triblockcopolymers [1], where PEG also prolongs blood circulation time [18][35]. PLGAs are also modified towards drug loading properties. Lysine is an effective additive that favours drug loading [26] and it can be relatively easily incorporated into PLGA hydroxyl end group [36]. For double emulsion technique NP preparation PEG acts as water soluble tail in later emulsion, while lysine functions as drug loading end in former emulsion [26].

2.2 Polymerization of PEG-PLA and PEG-PLGA

In polymerization of functionalized PLGA several choices are available. PEG is a well-known water soluble copolymer for PLGA and with methoxy and hydroxyl end groups,

methoxy group will remain passive in polymerization process, while hydroxyl group acts as an alcohol initiator for the polymerization [4][37, p. 263].

For small nanoparticles, less than 200 nm in diameter, polymer molar mass targeted is relatively small. Highly crystalline relatively short polymer less likely forms aggregates or takes highly coiled form. Because no surfactant is used in the polymerization process, nanoparticle size depends only on how the polymer will be processed to NPs [38]. Water soluble part (i.e. PEG) of block-copolymer needs to be long enough to form NPs, but on the other hand, since the glycolide ester bonds are the only effectively biodegradable parts of the polymer [2][31], as short as possible.

Nanoparticle uptake by the cells was found to be higher with 2 kDa than with 5 kDa PEG [39]. On the other hand, 6 kDa PEG was found slightly more effective to form small NPs than 1.5 kDa PEG [40], while Hirsjärvi et. al found that varying PEG size from 660 to 2000 Da does not have significant influence to the NP size [41]. So in order to get polymer best for the nanoparticles in target size range, choice needs to be made between 2 kDa and 5 kDa PEGs. Commercially available PEG-PLGA choices available in this class would be PEG 2kDa with 11.5 kDa 50:50 PLGA [42] and PEG 5kDa with 12 kDa 50:50 PLGA [43].

Choice of the catalyst depends, among many other factors, on reaction conditions required and reaction time. Beyond those factors, different catalysts tend to affect product's tacticity as well [4], which can be voided if only RR or SS stereoisomer is used to be paired with glycolide, in which case tacticity aspect does not limit the choice of catalyst.

Metal-free catalyst may be chosen according to features required, for example 11 choices presented in Suriano et al. [10]. Phosphines have shown even over 90 % LA conversions [11], but 135 ºC and 180 ºC temperatures were used and polydispersity indexes (PDI) were generally high. DMAP was used pure and as different salts, but polymer molar masses remain very modest at 25 ºC [12] and higher molar masses were achieved at solvent's boiling point. 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) was found widely used in cyclic polyester polymerizations, and in comparison with TBD and MTBD it gave smallest PDIs [13][14][15].

Room temperature and ambient pressure are favourable, and since DBU has been reported successful in polymerization of cyclic esters, giving high yields in short reaction times [4][14][15], even 87.5 % in 30 min. [4], DBU is best choice, even though DBU was reported to be very sensitive to carbon dioxide and water [16]. ROP in solution seems best suited for DBU, since DBU flash point is only 116 ºC [17]. The only special arrangement required is some assembly to keep the catalyst isolated from impurities, especially moisture and carbon dioxide.

Different methods to synthesize especially PLA have been developed over the years with wide variety of different catalyst and initiators. Some of the interesting schemas are shown in Table 1.

Table 1. *Selection of PLA synthetization methods.*

C	CC	I	Mech.	M:I:C:CC	T [ºC]	Solvent	Time [h]	Conv [%]	PDI	Ref.
DMAP	DMAP·HX	p-PhBzOH	An/Ca	20:1:1:1	rt	DCM	24	35-100	1.06-1.13	[12]
DMAP		EtOH	Nu/Li	300:10:1-15:1:2	35	DCM	96-24	na	1.08-1.13	[44]
TBD		PEO	Nu/Li	1000-5000:10-50:1	rt	DCM	20 - 60 s	99 - 95	1.19-1.11	[15]
MTBD		PEO	Nu/Li	100:1:1	rt	$CDCl_3$, DCM	0.5	92, 99	1.05, 1.10	[15]
DBU		PEO	Nu/Li	100-500:1:1	rt	$CDCl_3$	1 - 2	99-98	1.05-1.08	[15]
DBU		PEG	Nu/Li	700:20:1	rt	DCM	1	100	1.06	[4]
PPY		EtOH	Nu/Li	30:1:2	35	DCM	20	na	1.10	[44]
PBu_3		PhEtOH	Nu/Li	6-20:0.2:1	135-180		110 min	73-84	1.11-1.16	[11]
PBu_3		PhEtOH	Nu/Li	6-20:0.2:1	50	THF	110 min	60	1.18	[11]
PBu_3		PhEtOH	Nu/Li	12:0.2:1	94	Toluene	110 min	90	1.18	[11]
$Sn(Oct)_2$			Bulk	10000:0:1	180		3	84-93	na	[45]
$Sn(Oct)_2$	1-dodecanol		Bulk	10000:0:17-95:1	180		0-0.06	0-90	1-1.8	[46]
$Sn(Oct)_2$			Bulk	100-200:1	120		24	92-94	na	[47]
$Sn(Oct)_2$		BnOH	Bulk	200:0.2-2:1	120		na	78-95	na	[47]
		[Zn(Ac)2(L1)]	Co	50:1	110	Toluene	9, 21	95, 96	1.64, 1.33	[48]
		[Cu(Ac)2(L1)]	Co	50:1	110	Toluene	54	95, 96	1.23, 2.38	[48]

C catalyst, **CC** cocatalyst, **I** initiator, **Mech** mechanism, **M** monomer, **T** temperature, **p-PhBzOH** *p*-Phenylbentzyl alcohol, **An** Anionic, **Ca** Cationic, **Nu** Nucleophilic activation, **Li** Living polymerization, **Bu** butyl group, **Ph** Phenol group, **PEO** polyethylene oxide, **Oct** octanoate group, **X** Anionic group from methanesulfonic acid or *tri*-fluoromethanesulfonic acid, **na** not announced. Abbreviations are valid in this table only.

More reaction times, initiator and catalyst ratios are found from the references. Almost all of the PLGA polymerizations found were tin(II)octanoate catalysed and some of the syntheses lacked substantial documentation. Selection shown in Table 2 points out the difference in reaction times and PDIs between ROP in solution with DBU in comparison to bulk polymerization.

Table 2. *Selection of PLGA synthetization methods.*

C	I	Mech.	M:I:C:CC	T [ºC]	Solvent	Time [h]	Conv [%]	PDI	Ref.
$Sn(Oct)_2$		Bulk	50:0:1	140		10	na	na	[49]
$Sn(Oct)_2$	PEG	Bulk	na	na, vacuum		na	na	1.9-3.2	[18]
$C_6H_{10}O_6Zn$		Bulk	2800:280:1	130		8	na	na	[26]
DBU	PEG	Nu/Li	35:1:2.5	rt	DCM	0.5	100	1.08	[4]

C catalyst, **CC** cocatalyst, **I** initiator, **Mech** mechanism, **M** monomer, **T** temperature, **Nu** Nucleophilic activation, **Li** Living polymerization, **Bu** butyl group, **PEG** polyethylglycol, **Oct** octanoate group, **na** not announced. Abbreviations are valid in this table only.

Selection of some interesting ROP synthetization methods, which may be applicable for PLGA too, are shown in Table 3.

Table 3. *Selection of other ROP syntheses.*

M	C	I	Mech.	M:I:C:CC	T [°C]	Solvent	Time [h]	Conv [%]	PDI	Ref.
Bu-NCA		DBU	ZROP	25-400:1	50	THF	1-64	43-100	1.02-1.12	[13]
NBC		DBU	An/Bulk	25:1	100-120		1	68-53	1.49-1.48	[50]
NBC		DMAP	An/Bulk	25:1	120		5, 20	7, 52	1.25, 1.45	[50]
NBC		Dabco	An/Bulk	25:1	120		1- 20	7-70	1.26-1.43	[50]
ε-CL	TBD	PEO	Nu/Li	100-400:2:1	rt	C_6D_6	5-8	76-52	1.10-1.16	[15]
TMC		Hematin	Bulk	100-750:1	100		24	69-81	na	[51]

M monomer, **C** catalyst, **CC** cocatalyst, **I** initiator, **Mech** mechanism, **T** temperature, **Bu-NCA** N-butyl N-carboxyanhydride, **ZROP** Zwitterionic ROP, **An** Anionic, **NBC** 5,5-(bicyclo[2.2.1]hept-2-en-5,5-ylidene)-1,3-dioxan-2-one, **Dabco** Triethylenediamine, **ε-CL** ε-caprolactone, **PEO** polyethylene oxide, **Nu** Nucleophilic activation, **Li** Living polymerization, **TMC** Trimethylcarbonate, **na** not announced. Abbreviations are valid in this table only.

Bulk polymerization with DBU gives relatively short reaction time, but PDI is still rather high in comparison with the latter DBU entry in Table 2, which supports DBU in solution over the other proposed choices.

For basic catalyst acid is needed to terminate the reaction. Benzoic acid (PhCOOH) is easy to handle since it is solid, relatively safe [52] and as aromatic, easily detectable in both, NMR and IR [53].

Reaction equation for the polymerization process from initiation [54, p. 199] through ring opening polymerization [4], lactide as monomer, is shown in Figure 1. In principle this polymerization requires equal amounts of catalyst and initiator, since amount of active chain ends depends on amount of catalyst. In practice excess catalyst amount is needed in order to increase the probability of catalyst molecule presence while nucleophilic attack by the alcohol occurs and to increase the probability of the alcohol being deprotonated at the moment when orbitals of nucleophile and electrophile overlap. DBU probably activates the alcohol too, via hydrogen bonding [15].

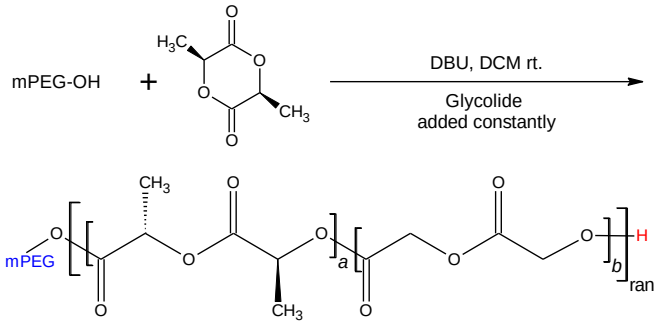


Figure 1. *PEG-PLGA polymerization with DBU. Redrawn from [4].*

Other reaction mechanisms have been proposed in literature too: DBU first activates the initiating alcohol [10][55] shown in Figure 2, or DBU's nucleophilic attack to monomer

[13] shown in Figure 3. Transesterification side reactions are expected to be rare, since no metal catalyst is used [54, p. 209][55][56].

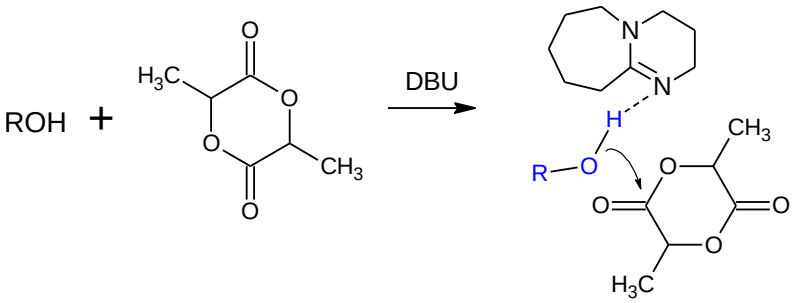


Figure 2. *Proposed activation of alcohol by DBU. Redrawn from [10].*

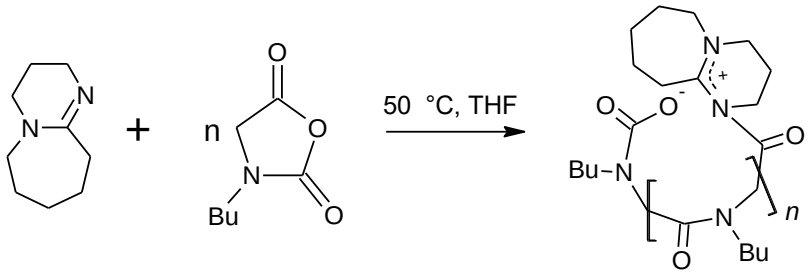


Figure 3. *Proposed DBU's nuclephilic attack to the monomer. Redraw from [13].*

Since glycolide is far more reactive than lactide [4][5][6], even three orders reported [4], glycolide concentration has to be far less than lactides. Thus, in order to achieve higher glycolide content, all the glycolide cannot be added once to the reaction or else long glycolide blocks appear immediately and precipitate, since PGA has poor solubility in all common organic solvents [48][57].

Previously dichloromethane (DCM) was used as solvent for PEG and LA, tetrahydrofuran (THF) for GA and 2-propanol (iPrOH) was used for precipitation [4]. DCM was found to be the most common solvent used in PLA and PLGA ROP, although chloroform ($CHCl_3$), THF and toluene was also used [13][15][58]. Acetone was excluded because of possible disturbance of the hemiketal formation [54, pp. 137-138]. No reports of polymerization in fluorinated solvents was found.

2.3 Synthesis of PEG-PLGA-LL

PLGAs are also modified towards drug loading properties. Lysine have been found effective additive in NP drug loading [59][60] and relatively easily attached to PLGA hydroxyl end group [25][26][36].

Straight forward incorporation of plain amino acid is not plausible since amine and carboxylic acid groups would react forming polyamide. Thus protected amino acid needs to be used. Depending on protective group of choice, either basic (fluorenylmethyloxycarbonyl, Fmoc) or acidic (*tert*-Butyloxycarbonyl, Boc) conditions are required

in deprotection stage [54, pp. 557-559][61]. For example *N*,*N*-di-Fmoc-L-lysine (Fmoc-Lys(Fmoc)-OH, Fmoc-LL) can be used as protected lysine.

The modification of the PEG-PLGA end group carried out using Fmoc protected lysine requires two synthesis steps. First protected lysine needs to be transformed to acyl chloride using thionyl chloride [54, pp. 214-215], then added to mPEG-b-PLGA-OH via acylation reaction in basic conditions (in DMF), halogenated amino acid as acylating agent. Weak base, for example trimethylamine (Et_3N) pulls the reaction towards products and keeps pH higher by removing hydrochloride formed in the reaction.

Deprotection of the protected amine has been carried out with piperidine in DMF [62][63][64], with piperidine in DCM [63][64], with piperidine in $CHCl_3$ [63] and with DBU in THF [61]. Fmoc cleavage was found to be zero after 15 min with piperidine in $CHCl_3$ [63]. In DMF both piperidine and DBU was found very effective, which is evident since DMF is weak base. All the reactions in the references were carried out in room temperature.

3. THEORETICAL APPROACH

Reaction mechanisms proposed, based on the information found in literature, are described in this section. This section also describes how and why, on the grounds of theory, decisions for the experimental part was made. Equations for the drop size and needle size are formulated from the volume flow and surface tension equations found from literature. Equations for reaction kinetics are formulated from the pseudo-first order reaction rate equation.

3.1 Polymerization of PEG-PLGA

Reaction equations for the polymerization process from initiation through ring opening polymerization [54, p. 199], lactide as first monomer and glycolide as second monomer, to termination with benzoic acid is shown in Figure 4. Proton exchange between catalyst and active chain end occurs in the reaction as well.

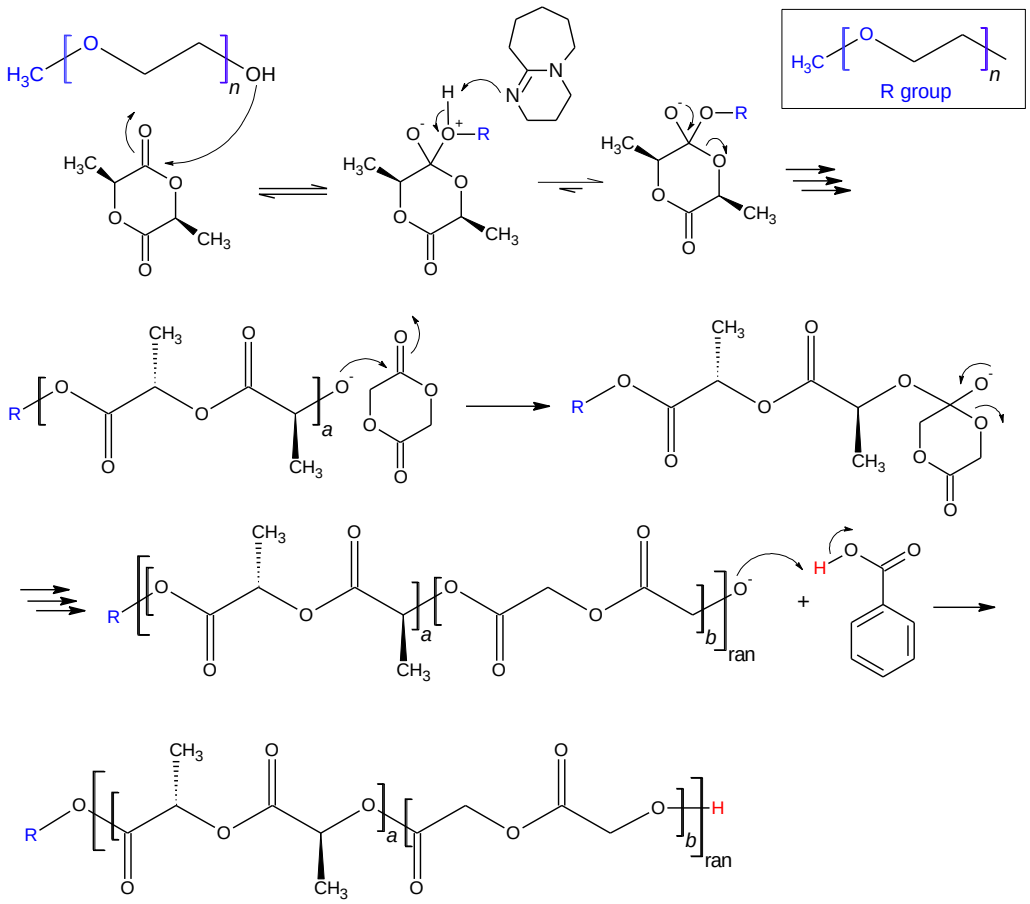


Figure 4. *PEG-PLGA polymerization with DBU. [65]*

The amounts of substances will be calculated from equation (4)

$$n = \frac{m}{M} \tag{4}$$

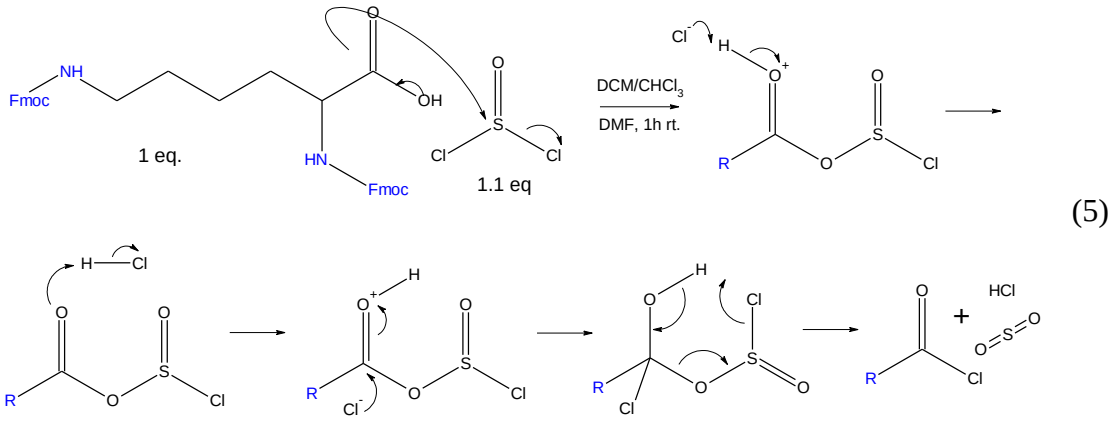
where *n* denotes the amount of substance, *m* denotes mass and *M* denotes molar mass.

3.2 Synthesis of PEG-PLGA-LL

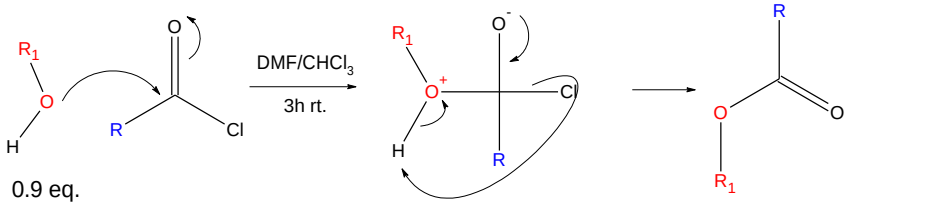
In this study a single L-lysine amino acid needs be attached to PEG-PLGA copolymer in order to increase NP efficiency towards entrapping anionic drugs.

3.2.1 Synthesis of PEG-PLGA-(Fmoc-Lys(Fmoc)-OH)

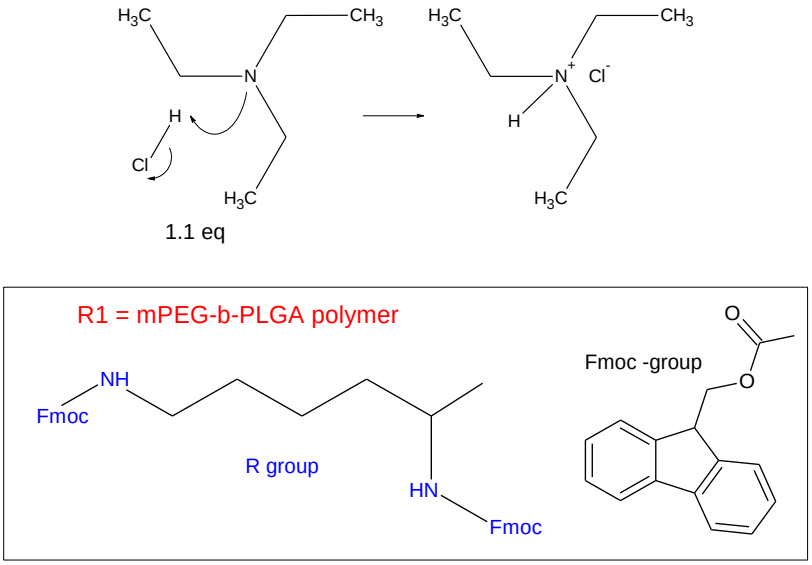
The modification of the PEG-PLGA end group carried out using Fmoc protected lysine requires two synthesis steps as found earlier in 2.2. First protected lysine needs to be transformed to acyl chloride using thionyl chloride [54, pp. 214-215], equation (5), then added to mPEG-b-PLGA-OH via acylation reaction in basic conditions (in DMF), equation (6), halogenated amino acid as acylating agent. Depending on polymer's solubility, either DCM or $CHCl_3$ maybe be used along with DMF (basic solvent). Weak base, in this case trimethylamine (Et_3N) pulls the reaction towards products and keeps pH higher by removing hydrochloride formed in the reaction, equation (7).



(5)



(6)



(7)

3.2.2 Deprotection of L-lysine

After product dries out, deprotection process follows: based on information gathered from [54, p. 559][62][63][64] and [61], 1:50:50 ratio of DBU:DCM:DMF or pre-determined 20% piperidine in DCM/DMF [64] will be used. Solvent choices will be made according to product solubility limitations and use of piperidine depends on whether product is soluble enough for the amount of piperidine available. Deprotection reaction is shown in Figure 5.

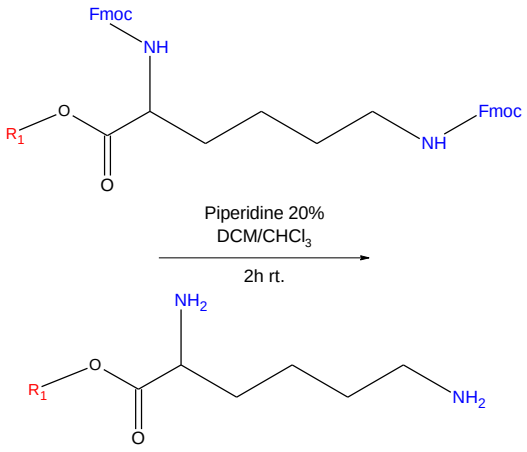


Figure 5. *Fmoc protected Lysine deprotection. [65]*

3.3 Implementation with Manual Feed

Reaction time was expected to be 30 min, so continuous manual syringe control was no option for addition of second solution. First option was dropping funnel, but the 25 ml piece available was rather large for the small scale reactions in the beginning. Also drop

size from dropping funnel tip was quite large. Thus optional way to control the second solution feed to the reaction vessel was designed.

Vertically positioned thin glass syringe with needle would allow small droplet size and perhaps automatically adjusting drop rate, based on viscosity, surface tension and gravity, Figure 6. Syringe could be used with or without piston, so back-up for possible instances when needle gets clogged would be at hand. Idea with the syringe and needle is to keep the flow rate in control by controlling drop frequency rather than following a rough scale. Syringe can be replaced with any other glass ware or device with ability to connect to the syringe needle.

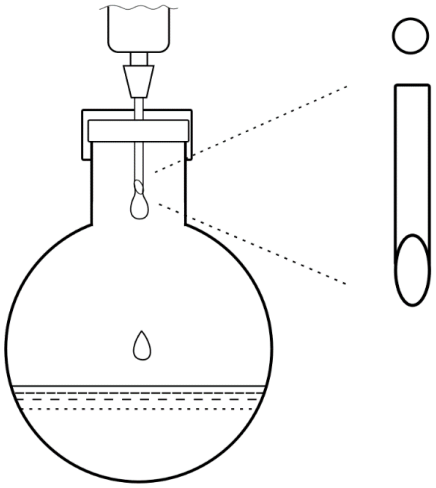


Figure 6. *Schematic picture of reaction assembly with syringe needle. Needle magnification with horizontal projection on the right.*

Volume flow through a narrow duct [66] is

$$Q = \frac{\pi d^4}{128 \eta l} \Delta p \tag{8}$$

where Q denotes volume flow, d denotes duct diameter, η denotes dynamic viscosity, l denotes duct length and Δp denotes pressure difference between duct ends and dynamic viscosity is equal to product of kinematic viscosity ν and density ρ. Pressure difference between duct ends is thus

$$\Delta p = \rho g l \tag{9}$$

where ρ denotes density and g denotes gravity. If second monomer (in solvent 2) is 1000 times more reactive than first one, drop size should not exceed 1:1000 of the solvent already in the reaction mixture (solvent 1 in the beginning). With 2 ml of solvent, assuming equal concentrations as first approximation, drop size should not exceed 2 µl. Drop size depends on surface tension, density and needle diameter [66, pp. 11-20], as found in following derivation.

$$\Delta p = \frac{2\gamma}{r} = \rho g h \tag{10}$$

where γ denotes surface tension, r denotes needle radius and h denotes column height. Minimum needle length, restricted by the septum and needle tip visibility (since drop rate needs to be controlled), is approximately 15 mm, which yields to the column minimum height approximately 25 mm. THF density is 889 kg/m^3 and surface tension is 26.40 mN/m in rt. [68]. Circular shape needle tip was used to approximate circumference, since that is the circumference in the horizontal projection and pressure is force per perpendicular area. Surface tension originated retarding force [66, pp. 11-20] equals gravity when the drop size is in its maximum

$$2\pi r\gamma = mg = \rho Vg \tag{11}$$

where m denotes mass and V volume of the droplet. Solvent density used as solution density is accurate enough at this stage. Equation (11) gives the needle radius as

$$r = \frac{\rho Vg}{2\pi\gamma} = \frac{889\,\frac{\mathrm{kg}}{\mathrm{m}^3}\cdot 2\cdot 10^{-9}\mathrm{m}^3\cdot 9.81\,\frac{\mathrm{m}}{\mathrm{s}^2}}{2\pi\cdot 26.40\cdot 10^{-3}\,\frac{\mathrm{N}}{\mathrm{m}}} = 1.05\cdot 10^{-4}\mathrm{m} \approx 0.1\ \mathrm{mm}$$

Putting in this result to equation (10) yields to the minimum height of the solution column

$$h = \frac{2\gamma}{\rho gr} = \frac{2\cdot 26.40\cdot 10^{-3}\,\frac{\mathrm{N}}{\mathrm{m}}}{889\,\frac{\mathrm{kg}}{\mathrm{m}^3}\cdot 9.81\,\frac{\mathrm{m}}{\mathrm{s}^2}\cdot 1.05\cdot 10^{-4}\mathrm{m}} = 58\ \mathrm{mm}$$

which means that column shorter than 58 mm would not pass any THF with 0.2 mm diameter (Ø) needle attached. Combining these results with equations (8) and (9) gives volume flow rate, with 15 mm needle length and kinematic viscosity ν of THF 5.29·10^{-7} m^2/s [69]

$$Q = \frac{\pi d^4}{128\eta l}\rho gh = \frac{\pi d^4}{128\upsilon\cancel{\rho} l} g\cancel{\rho} h$$

$$= \frac{\pi\cdot(1.05\cdot 10^{-4}m)^4}{128\cdot 5.29\cdot 10^{-7}\,\frac{m^2}{s}\cdot 15\cdot 10^{-3}m}\cdot 9.81\,\frac{m}{s^2}\cdot 58\cdot 10^{-3}m = 2.14\cdot 10^{-10}\,\frac{m^3}{s}$$

$$= 13\,\frac{\mu l}{min}$$

which means that Ø 0.2 mm needle is far too small to pass enough (1 ml in 10 min), even pure solvent. Table 4 shows corresponding evaluations for selected needle sizes. Results reveal that already Ø 0.3 mm would pass enough and be otherwise perhaps optimal, but since it requires 26 mm extra height, lots of solution will be lost even with the minimum size syringe. On the other hand, the evaluated flow rate is the minimum rate, as liquid column height increases, so does the flow. So unless viscosity does not increase significantly with the glycolide in, already Ø 0.3 mm needle passes too fast.

Table 4. *Needle sizes and corresponding column heights, needle lengths, flow rates and drop sizes for THF.*

Needle diameter *d*, [mm]	**Column height *h*, [mm]**	**Duct length *l*, [mm]**	**Flow *Q*, [µl/min]**	**Drop size *V*, [µl]**
0.3	41	15	320	2.9
0.4	30	20	1900	3.8
0.5	24	30	1400	4.8
0.7	17	50	2200	6.7

For a bigger batch larger needles may work excellent, but possible needle clogging concerns with the small diameter needles. Drop sizes and drop rates with different column heights need to be measured in practice with the glycolide in and re-evaluate if the surface tension drags enough yielding Q(h) with proper nonlinearity. Otherwise syringe needle needs to be connected to dropping funnel in order to achieve small drop size together with perhaps plausible flow control.

3.4 Reaction Kinetics

As found in [4], GA rich chains tend to precipitate early. Thus avoiding long GA blocks is essential and GA's reaction rate should match LA's reaction rate as well as possible. Lactide ring opening polymerization rate depends on lactide rate constant, DBU concentration, active chain end concentration and monomer concentration [4]

$$v_L = \frac{d[LA]}{dt} = k_L[DBU]^x[OH]^y[LA]^z \tag{12}$$

where v_L denotes the lactide polymerization rate, *d[LA]/dt* means the first time derivative of lactide concentration *[LA]*, k_L denotes lactide rate constant and *[OH]* denotes concentration of active chain ends. Exponents *x*, *y* and *z* denote reaction order relative to corresponding concentration. According to [4][12][58] each is first order. Because DBU and active chain concentrations are time-invariant, reaction is pseudo-first order with only lactide concentration as time-dependent term. Integration of equation (12) with positive value rate constant

$$\frac{d[LA]}{dt} = -k_L[DBU]\,[OH]\,[LA]$$

$$\Leftrightarrow \frac{\frac{d[LA]}{dt}}{[LA]} = -k_L[DBU]\,[OH]$$

$$\Leftrightarrow \int_0^t \frac{\frac{d[LA]}{dt}}{[LA]}dt = -\int_0^t k_L[DBU][OH]dt$$

$$\Leftrightarrow \ln[LA] - \ln[LA]_0 = -k_L[DBU][OH]\mathrm{t}$$

$$\Leftrightarrow \ln\frac{[LA]}{[LA]_0} = -k_L[DBU][OH]\mathrm{t}$$

$$\Leftrightarrow \frac{[LA]}{[LA]_0} = e^{-k_L[DBU][OH]t} \tag{13}$$

gives relation between measured degrees of conversion and rate constant. Glycolide rate constant could be expressed by similar manner, but since glycolide in THF is added to reaction dropwise, glycolide rate constant in THF does not apply Since glycolide rate constant was found to lie even at around 1000 times to lactide's rate constant, that value will be applied in first approximation. PGA polymerization is rarely reported (in comparison to PLA), but since other than lactide ROP has also been reported to be pseudo first order, for example *N*-alkyl *N*-carboxyanhydride [13] and PGA [4], polymerization of PGA will be calculated according pseudo first order reaction kinetics.

In the PLGA synthesis total amount of solution varies throughout the process since glycolide in THF solution is added constantly. Total volume is thus

$$V = V_0 + \int_0^t Q(t)dt \tag{14}$$

where V denotes total volume, V_0 the initial volume, equal to solvent used for lactide and initiator solution and Q denotes glycolide in THF solvent volume flow rate. Actual lactide concentration at time t is then the remaining amount of lactide in moles per total volume, putting in equation (14) to equation (12) gives

$$[LA] = \frac{[LA]_0V_0 - \int_0^t \frac{d[LA]}{dt}\left(V_0 + \int_0^t Q dt\right)dt}{V_0 + \int_0^t Q dt} \tag{15}$$

Adding another solvent affects catalyst and initiator concentrations respectively: concentration of each dilutes with the initial volume and total volume ratio

$$[DBU] = [DBU]_0\frac{V_0}{V_0 + \int_0^t Q dt} \tag{16}$$

$$[OH] = [OH]_0\frac{V_0}{V_0 + \int_0^t Q dt} \tag{17}$$

where subscript *0* refers to initial state. Combining equations (12) − (17) gives lactide polymerization rate equation

$$\frac{d[LA]}{dt} = -k_L\frac{[DBU]_0[OH]_0V_0^2}{\left(V_0 + \int_0^t Q dt\right)^3}\left([LA]_0V_0 - \int_0^t \frac{d[LA]}{dt}\left(V_0 + \int_0^t Q dt\right)dt\right) \tag{18}$$

where lactide concentration at time t, $[LA]_t$, is

$$[LA]_t = \frac{[LA]_0V_0 - \int_0^t \frac{d[LA]}{dt}\left(V_0 + \int_0^t Q dt\right)dt}{V_0 + \int_0^t Q dt} \tag{19}$$

Denoting denominator *V* and combining equation (19) to (18) gives differential equation form

$$\frac{d[LA]}{dt} = -k_L \frac{[DBU]_0[OH]_0 V_0^2}{V^2} [LA]_t \tag{20}$$

If lactide concentration is measured at known times, rate constant would be resolved graphically. Obviously, when added to following glycolide expression, graphical solution is not plausible anymore.

Reaction equation for glycolide forms respectively, except glycolide concentration at time *t*, $[GA]_t$. In glycolide's case, glycolide's concentration at time *t*, $[GA]_t$, depends on difference of glycolide fed and glycolide consumed in reaction

$$[GA]_t = \frac{[GA]_0 \int_0^t Q dt - \int_0^t \frac{d[GA]}{dt}\left(V_0 + \int_0^t Q dt\right) dt}{V_0 + \int_0^t Q dt} \tag{21}$$

where $[GA]_0$ denotes glycolide initial concentration in THF. Thus the final reaction rate equation for glycolide polymerization rate becomes

$$\frac{d[GA]}{dt} = -k_G \frac{[DBU]_0[OH]_0 V_0^2}{\left(V_0 + \int_0^t Q dt\right)^3} \left([GA]_0 \int_0^t Q dt - \int_0^t \frac{d[GA]}{dt}\left(V_0 + \int_0^t Q dt\right) dt\right) \tag{22}$$

where k_G denotes glycolide rate constant. Depending on application, equations (18) and (22) need to be solved either manually using Taylor series and iteration, or simulation, to solve GA solution volume needed to be fed at given time to match consumed LA and GA to target ratio.

4. MATERIALS AND METHODS

4.1 Chemicals

All the chemicals used in this study, with purities and reference for detailed information are provided in Table 5.

Table 5. *Chemicals used in the research.*

Chemical	Grade	Purity	Remarks	Reference
Acetone	Technical	ND		[70]
Benzoic Acid		99.5%		[71]
Chloroform		99.8 %	anhydrous	[72]
Chloroform-d		99.8 %	0.03 V-% TMS	[73]
DBU		98 %		[74]
Dichloromethane		99.8 %	anhydrous	[75]
Diethyl ether (Lab-scan)	Analytical	water <0.2 %	expired 10/2005	
L-lactide		98 %		[76]
Glycolide		> 99 %		[77]
Ethanol		96 %		
Ethyl acetate	Synthesis	99.5 %	ACS reagent	[78]
Hexane	Laboratory	95%		[79]
Methanol	Analytical	ND		[80]
N,*N*-Dimethylforamide	Reagent	99.8 %	anhydrous	[81]
N,*N*-di-Fmoc-L-lysine		98%		[82]
Piperidine		99%		[83]
PEG 2k and 5k		ND		[84]
2-propanol	Technical	ND		[85]
Tetrahydrofuran		99.8 %	anhydrous	[68]
Thionyl chloride		≥ 99 %,		[86]
Toluene		≥ 99 %,	expired 9/2014	[87]
Trimethylamine	Synthesis	99.5 %		[88]

Additional chemical properties important for this study for the chemicals in Table 5 are provided in Table 6.

Table 6. *Molar masses (M), densities (ρ) and enthalpies of vaporization (ΔH^{vap}) at 25 °C for selected chemicals. Molar masses and densities are from Table 5 references.*

Chemical	**M [g/mol]**	**Density [g/cm^3]**	**(ΔH_{vap}) [kJ/mol]**	**Reference page in [89]**
Acetone	58.08	0.792	30.99	6-132
Benzoic Acid	122.12			
Chloroform	119.38	1.492	31.28	6-144
DBU	152.24	1.018		
Dichloromethane	84.93	1.325	28.82	6-135
L-lactide	144.13			
Glycolide	113.07			
Ethyl acetate	88.11	0.902	35.60	6-137
Hexane	86.18	0.659	31.56	6-139
Methanol	32.04	0.7198	37.43	6-140
N,*N*-Dimethylformamide	73.09	0.944	46.89	6-136
N,*N*-di-Fmoc-L-lysine	590.66			
Piperidine	85.15	0.862	39.29	6-143
PEG 2k/5k	2050/5000			
2-propanol	60.10	0.786	45.39	6-143
Tetrahydrofuran	72.11	0.889	31.99	6-143
Thionyl chloride	118.97	1.631	31	6-131
Toluene	92.14	0.867	38.01	6-144
Trimethylamine	101.19	0.726	34.84	6-144
Water			43.98	6-132

4.2 Instrumentation and Software

New Era NE-500 programmable syringe pump [90] was used in automatic feed implementation. Besides common laboratory ware, Mettler Toledo AG245 (±0.01 mg accuracy) and Mettler PM400 (±1 mg accuracy) scales were used in weighing, FinnSonic m12 ultrasonicator for accelerating solubility and Hettich Universal 320R centrifuge was used in centrifugations. Instrumentation used for characterization are listed in Table 7.

Table 7. *Instrumentation used for characterization.*

Technique	**Instrument**
NMR	Varian Mercury 300 MHz NMR Spectrometer
DSC	TA Instruments DSC Q1000

Software used in this research with respect to the applications are listed in Table 8. High computing performance laptop was used in data processing and simulations.

Table 8. *Software used with respective applications.*

Software	Application
MS Office 2016	Reporting and data processing
Inkscape 0.91	Drawings
IrfanVieW 4.42 Gimp 2.8	Image processing
TA Universal Analysis	TA Instrument DSC analysis and reporting
ACD Labs 1D/2D NMR Processor	NMR spectrum analysis and reporting
Matlab R2016a	Syringe pump control, linear algebra operations
Simulink R2016a	Reaction kinetics simulations, flow demand curve source

Suitable valve for the nitrogen atmosphere was found from Numatics, model R880G02A [91]. Final assembly is shown in Figure 7, valve specifications in Appendix A and nitrogen atmosphere design without glovebox in Appendix B.

Figure 7. *Nitrogen control system assembly.*

Valve's maximum supply pressure is 17 bar and maximum outlet pressure 113 mbar with 0,6 mbar sensitivity. Due to the hose connecting valve to experiment instrumentation the expected outlet pressure needed from the valve lies around 1-2 mbar. Valve and other parts supplied Sitek-Palvelu Oy from Jyväskylä.

4.3 Simulink Model of the Reaction Kinetics

To begin with, lactide simulation model should give the same result as equation (13). For that purpose, PEG-PLA polymerization is essential. Simulations have been carried out in Matlab® Simulink®. For simulation model equation (13) needs to be expressed

$$\frac{d[LA]}{dt} = k_L[DBU]\,[OH]\,[LA]$$

$$\Leftrightarrow \frac{d[LA]}{dt} = k_L[DBU]\,[OH]\,([LA]_0 - [LA]_{reacted})$$

$$\Leftrightarrow \frac{d[LA]}{dt} = k_L[DBU]\,[OH]\left([LA]_0 - \int_0^t \frac{d[LA]}{dt}\,dt\right) \tag{23}$$

For future purposes conversion

$$c = \frac{n}{V} \Leftrightarrow n = cV \tag{24}$$

where *c* denotes concentration, *n* denotes the amount of substance (in moles) and *V* denotes volume has been built in the simulation model, Figure 8.

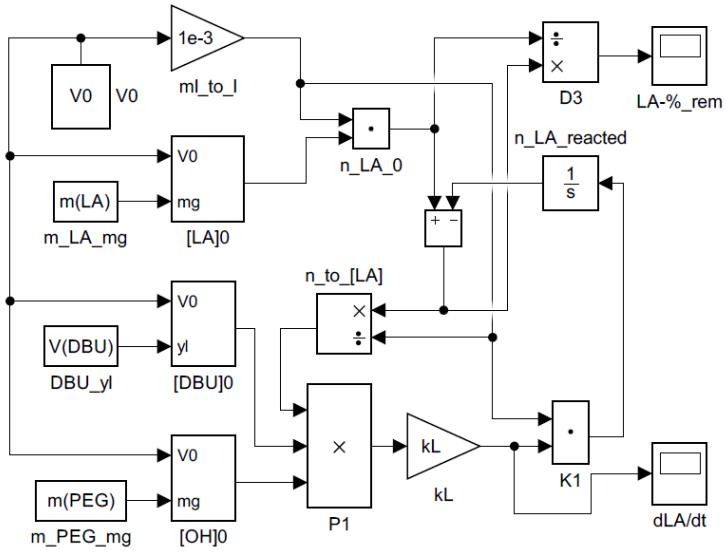


Figure 8. *Simulation model for PEG-PLA polymerization.*

Each subsystem in the model converts quantity of the substance as input to concentration in moles per litre as output, lactide as an example, Figure 9, where *M(LA)* denotes lactide's molar mass.

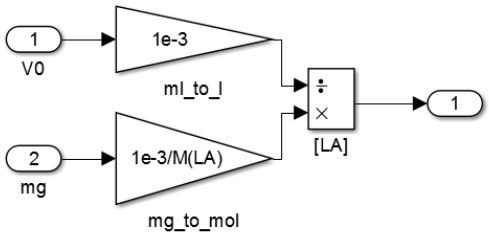


Figure 9. *Subsystem [LA]0, lactide initial concentration in mol/l from lactide mass in mg, lactide molar mass in g/mol and initial solvent volume in ml.*

Simulation model for PEG-PLGA polymerization combines equations (18) and (19). To study the influence of added THF to the lactide rate constant, manual feed rate needs to be used as volume flow input. Simulation model of the system is shown in Figure 10.

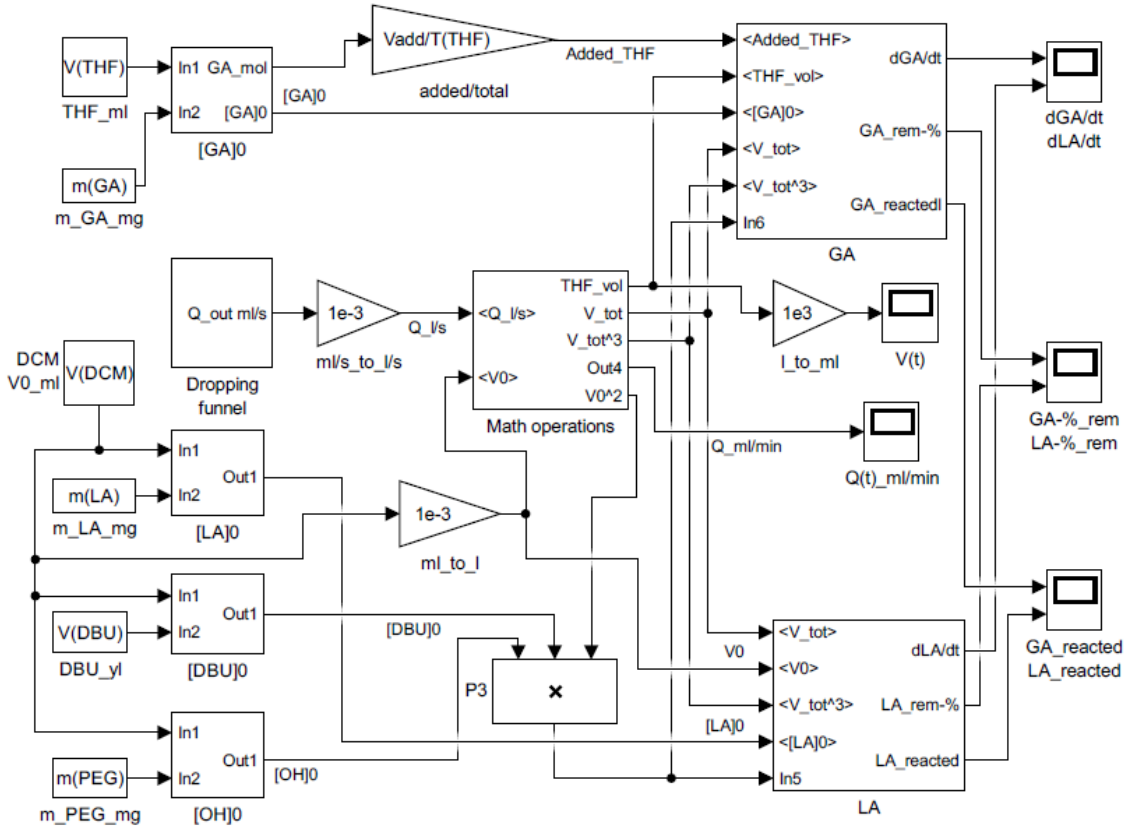


Figure 10. *Simulation model for PEG-PLGA polymerization with manual glycolide in THF feed.*

Simulation model for glycolide polymerization, the GA subsystem, is shown in Figure 11 and for lactide polymerization, the LA subsystem, is shown in Figure 12.

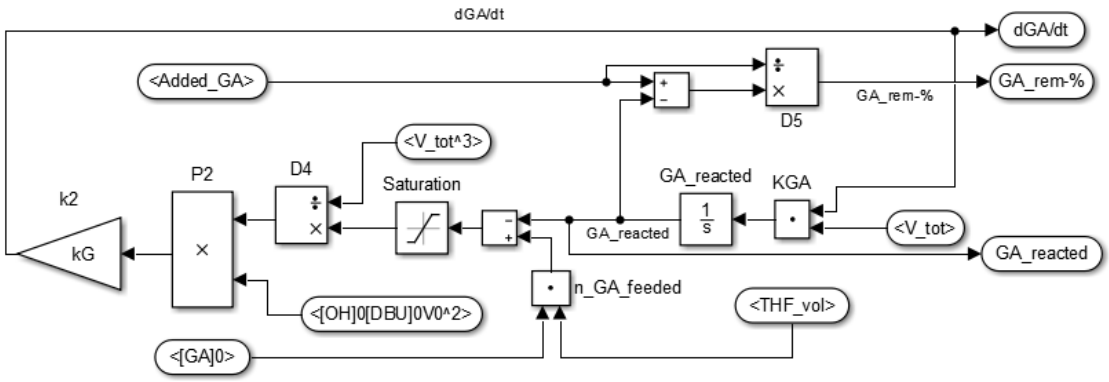


Figure 11. *Simulation model for glycolide polymerization, the GA subsystem.*

Glycolide system has saturation in order to avoid reacted amount overcoming available amount and if such situation occurs, any output alarms operator. Math operations subsystem in shown in Figure 13 and manual feed via dropping funnel in Figure 14. These models need to be verified experimentally, especially solvent composition weighted glycolide rate constant might be necessary instead of simple concentration dependency.

After rate constants have been verified and possible non-unity order reactants revealed, model will be ready for optimum glycolide in THF feed rate, or volume flow rate, simulation. For that purpose, dropping funnel subsystem needs to be replaced with virtual controller which compares and balances lactide and glycolide polymerization rate by adjusting glycolide in THF feed rate, Figure 15. In Simulink discrete systems need Z-transform and continuous systems need Laplace transform. Systems simulated in this thesis are continuous. Laplace transform allows complex variables, which is often required in some step when simplifying real word systems to equations. In the simulation models all the integrations are *1/s* simply because Laplace transform of integration is *1/s*.

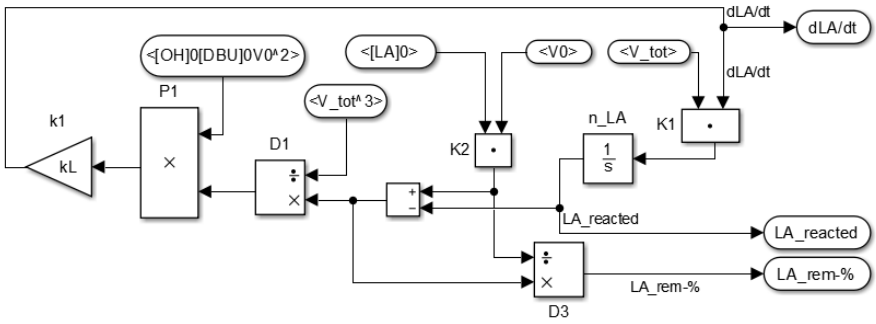


Figure 12. *Simulation model for lactide polymerization, the LA subsystem.*

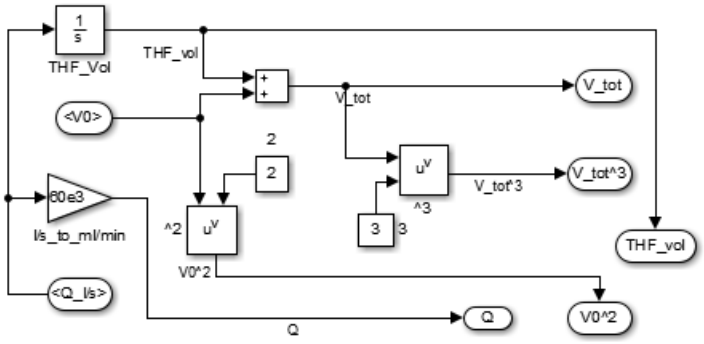


Figure 13. *Math operations subsystem.*

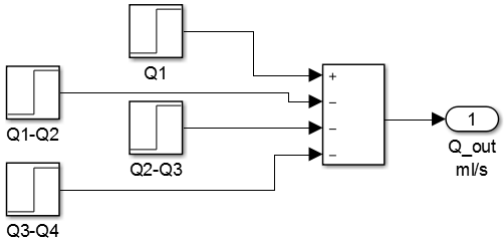


Figure 14. *Dropping funnel subsystem*

Figure 16 shows virtual controller in the PEG-PLGA model. Virtual controller output units are s^{-1}, thus unit conversion block has been removed. Before taking the flow rate to Matlab Workspace and further to syringe pump program, signal needs unit conversion to µl/min and sampling, shown in Figure 16 as well.

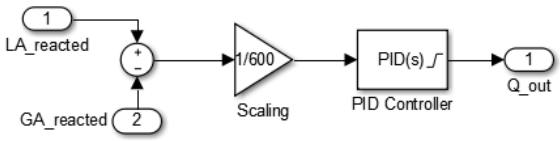


Figure 15. *Virtual controller subsystem for feed rate*

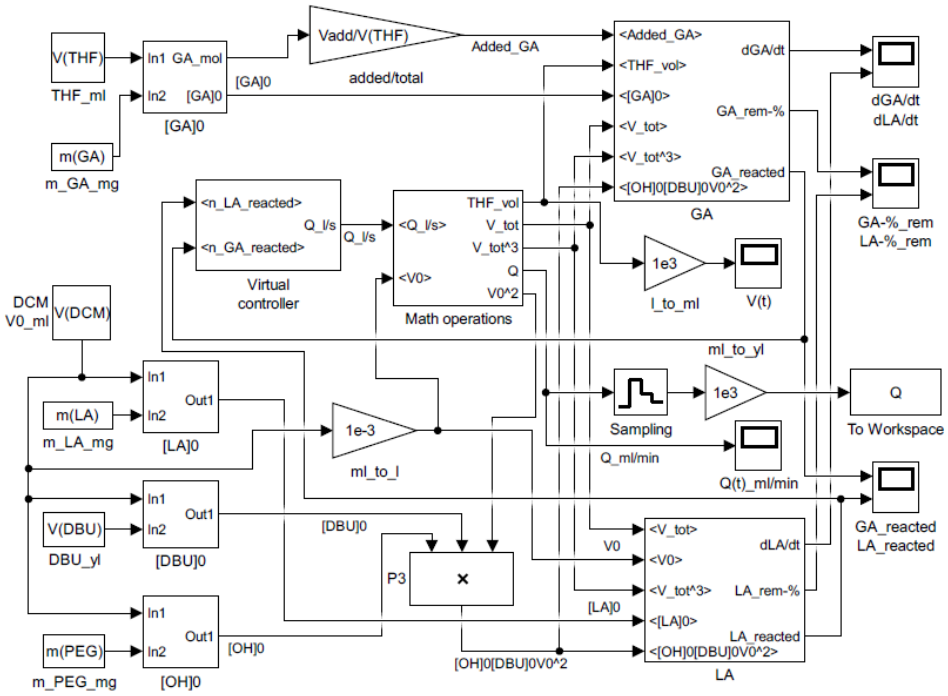


Figure 16. *Simulation model for glycolide in THF solution feed rate setting to syringe pump in PEG-PLGA polymerization.*

Reaction time chosen was 3600 seconds and sample rate 1/60 Hz. Drop size and rate model would cause oscillations, thus glycolide feed was modelled as continuous flow. Continuous flow is still more accurate than actual flow rate control, since flow rate demand cannot be continuous.

4.4 Syringe Pump Control

Syringe pump in question has no user interface of its own and the control program provided by the manufacturer was not applicable, thus Matlab was the only available choice for the pump control. Most of the pump m-file control code needed was provided together with pump, only variable flow rate was needed to set up.

No arithmetic operations are executable in the pump, neither time-variant functions are accepted, only scalar inputs and constant period are allowed. The code for varying flow rate command, generated from the simulation model, to be used is thus

```
rate = [y']; % uses transpose of y from workspace as flow rate command
             % vector
intervaltime = 1; % period set to one minute
  P = 1;    % starts at step 1
  for i = 1:length(rate); % repeat until index reaches length of y'
  P = pump(s, diameter,rate(i),rateunit,rate(i)*intervaltime,volumeunit,
     'INF',P,0); % next step, next rate
  end
```

Simulated flow rate need for the 3600 s reaction time was sampled at 60 s intervals, which generated 60 values long flow rate command vector *y*.

4.5 Characterization

After any chemical reaction or reaction series, the product needs to be verified somehow. This process, characterization, is based on material properties like melting point or behaviour, bond resonances or nuclear resonances. Each property of interests can be measured with designated device or system and the methods primary in this study are described in the following sections. No method alone gives complete information about the product, but cross comparison between the results excludes possibilities, hopefully yielding to a single solution.

Yield gives the first approximation of the possible content, especially when solubility of different chain lengths, with respective block contents, is known in the precipitation or washing solvent used in the step in question. Analysis of the liquid phase or filtrate gives further information. Solubility tests of the starting materials and product gives hints of the structure at hand and solubility tests in comparison with known samples offers further information about possible content.

4.5.1 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance spectroscopy (NMR) is based on nucleus spin. Only nucleus with total half integer spin is visible in NMR. Chemical environment of the nucleus affects to the effective field each nucleus experiences, which affects to the resonance, and thus to the detected frequency. Each chemical shift detected is then compared to the reference chemical shift and results are detected shifts relative reference shift [92].

Because NMR gives exact relative chemical shifts regardless of magnetic flux density used, spectrums measured by different devices are comparable between different references. NMR is sensitive to only the measured nucleus, so all other parts of the measured system give no arise of signal and signal strength is directly proportional to the amount of shift detected, making NMR not only qualitative but quantitative measurement method as well [92].

In proton, or ^{1}H, NMR most of the hydrogens are visible but in carbon, or ^{13}C, NMR only one per mil of the carbons are visible and thus signal to noise ratio (SNR) tends to rise, making dilute samples impossible to measure. Spectrum peak areas are directly proportional to the number of protons experiencing that shift is present in the sample.

In the case of PEG-PLGA, the basic structure consists of initiator block bonded to more or less random series of PLA and PGA blocks. Thus the peaks expected to appear come

from methoxy (-O-CH_3) and hydroxyl (-OH) terminal groups, and methylene (-CH_2-) backbone from PEG, methine (=CH-) and methyl (-CH_3) groups from PLA and methylene group from PGA.

2-propanol residues are expected to show peaks from methyl groups, methane group and hydroxyl group. Methanol residues are expected to show peaks from methyl and hydroxyl groups. DMF residues are expected to show peaks from methyl groups (2) and carbonyl hydrogen. DCM and $CHCl_3$ are not expected to be found due their high volatility.

L-lysine is expected to show peaks from the backbone methine and methylene (4) groups. Fmoc is expected to show peaks in the aromatic region as benzoic acid. Et_3N residues are expected to show peaks from methyl and methylene groups. In the studies of degree of conversion peaks originated from benzoic acid benzene ring hydrogens (3 different shifts) and some residues of un-evaporated THF (2 different shifts), as well as from catalyst (several shifts) are expected.

Expected chemical shifts, peak shapes and number of equal protons are collected to Table 9. Since each 2 kDa PEG in the sample includes 180 equal protons, when PEG peak area is set to 180 as reference, all other peak areas need to be divided by their respective number of equal protons in order to assign their relative amounts (moles) in the sample. Multiplicity is based on the chemical structure and/or reference. In DBU's case all the peaks should be max 1:2 in height. In the NMR spectrum integrals PEG relative area were set to 180, as reference.

Samples for NMR were weighed to 1 ml glass bottles up to 30±1 mg/0.7 ml $CDCl_3$, unless sample solubility became limiting factor. ^1H NMR samples were weighed approximately 6±1 mg/0.7 ml $CDCl_3$ or D_2O. NMR solvents were taken from the reagent bottles with syringe and needle. Before taking sample solutions with Pasteur pipettes from 1 ml glass bottles to NMR tubes, sample solutions were sonicated.

NMR tubes were washed five times 30 s in ultrasonicator with $CHCl_3$, then three times with technical ethanol and oven dried overnight in 85 ºC after every use. In NMR analysis instrument output data was first Fourier transformed, TMS reference peak was set to 0 ppm, then phase was adjusted against the right-most intense peak and baseline was drawn.

Table 9. *Expected ppm values and peak shapes for different hydrogens in NMR spectrum with corresponding references'. Y and Z refers to PEG_X-PL_YG_ZA, s to singlet, d to doublet, t to triplet, q to quartet, sep to septet, m to multiplet and b to broad. ND refers to Not Defined.*

Chemical abreviation	**Proton**	**Shape and shift [ppm]**	**Equal protons**	**Reference**
PEG	CH_2	s 3.65	4x45	ND
	MeO	s 3.37	3	ND
PLA	CH	q 5.16	2Y	[6]
	CH_3	d 1.57	6Y	ND
LA	CH	q 5.08	2	[76]
	CH_3	d 1.65	6	[76]
PGA	CH_2	m 4.82	4Z	[6]
GA	CH_2	t 4.65	4	ND
LL	α CH	t 3.75	1	[93]
	β CH_2	t 1.90	2	[93]
	γ CH_2	t 1.47	2	[93]
	δ CH_2	t 1.72	2	[93]
	ε CH_2	t 3.01	2	[93]
	α NH_2	2.22	1	[93]
	α NH_2	0.72	1	[93]
	ε NH_2	0.84	1	[93]
	ε NH_2	0.32	1	[93]
PhCOOH	meta	t 7.45	2	[53]
	orto	d 8.12	2	[53]
	para	t 7.62	1	[53]
DBU	ND	d 1.61	ND	[74]
	ND	m 1.79	ND	[74]
	ND	d 2.38	ND	[74]
	ND	m 3.20	ND	[74]
	ND	t 3.28	ND	[61]
DMF	CH	s 8.02	1	[94]
	CH_3	s 2.96	3	[94]
	CH_3	s 2.88	3	[94]
THF	CH_2	m 1.85	4	[94]
	CH_2O	m 3.76	4	[94]
iPrOH	CH_3	d 1.22	6	[94]
	CH	sep 4.04	1	[94]
MeOH	CH_3	s 3.49	3	[94]
Et_3N	CH_3	t 1.03	9	[94]
	CH_2	q 2.53	6	[94]

PEG, LA, GA and Fmoc-LL reference spectrums are shown in Appendix C. GA was dissolved in D_2O.

4.5.2 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measures sample enthalpy changes as a function of temperature. Glass transition temperature (T_g) is the temperature where amorphous polymer starts to transform from rigid to soft and gradually up to liquid form. In the case of semicrystalline polymer, the amorphous regions of the polymer soften at T_g and at higher temperatures the crystalline regions start to melt. The melting is seen as exothermic peak in DSC and melting exotherm peak temperature is the melting temperature (T_m). Melting exotherm peak temperature is the T_m. With known reference samples, integration of melting exotherm would give information about the sample's composition. [95, pp. 1-14]

PEG-PLGA copolymer T_g is approximately weighted average of T_gs of copolymer blocks. T_gs of PEG, PLLA and PGA homopolymers are -40, 60 and 35 ºC [4] [96, p. 199] and T_ms of the homopolymers are 63, 180 and 220–230 ºC respectively [4][96, p. 199][97]. T_m broadens with increasing share of GA, eventually vanishing at approximately 30 % share [98]. Typical forms of PLGA DSC curves are found also from [6]. Sample needs melting for relaxation of stress residues before actual DSC curve can be measured, especially if polymer is collected by evaporating the solvent from the polymer solution. Since 10 ºC/min is best suited slope for T_m measurement and 20 ºC/min is best suited slope for T_g measurement [95, pp. 33-34], slope needs to be changed during the run.

For the DSC run 6.43 mg of the product was weighed to 20.20 mg standard aluminium pan and lid, -20 – 250 ºC 20 ºC/min relaxation run was executed first. The actual measurement run was executed -50 – 130 ºC 10 ºC/min and continued to 250 ºC 20 ºC/min. T_m was not expected to show up. Standard aluminium reference pan mass was 20.370 mg and nitrogen purge flow was 50 ml/min.

4.6 Experimental Research Methods

Until permission to purchase the nitrogen system, syntheses were tried by using the nitrogen flow from gas cylinder pressure reducing valve, with an internal flow restrictor and DBU was used directly from manufacturer's screw cap bottle. Sometimes nitrogen flow stopped during the reaction, sometimes solvent excess evaporation occurred due to the increased nitrogen flow. Reactions were a little successful, if at all. With nitrogen system installed it became clear that DBU is too sensitive to atmospheric impurities and once opened, the remaining catalyst effectivity decreases significantly. Thus one 25 ml bottle of DBU was divided to small bottles, enclosed with septum, in glovebox. After that PLA polymerizations became successful.

4.6.1 Glycolide Chain Solubility Limit

Glycolide chain solubility limit was studied too. PEG-PGA solubility was assumed to restrict chain growth to glycolide rich. 1,5 ml THF was added to 40,87 mg of glycolide and 1,5 ml of DCM was added to 12,55 mg of 2k PEG. Both solutions were transferred to 50 ml 3-neck bottle, stirred for a couple of minutes under nitrogen and then the reaction was activated with 3 µl of DBU.

4.6.2 Manual Feed Setup

In manual feed setup dropping funnel was used for GA in THF solution, or GA in solvent mixture, feed to the reaction mixture.

First tests were carried out with 10 ml burette and pure THF, but the burette stopcock was found impossible to set to same, almost closed, setting twice. Cock fully open with a needle as restrictor, giving automatic decrease on hydrostatic pressure among time, was tested. Column in the half-empty 10 ml burette is a little higher than in full 25 ml dropping funnel, but employing the burette offered better resolution.

Dropping funnel's stopcock had at least some adjustability for restricting the flow in comparison to that in burette and thus testing for both, smaller test batches and bigger batch in mind, was carried on with the 25 ml pressure-equalizing dropping funnel. Testing was carried out with pure mixture of solvents: 10 ml of THF and 10 ml of $CHCl_3$. 1 ml pipette tip, cut both ends, was used to fit needle inlet to the dropping funnel outlet.

Schematic picture of the installation used in manual feed experiments is shown in Figure 17. Either 25 or 50 ml 3-neck bottle (ground joints) with closed septum was placed over the magnetic stirrer. Extension was used between 3-neck bottle and dropping funnel in order to increase the distance between dropping funnel outlet and reaction mixture surface. That was necessary due to the cut 1 ml pipette tip and needle attached to the dropping funnel outlet. Needle sizes used varied from Ø 0.3-0.7 mm, based on what was found in 3.3. When bigger batches were synthesized, 1 ml pipette tip was only cut to tightly fit into the dropping funnel outlet, tip outlet was not cut. Dropping funnel inlet was closed with septum as well, needle was used to provide outlet for the nitrogen. Rightmost neck of the 3-neck bottle was used to feed in the nitrogen with needle as well. Leftmost neck was used for sampling and dosing the catalyst.

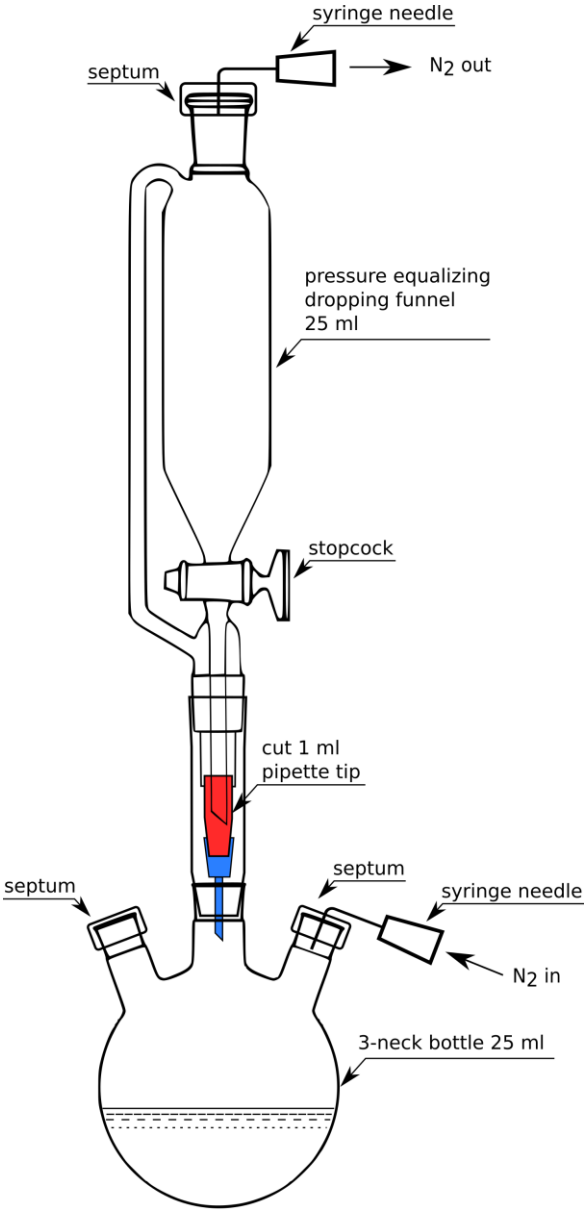


Figure 17. *Schematic picture of the installation employed in the manual feed experiments.*

Needle size was first chosen based on what was found in 3.3. Drop sizes were then measured and based on the actual drop size, drop rate needed to hit the target feed rate was then calculated. Target drop frequency was first based on "procedure 1"[4]:

1º	50 %	during 1st	10 min
2º	25 %	during 2nd	10 min
3º	12.5 %	during 3rd	10 min

After the formulated equations of the reaction kinetics (3.4) were manually solved at 0, 5, 10, 20 and 30 min, this procedure was found too slow in the beginning. Based on those solutions, GA feed procedure was updated to "procedure 2":

1º	60 %	during 1st	10 min
2º	27 %	during 2nd	10 min
3º	13 %	during 3rd	10 min

Procedure was further developed, this time equations of the reaction kinetics (3.4) were manually solved at 0, 1, 2, 3, 4, 5, 10, 15, 20 and 30 min. GA feed procedure was updated to "procedure 3":

1º	38 %	during 1st	5 min
2º	23 %	during 2nd	5 min
3º	24 %	during	10–20 min
4º	15 %	during	20–30 min

For bigger batch, plan was to dissolve 800 mg GA in 20 ml THF and control the feed with 25 ml pressure-equalizing dropping funnel. 326 mg of possibly contaminated GA was used to test solubility as well as free flow rate and drop size through different needle sizes. 1.5 ml of THF was found just too little to dissolve 326 mg of GA, 2.0 ml was enough. Since 326 mg is 40.75 % of 800 mg, 40.75 % of 20 ml of THF, or 8.15 ml, was used for flow tests. Solution was poured to 10 ml burette with Ø 0.5 mm needle attached to the tip and stopcock was opened.

4.6.3 Lactide Rate Constant Assay in Dichloromethane

Lactide rate constant was assayed first based on equation (4). Lactide and initiator were dissolved to 1,5 ml DCM and transferred to a 25 ml 3-neck bottle with 1 ml pipette. Lactide bottle was then rinsed with 0.5 ml DCM, which was then transferred with 1 ml pipette to the 3-neck bottle as well. Nitrogen flow was adjusted with bubble bottle, where remaining solvent was poured, and flow was kept constant while stirring the mixture for a few minutes before starting the reaction. 4,5 µl of DBU was added to start the reaction. Reaction was sampled in three portions, at 15, 28 and 45 min. Each sample was taken with a syringe and needle, added to approximately three times more 2-propanol, centrifuged in freeze (since in room temperature this polymer was found to behave like having same or slightly lower density than 2-propanol) and finally dried product was weighed in pre-weighed watch glasses.

4.6.4 Automatic Feed Setup

In automatic feed setup dropping funnel and extension was replaced with closed septum and syringe pump with needle, placed vertically above the reaction vessel and supported with two clamps. Syringe pump was employed with 10 ml glass syringe and Ø 0.4 mm syringe needle was used for GA in THF solution feed to the reaction mixture. Additional solution was needed due to the pump, since stepping motor of the pump is strong

enough to brake the syringe if it reaches the end of the stroke and on the other hand, due to the flexibility of the belt drive and mechanical clearances of the pump assembly.

GA in THF solution was mixed in the 20 ml glass bottle and sucked into the glass syringe with Ø 1.2 mm syringe needle. When the solution was in the syringe, Ø 1.2 mm syringe needle was replaced with Ø 0.4 mm syringe needle. This procedure was needed in order to avoid air entering to the syringe since the syringe was not gas proof. Syringe was then placed to the syringe holder in the syringe pump and syringe piston was attached to the drive mechanism.

Pump protocol was first started and in the first experiment DBU was added within a second. In the second experiment DBU was added after 5 s since the GA rate constant was found to be significantly lower than expected.

4.6.5 Lactide Rate Constant Assay in Chloroform

Because of the short chains, thus probably soluble even in 2-propanol, were expected and degree of conversion was under scope as well, none of the sample content should not be lost and thus all the samples were simply dried under fume hood and then examined with NMR.

Solution for the synthesis was

- 363.85 mg (2.52 mmol) LA and
- 85.65 mg (0.0418 mmol)PEG in 2.8 ml of DCM
- 5 mg of benzoic acid in 0.1 ml, 6 solutions

Catalyst amount was 36.3 µl, (0.243 mmol).

0.35 – 0.4 ml samples were taken and terminated at 450, 900, 1350, 1800, 2700 and 3600 s. Samples were unsuccessfully precipitated in 1.5 ml of 2-propanol each, then left to evaporate on petri dishes. After samples dried, all samples from petri dishes were carefully peeled with surgical knife blade to mini bottles, dissolved to $CDCl_3$ and transferred to NMR tubes with Pasteur pipettes.

4.6.6 Solubility Tests

In order to remove all the monomers from the product, reactants' solubilities in various solvents were tested. 5-6 mg of each reactant was accurately weighed to 3x9 mini bottles and then 100 µl of each solvent pipetted in. If the reactant dissolved in the solvent within 15 minutes, it was classified very soluble, within 2 hours soluble, during the weekend slightly soluble and otherwise insoluble.

THF and DCM was found normally chosen as solvent pair, rather than THF and $CHCl_3$, but since NMR samples were dissolved to $CHCl_3$, and it seemed to be better solvent to

the products, comparing solubility tests were carried out. Thus each of the manual feed samples left weighed in 15-20 mg to mini bottles in two rows, date order and DCM was added to other row, $CHCl_3$ to another. Solvents were added to the samples parallel, 0.2 ml per round. Sticky samples dissolved already at first round but even those cases faster to $CHCl_3$. None of the samples dissolved to smaller amount of DCM than $CHCl_3$, but both the average 39 and 42 monomer long samples dissolved to $CHCl_3$ one round or two rounds before than to DCM. Because DCM is more volatile (noticed and also calculated based on data presented in Table 6), very small diameter bottles were used and amount of solvent was checked before final conclusions.

4.6.7 Synthesis of PEG-PLGA-(Fmoc-Lys(Fmoc)-OH)

First synthesis was carried out in pre-determinated way: product was tried to dissolve in 5 ml DCM and transfer to 25 ml 3-neck bottle, 1.5 eq. of Fmoc-LL was dissolved to 1 ml DMF and added to polymer solution, 1.75 eq. Et_3N was added to solution and 1.75 eq. of $SOCl_2$ was dissolved to 1.5 ml $CHCl_3$ and added to polymer solution in ice water bath during 30 min via 25 ml dropping funnel and let the reaction continue for 24 h in rt under stirring. $CHCl_3$ was added until product dissolved completely.

Second synthesis was carried out according to 3.2.1. Protected amino acid (Fmoc-LL) was halogenated in a 25 ml 3-neck bottle with closed septum at 0 ºC. Fmoc-LL was dissolved to 1 ml DMF and 1 ml $CHCl_3$ and reaction vessel was placed in ice water bath over the magnetic stirrer before $SOCl_2$ was added. After 30 min ice water bath was removed and after next 30 min mixture was transferred via syringe and needle into a 50 ml 3-neck bottle with closed septum, containing the PEG-PLGA solution.

Polymer was dissolved in $CHCl_3$, DMF and DCM in 3 ml phases. After each 3-8 min vortexing and sonication, dissolved fraction was transferred to 50 ml 3-neck. Before sucking the halogenated amino acid out, nitrogen outlet was closed and inlet moved to 50 ml bottle, then 50 ml bottle nitrogen outlet was opened.

Thionyl chloride was used in excess ratio, i.e. 1.1 eq. was used with respect to L-lysine, Et_3N 1 eq. with respect to thionyl chloride and polymer 0.9 eq. with respect to L-lysine. Reaction vessel was opened 24 h later and sample was collected via centrifugation. Polymer attached to protected lysine was precipitated twice in 2-propanol and centrifuged, in order to remove the salt formed in the reaction. Product was then dried in vacuum.

4.6.8 Deprotection of the PEG-PLGA-(Fmoc-Lys(Fmoc)-OH)

First method used, with DBU, was: 51.41 mg of protected polymer was dissolved to 4 ml $CHCl_3$ and 4 ml DMF. Based on densities found in Table 6, average density of solvent mixture is about 1.2 g/ml while DBU's density is 1.018 g/ml. 1/100 DBU is thus 96 mg, or 0.63 mmol, when amount of the polymer (based on yield) was

51.41 mg/(9180 + 590) g/mol, which equals to 5.26 μmol. DBU was decided to add 4 times the assumed amount (moles) of the polymer, that is 21 μmol, or 3.21 μl. Reaction mixture was stirred 24 h rt, precipitated in 2-propanol, collected from filter paper and dried in vacuum.

Second method used, with piperidine, was: Protected polymer was dissolved in similar manner to $CHCl_3$, DMF and DCM than in acylation stage, total 24 ml, in 50 ml 3-neck bottle. Reaction mixture was placed over magnetic stirrer and 350 rpm stirring was started. Total 5 ml (5 times 1 ml) of piperidine was added (20 %, based on 3.2.2, would be 8 ml). After 2 h in *rt* product was collected via centrifugation.

4.6.9 Standard Research Procedures

Standard research procedures were as follows and any exceptions are described when occurred.

Weighing procedure for simultaneous qualitative and quantitative analysis was carried out by setting the sample into a pre-weighed glass dish when yield was used as first approximation of degree of polymerization. When degree of conversion was determined, reaction mixture was poured into a petri dish or watch glass to evaporate solvents. Starting materials were weighed in the weight-in boats and transferred quantitatively forward. Starting materials were taken into room temperature and let to warm up before opening packages. After weighing starting material packages were treated with the nitrogen and closed, before stored back to freezer or refrigerator. Multiple times opened starting material packages were dried in vacuum overnight twice during this study.

All synthesis steps were carried out in the heat gun or oven dried reaction vessels and all the glassware used to handle anhydrous chemicals were dried by similar manner before use. Catalyst was dosed to reaction either directly from manufacture's screw cap bottle by mechanical pipette or with new needle through septum in both, catalyst bottle and reaction vessel.

Lactide and initiator were dissolved to major part of solvent 1, DCM or $CHCl_3$, in one suitable size glass bottle. Rest of the solvent was used to rinse the bottle in order to move all the monomer and initiator to reaction vessel as completely as possible. Glycolide was dissolved to solvent 2, THF or mixture of solvents, then transferred to glassware used in the GA feed (burette, dropping funnel or glass syringe). Samples from active reactions were taken through septum with new needle and syringe.

Reactions were terminated approximately 10 % PhCOOH relative to mass of PEG, dissolved to small amount of $CHCl_3$. Solution was transferred to active reaction mixture via Pasteur pipette.

Nitrogen flow was adjusted with bubble bottle to approximately 5-10 bubbles per second, through Ø 0.8 mm syringe needle. Bubble bottle was a 7 ml glass bottle and solvent was either THF, DCM or $CHCl_3$.

Products were collected either via centrifugation or filtration. In both processes reaction mixture was transferred to three to four times larger volume 2-propanol via Pasteur pipette. In centrifugation procedure solution was centrifuged 20 min 5000 rpm in -15– 20 °C, liquid phase was poured out from the tube and then dissolving the product to $CHCl_3$ and pouring the product to a petri dish or a watch glass to evaporate $CHCl_3$. In the filtration procedure the solution was poured to filter paper in funnel and the product was collected from filter paper with spatula to a petri dish or watch glass. After solvent evaporation products were dried under vacuum at least 24 h. Vacuum dryings were carried out in the glass bottles with two holes in the cap, bottles covered by highly permeable fabric.

Products were washed with either 2-propanol or MeOH, depending on product's solubility. 2-propanol was used to remove LA monomers, MeOH was used to remove GA and LA monomers. Since some of the products were soluble to MeOH, 2-propanol was mandatory even though it wasn't effectively removing GA or PhCOOH residues.

5. RESULTS AND ANALYSIS

5.1 Test Polymerizations, Manual Feed

5.1.1 Polymerization of Polylactide, Rate Constant Assay

Target polymer chemical formula with 2 kDa PEG was set to PEG-PL_{56}A. Equation (4) employed to Table 3 properties for calculating PEG amount needed for 100 mg of lactide gives

$$n(LA) = \frac{m(LA)}{M(LA)} = \frac{100mg}{144.13\frac{g}{mol}} = 693.82\ \mu mol$$

$$m(PEG) = n(PEG) \cdot M(PEG) = \frac{n(LA)}{56} M(PEG)$$

$$= \frac{693.82\ \mu mol}{56} \cdot \frac{2050g}{mol} = 25.399\ mg$$

Inputting weighed lactide 116.69 mg and PEG 28.13 mg to equation (4) gives polymer chemical formula PEG-PL_{59}A. PEG-PLA was polymerized according to 4.6.3. Sample volumes and yields are shown in Table 10 with respective reaction times.

Table 10. *PEG-PLA synthesis for determining lactide polymerization rate constant.* *) *all, app. 0.15 ml could not be collected.*

Reaction time [min.]	Sample volume [ml]	Yield [mg]
15	0.56	24.99
28	0.35	28.03
45	0.35*)	37.47

Total volume of the samples is thus 1.41 ml and comparison with the original 2.0 ml started with reveals that 0.59 ml of DCM evaporated during the reaction. Thus some rough approximation of the evaporation rate during the process has to be made. Approximation method is presented in detail in Appendix D, resulting correlation constants $k^{i'}$ for all the concentrations in corresponding time interval t_i as well as volumes V_i prior to each sampling are shown in Table 11.

Table 11. *Correction constants for concentrations and initial volumes prior to each sample for rate constant calculations.*

t_i	$k^{i'}$	V_i [ml]
t_1	1.08621	1.68252
t_2	1.39342	0.94433
t_3	1.99210	0.49999

Equation (4) employed for the initial amounts gives

n(OH) = 13.727 µmol
n(DBU) = 30.09 µmol
n(LA) = 0.80962 mmol

Employing equation (24) to the amounts of a substances (moles) gives initial concentrations of each substance

$$[OH] = \frac{n(OH)}{V_0} = \frac{13.727\ \mu mol}{2.00\ ml} = 6.8635\ mM$$

$$c[DBU] = \frac{n(DBU)}{V_0} = \frac{30.09\ \mu mol}{2.00\ ml} = 15.045\ mM$$

$$[LA]_0 = \frac{n(LA)}{V_0} = \frac{0.80962\ \mathrm{m}mol}{2.00\ ml} = 404.81\ mM$$

At 15 min, 0.56 ml sample taken, left 1.12252 ml out of 1.68252 ml in reaction mixture. Thus sample was 33.283 % of the original volume, 0.80962 mmol, and 100 % conversion would give 33,283 % out of the sum of original mass of LA and PEG, 0,33283·144.82 mg which equals to 48.200 mg. Samples 2 and 3 are calculated respectively and shown with sample 1 in Table 12 together with remaining concentrations: according to yield, $[LA]_1$ should be thus (100-51.8) % of 404.81 mM, that is 195.12 mM.

Table 12. *Sample shares of total mass inserted into the reaction, yields and remaining concentrations.*

Time [min]	Sample LA+PEG [mg]	Yield [mg]	Yield-%	[LA] [mmol/l]
15	48.200	24.99	51.8	195
28	35.811	28.03	78.3	87.8
45	42.566	37.47	88.0	48.6

Combining measured concentrations and corrections due to evaporation should give equal rate constants from equation (13)

$$\frac{[LA]}{[LA]_0} = e^{-k_L[DBU][OH]t}$$

$$\frac{[LA]_1}{k^{1\prime}[LA]_0} = e^{-k_L(k^{1\prime})^2[DBU][OH]t}$$

$$\Leftrightarrow \ln\left(\frac{[LA]_1}{k^{1\prime}[LA]_0}\right) = -k_L(k^{1\prime})^2[DBU][OH]t$$

$$\Leftrightarrow -k_L = \frac{\ln\left(\frac{[LA]_1}{k^{1\prime}[LA]_0}\right)}{(k^{1\prime})^2[DBU][OH]t}$$

$$\Leftrightarrow k_{L1} = \frac{\ln\left(\frac{195\text{mM}}{1.08621 \cdot 404.81\text{mM}}\right)}{-(1.08621)^2 \cdot 15.045\,mM \cdot 6.8635\,mM \cdot 900s} = 7.42\ \frac{l^2}{mol^2 s}$$

$$\frac{[LA]_2}{k^{2\prime}[LA]_1} = e^{-k_L(k^{2\prime})^2[DBU][OH]t}$$

$$\Leftrightarrow k_{L2} = \frac{\ln\left(\frac{87.8\text{mM}}{1.39342 \cdot 195\text{mM}}\right)}{-(1.39342)^2 \cdot 15.045\,mM \cdot 6.8635\,mM \cdot 780s} = 7.22\ \frac{l^2}{mol^2 s}$$

$$\frac{[LA]_3}{k^{3\prime}[LA]_2} = e^{-k_L(k^{3\prime})^2[DBU][OH]t}$$

$$\Leftrightarrow k_{L3} = \frac{\ln\left(\frac{48.6mM}{1.99210 \cdot 87.8\text{mM}}\right)}{-(1.99210)^2 \cdot 15.045\,mM \cdot 6.8635\,mM \cdot 1020s} = 3.06\ \frac{l^2}{mol^2 s}$$

Apparently first two are in good agreement but the last one differs significantly. Main reason for this lies in the difficulty to determine accurately the amount of solution left behind, even though it is easy to draw a line to the bottle, wash it and fill it through 50 µl steps to the line drawn, as was done here. But the reaction may not continue further when monomer concentration decreases below certain value and thus the final step needs to be taken into account. Time weighted average of the rate constants values is thus 5.77 and will be used from now on.

5.1.2 Polymerization of Polyglycolide

PEG-PGA was polymerized according to 4.6.1. Reaction was started and precipitation started immediately and after first seconds no visually detectable mutations appeared.

Reaction was terminated at 30 min and clear precipitation from 2-propanol was collected with centrifuge and washed twice with 2-propanol. Product was dried overnight under fume hood and weighed next day. Yield was 16.43 mg out of original 53.41 mg, which is only 3.89 mg more than original mass of PEG, which is not soluble to 2-propanol. Thus the PGA average block length from equation (4) and Table 6 is

$$\frac{n(GA)}{n(PEG)} = \frac{\frac{m(GA)}{M(GA)}}{\frac{m(PEG)}{M(PEG)}} = \frac{\frac{3.89\text{mg}}{116.07\frac{\text{g}}{\text{mol}}}}{\frac{12.54\text{mg}}{2050\frac{\text{g}}{\text{mol}}}} = 5.48$$

which means, in practice, that solubility will be the limiting factor of glycolide share and glycolide is very determining member in product solubility since PEG was very soluble to THF/DCM combination even though it is solubility in pure THF is quite limited. Glycolide reaction rate needs to be calculated based on PEG-PLGA polymerizations and characterizations in order to optimize the process, until that reactivity found in [4] will be assumed valid and adequately accurate.

Starting materials for PEG_X-PL_YG_ZA polymer are added into the reaction in corresponding amount (in moles) ratios, starting with PEG_1-$PL_{28}G_{28}A$. Using equation (4) mole ratios are converted to mass ratios:

mass of LA should be equal to 1.97 times PEG mass
mass of GA should be equal to 1.59 times PEG mass

5.2 Manual Feed Experiments

Based on the reported feed rates in [4], the goal was set to drop 3 ml of GA in THF solution to reaction mixture according to "procedure 1" in 4.6.2:

1º	1.5 ml	during	1st 10 min
2º	0.75 ml	during	2nd 10 min
3º	0.375 ml	during	3rd 10 min

Burette was tested with pure THF. Even with almost empty burette and Ø 0.3 mm needle the flow rate exceeded the target rate approximately 100 %. This result was, once again, in good agreement with the theoretical value found in Table 4.

GA in THF was then tested according to bigger batch procedure in 4.6.2. Starting points, end points, elapsed times, calculated number of drops and average drop sizes are shown in Table 13. GA concentration was calculated from equation (4) and (21)

$$c(GA) = \frac{n(GA)}{V(THF)} = \frac{\frac{m(GA)}{M(GA)}}{V(THF)} = \frac{\frac{326\text{mg}}{116.07\text{g/mol}}}{8.15\text{ml}} = 0.345\ \text{M}$$

Table 13. *0.345 M GA in THF flow rate and drop size data with 10 ml burette and Ø 0.5 mm needle.*

Start [ml]	Stop [ml]	Elapsed time [s]	Number of drops	Ave. drop size [µl]
4.55	4.95	28.6	112	3.57
9.00	9.30	39	73	4.11
9.35	9.75	60	85	4.71

Half-empty 10 ml burette column is little higher than in full 25 ml dropping funnel, but employing the burette offered better resolution. Flow rate over doubled from half-empty to almost empty burette and drop size approached the theoretical value in Table 4. Flow rates are significantly lower than theoretical and increase in the THF viscosity due to GA cannot explain that. Drop formation and back pressure caused by solution surface tension restrict the flow and play the key role in flow rate as far as minimum column height is exceeded. In the beginning flow rate was a bit too slow, but some of the solvent may be replaced with chloroform to fix that and the principle seems plausible for bigger batch. Surface tension seems a little increased since the drop size is so close to the theoretical value already at 0.4 ml/min (while all the dynamic effects were excluded in the theoretical part).

Stop cock adjustability was so poor that burette was replaced by dropping funnel. Dropping funnel was tested as described in 4.6.2, with THF/$CHCl_3$ solvent mixture. Measured drop frequency sequences', drop frequency averages, volumes and corresponding elapsed times together with calculated average drop sizes are shown in Table 14 for Ø 0.3 mm, Ø 0.4 mm and Ø 0.5 mm needles.

Table 14. *Measured drop frequency sequences', drop frequency averages, volumes, corresponding elapsed times and average drop sizes with Ø 0.3 mm, Ø 0.4 mm and Ø 0.5 mm needles, THF/$CHCl_3$.*

Needle size [mm]	Measured frequency sequence # refers to drop, [s/30#]	Drop frequency average [#/s]
0.3	47, 44, 50	0.6383
0.4	19, 20, 21, 18,5	1.529
0.5	9, 9, 10, 9, 9	3.261

	Volume [ml]	Elapsed time [s]	Ave. drop size [µl]
0.3	1.5	595	3.95
0.4	1.5	430	2.28
0.5	2	135	4.54

Theoretical approach clearly gives good guideline to begin with in practical part, even though drop sizes for solvent mixtures were not computed, but problems with Ø 0.3 mm

needle occurred already without solute. Drop rate differed a lot, for example even change in the fume hood front glass position caused 2 to 5 seconds change for 30 drops.

Burette result for 0.345 M GA in THF from surface at 4.55 ml was 28.6 s for 0.4 ml, which is 0.84 ml/min when this test result was 0.89 ml/min. Column heights were measured 220 mm and 160 mm respectively, thus the GA in THF flow rate from 160 mm would approximately equal to 0.61 ml/min and with Ø 0.4 mm needle GA in THF flow rate would approximately start from

$$Q = \frac{1.5\text{ml}\cdot 60\text{s/min}}{430\text{s}} \cdot \frac{0.63\,\frac{\text{ml}}{\text{min}}}{0.89\,\frac{\text{ml}}{\text{min}}} = 0.15\text{ml/min}$$

Duct length below the tap will be more than adequate to empty the dropping funnel if viscosity is increased in 0.89/0.63 ratio, since originally only 30 mm duct length was required. Thus Ø 0.4 mm needle with solvent mixture was chosen for a small test batch use and solvent amounts assure glycolide content in a single drop small enough.

First attempt with this setup was promising, but cock was first opened slightly too much and some precipitation appeared immediately. Based on later stage characterizations stickiness of the product was due to the short chain length. Next attempt failed since a gas bubble almost blocked the duct below dropping funnel cock and following attempt interrupted by the clog in the needle. Remaining GA (80.95 mg) in THF/$CHCl_3$ (3 ml/4 ml) solution was used to determine drop size with Ø 0.7 mm needle. Average drop size was 3.77 µl, which is slightly surprising as being smaller than found with Ø 0.5 mm needle with pure solvent mixture, but decrease in the drop size was on the other hand expected due to increased chloroform share.

Several syntheses were tried and PEG share varied, also with initiators excluded from this thesis were tested, but only a few attempts with PEG yielded to polymer that looked like and felt like possibly usable product and even those were lost since the glass centrifuge tubes broke down already in the beginning of centrifugation. In the way some observations were made anyway:

- Increasing the amount of catalyst improves the product, making it less sticky
- Decreasing PEG share yielded less sticky product too
- Using 5k PEG gives even less yield than 2k
- 30 min reaction time yields were no more than 75 %
- “procedure 1” do not match with reported rate constant

5.2.1 First Manual Feed Results

Based on approximate manual solution of equations (18) and (22), with rate constants found in the report this study was based on [4], the goal drop rate was re-set to favour

more GA rich reaction mixture according to “procedure 2” in 4.6.2. Reactants, reagents and solvents used were, based on the yields got earlier and the target polymer formula:

- LA 125.58 mg
- PEG 2k 47.01 mg
  Both in 2,0 ml DCM + 0.5 ml rinse
- GA 102.70 mg in 3 ml THF + 2 ml DCM + 2 ml $CHCl_3$, Ø 0.7
- DBU 14.4 µl

GA drop rate was set to 0.5 Hz, DBU added and drop rate increased to 2.7 Hz. In two minutes’ rate already decreased to 2.5 Hz but since that small changes are not adjustable, the mistake to try was not made anymore. After 7 min. drop rate decreased under 2 Hz and cock was gently tapped. At 10 min. the rate was 2 Hz again and since the feed rate was slower in the beginning, nothing was done and rate slowed down itself to 0.7 Hz during the second third of the reaction time. During the last third drop rate was adjusted in the beginning to 0.5 Hz and it slowed down to 0.25 Hz within 6 min. Remaining GA was fed into the reaction during the next two minutes when GA finished, two minutes early. Reaction was terminated at 30 min and collected via filtration as described in 4.6.9. After filter paper dried, the product was easily detachable, little rubbery but felt dry and was thus transferred to mini bottle with holes in the cap and placed in the vacuum drier over-night.

After vacuum the product was weighed, revealing 203.99 mg yield when total amount of reactants was 275.29 mg, so the yield was 74 %. Mole ratios in the reaction were, based on masses and molar masses

$$\frac{47.01}{2050}:\frac{125.58}{144.13}:\frac{102.70}{166.07}=1:\frac{125.58}{144.13}\frac{2050}{47.01}:\frac{102.70}{116.07}\frac{2050}{47.01}=1:38.0:38.6$$

so 74 % yield would mean, if product is 50:50, 1:28:28. Next step was to examine the product with NMR according to 4.5.1. NMR spectrum, shown in Figure 18, revealed some benzoic acid residues. Relative peak area of the left-most benzoic acid residue peaks is 4.27, which comes from two protons in orto positions. In comparison with the 180 (PEG reference), 2.135 benzoic acid molecules are present for each polymer, which is approximately the ratio of benzoic acid used in the first place and reveals that 2-propanol did not remove it.

Small amounts of GA and LA monomers or oligomers was found from the sample, Figure 19. On the left part a broad peak above 5.16 ppm overlaps PLA and tiny signal from GA monomers and oligomers is present at 4.65 ppm. On the right part LA oligomers and monomers appear at 1.67-1.68 ppm. Polymer formula PEG_1-$PL_{28}G_{14}A$, calculated from integrals is shown in Figure 20. Measured reference spectrums for PEG, LA and GA are presented in Appendix G

.

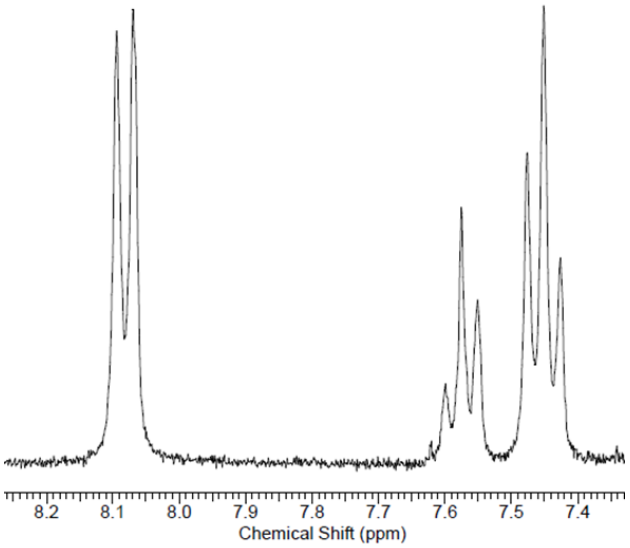
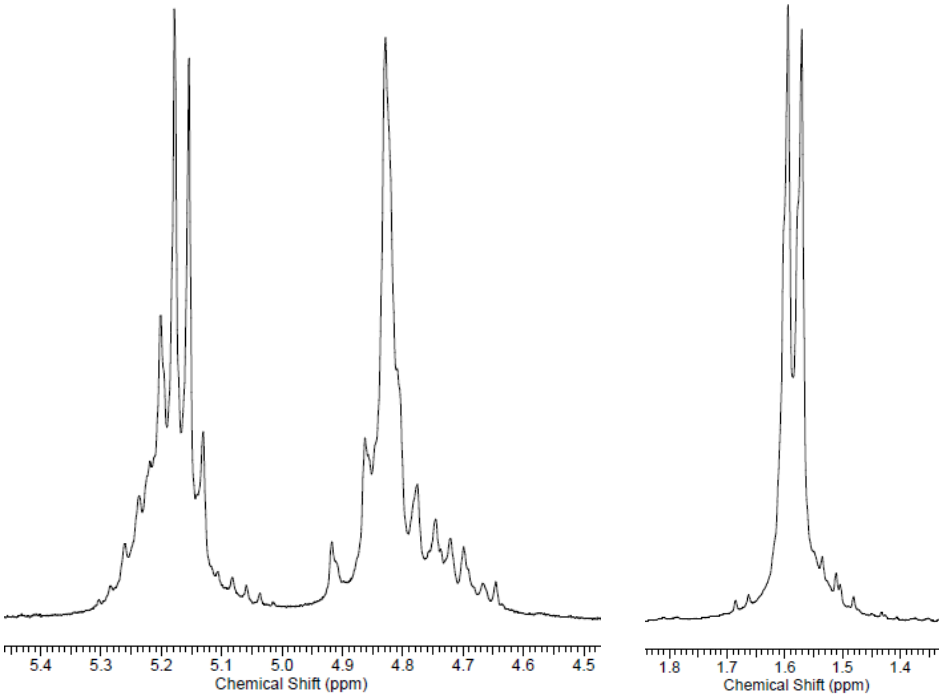


Figure 18. *First NMR results of PEG-PLGA, benzoic acid peaks*



PGA peak at 4.82 ppm and PLA peak at 5.16 ppm PLA peak at 1.57 ppm

Figure 19. *First NMR results of PEG-PLGA, PGA and PLA regions.*

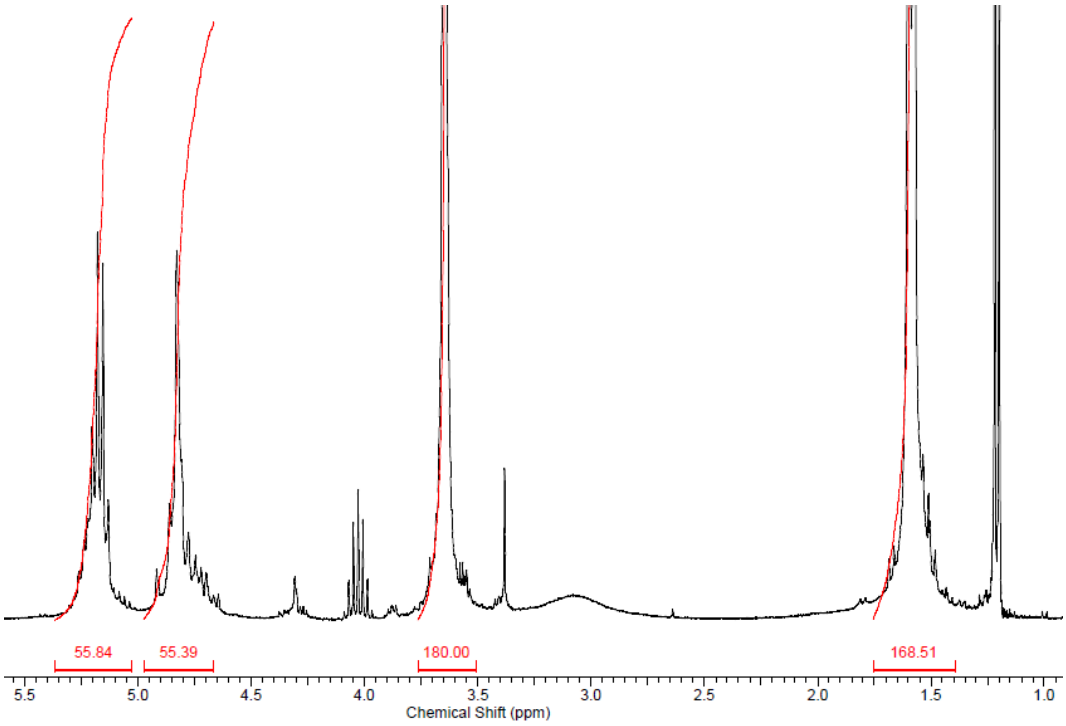


Figure 20. *First NMR results of PEG-PLGA, PEG-PLGA molecular formula integration.*

PLA peaks give both approximately same 28 units of LA, PGA peak gives 14 units of GA. Some LA monomers are found at the 5.05 ppm and methoxy end group peak at 3.37 ppm. Multiplets of multiplets above 4 ppm, broad peak above 3 ppm, sharp peak at 2.8 ppm and doublet at 1.21 ppm belong probably to 2-propanol, revealing that drying time was not adequate.

Due to the residues solubilities of the starting materials were tested in the various solvents according to procedure described in 4.6.6. Results are shown in Table 15 with earlier tested solubilities in CDM and $CHCl_3$ included.

Table 15. *Solubility results of reactants to different solvents. Hex refers to hexane, Ace to acetone.*

	THF	iPrOH	EtOH	Hex	Ace	H_2O	Et_2O	EtAc	MeOH	DCM	$CHCl_3$
PEG	s	i	vs	i	vs	vs	i	vs	vs	vs	vs
LA	vs	vs	vs	i	vs	i	i	s	s	vs	vs
GA	vs	i	ss	i	vs	s	i	vs	s	i	i

vs= very soluble, s=soluble, ss= slightly soluble, i= insoluble

Acetone seemed very tempting for washing, but all the products got this far were soluble in acetone, as well as ethyl acetate and mostly to methanol too. Benzoic acid solubility in methanol was also found to be better than to 2-propanol. PEG was only

slightly soluble when it was tested to 50 µl THF and 5k PEG was practically insoluble in 50 µl THF.

Reproducibility was such poor when dropping funnel stopcock was tried to use to restrict the GA solution feed, that bigger batch was the only possible way to improve that as long as this study was limited to manual feed. Bigger batch made also more concentrated solutions applicable, which automatically improves reaction rate. Catalyst amount was chosen five times more than initiator amount in order to improve the probability of active chain end to attack monomer. The amount of PEG was chosen 1:35 of lactide, and glycolide, amount (mole) in order to get longer chains with the yields seen this far. LA mass target was set 504 mg (3.50 mmol), thus GA target was 406 mg (3.50 mmol) and PEG 205 mg (0.100 mmol).

With more concentrated GA solution, risk to needle getting clogged increases so it was decided to abandon the needle and use only the 1 ml pipette tip at the dropping funnel outlet. 0.43 M GA in THF solution was used in the drop size test and drop size average was found to be 15.2 µl. Plan was, based on "procedure 3" in 4.6.2:

1º	0.667 Hz	from 0 to 5 min
2º	0.4 Hz	from 5 to 10 min
2º	0.4 Hz	from 10 to 20 min
3º	0.13 Hz	from 20 to 30 min

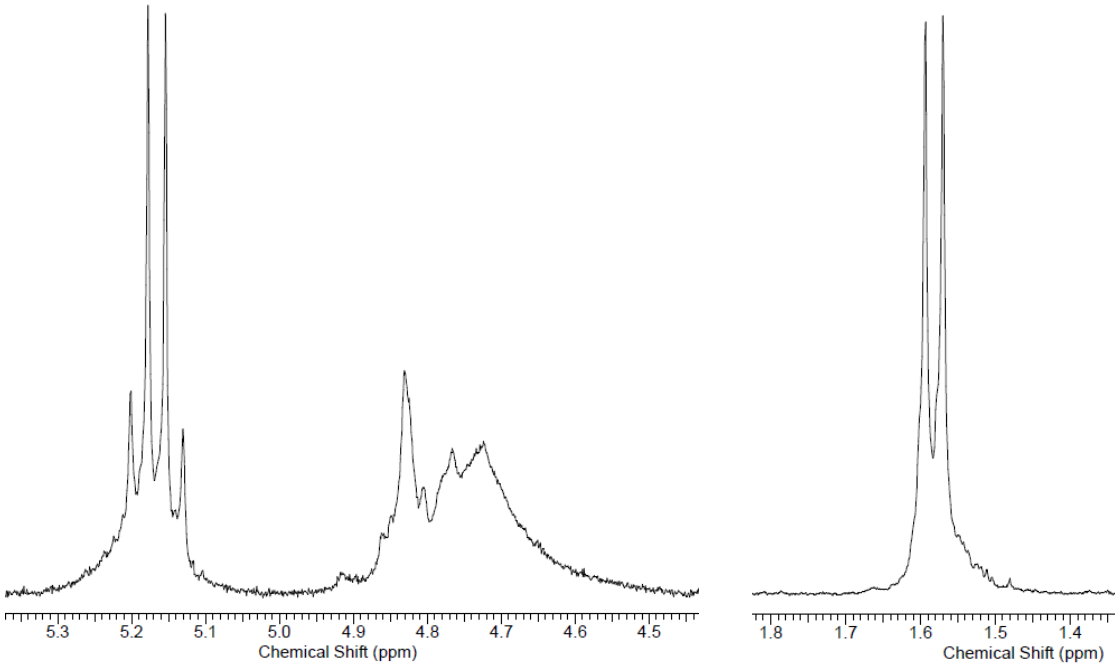
Solutions for the synthesis were

- 508.61 mg (3.53 mmol) LA and 207.28 mg (0.101 mmol) PEG in 6 ml of DCM
- 406.42 mg (3.50 mmol) GA in 8 ml THF

Catalyst amount was set to 74.7µl, or 0.500 mmol.

GA dropping started, after 3 drops DBU was added and GA drop rate adjusted to target. But once again rate slowed down and could not be kept steady. Two second per drop was thus kept from 8 to 12 min. At 30 minutes from start reaction was terminated. 0.5 ml, or 1/16, GA in THF was left, precipitated twice in 2-propanol and filtered both times. Sample was on watch glass in fume hood 20 hours and after dried in vacuum. Yield was 927.56 mg out of 1096.9 mg put into reaction (1/16 of GA subtracted), which is 84.6 %. Small amount was washed with methanol: all went through the filter paper.

NMR results revealed neat PLA peaks with practically no monomers present, while PGA peak was rather small and only a fraction of GA monomer peak, as shown in Figure 21.



PGA peak at 4.82 ppm and PLA peak at 5.16 ppm PLA peak at 1.57 ppm

Figure 21. *NMR results of PEG-PLGA polymerization 1.6.2016, PGA and PLA regions.*

On the left part a broad peak above 5.16 ppm overlaps PLA and strong signal from GA monomers and oligomers overlap the PGA peak. On the right part neat PLA peak is shown, no oligomers or monomers appear. As expected, benzoic acid was there. Polymer peaks integrated in Figure 22 reveals polymer formula PEG_1-$PL_{31}G_8A$. Peaks at 5 ppm range reveals approximately 2:1 GA:PGA ratio. A broad peak between PEG and lactide methyl peak appears this time below 2.5 ppm, with only weak doublet from 2-propanol's methyl groups at 1.21 ppm. Hydroxyl group may appear in various shifts depending on chemical environment, and is often broad, thus the 2.45 ppm peak is assumed to become from 2-propanol. PEG methoxy end group area is about 3 as expected.

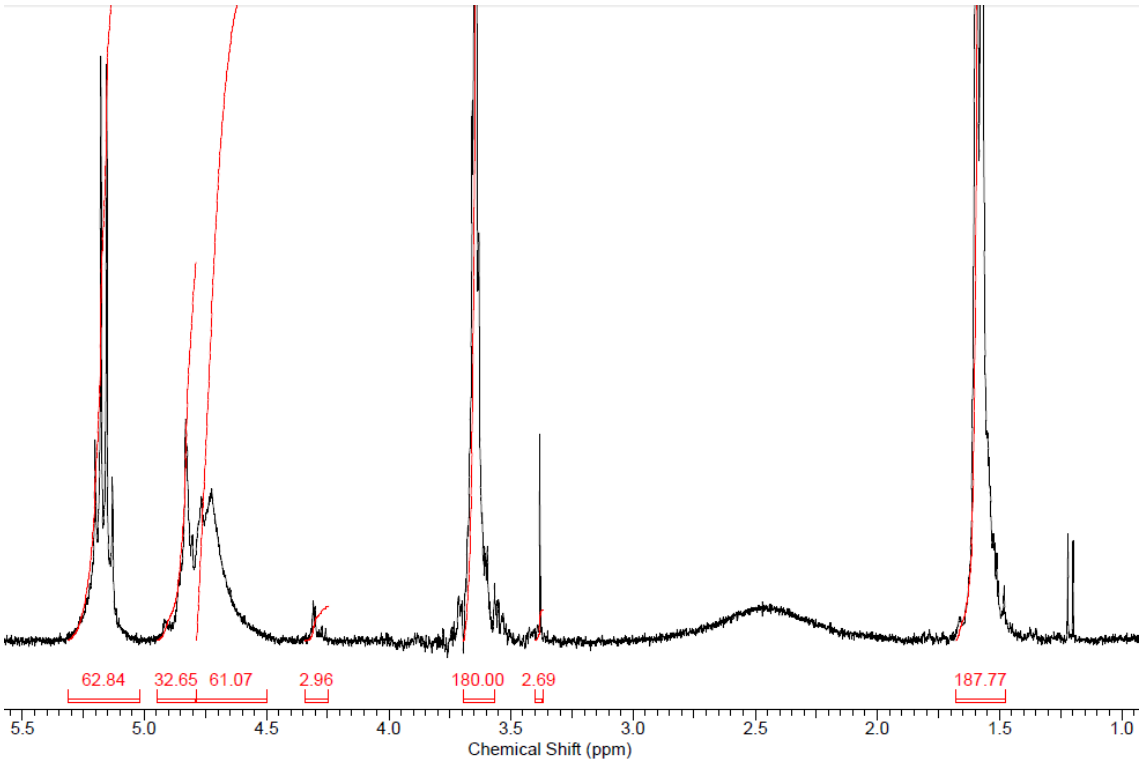


Figure 22. *NMR results of PEG-PLGA polymerization 1.6.2016, PEG-PLGA molecular formula integration.*

Regardless of glycolide feed rate, chains do not seem to grow more glycolide rich than 2:1 LA:GA or chains precipitate and product is very short chain polymer. Glycolide feed rate cannot be repeated exactly same within 10 % accuracy, probably not even within 20 %. Only machine-operated feed might make 50:50 PLGA solution ROP polymerization possible unless target chain length is very short.

5.3 Automatic Feed Experiments

The first step was to verify the model with the chemical amounts same as in manual feed implementations and feed as accurately as possible. Solubility was intentionally left out from the model since time was almost up, so only conversion kinetics was modelled and conversions compared.

5.3.1 Model Tests with Manual Feed Results

Model verification started with LA rate constant test, with 15 min and 28 min points simulated, since the last one was too arbitrary due to unknown share remaining in the reaction vessel at the end. Rate constant value 7.42 applied to the first stage and 7.22 to the second gave exactly the same remaining percentages. Thus average, 7.32, was used in PLGA part verification. Apparently math has been modelled correctly.

In the manual feed implementation there was two syntheses of 500 mg LA, the last one described in the section 5.2 and one after that, which precipitated early and was terminated at 20 min. In both the drop rate was, at each stage, at least successfully kept over the starting level of the following stage. For that reason, those were the best suited for PLGA model verification.

In the first case average polymer formula was PEG_1-$PL_{31}G_8A$, while the 100 % polymerization would mean, out of 508.61 mg LA, 406.42 mg GA and 207.28 mg PEG, PEG_1-$PL_{35}G_{32}A$ (glycolide leftovers retracted, thus 381.02 mg GA taken into account). Lactide monomers were mainly lost in precipitations but glycolide should be present and that is not the case since GA and PGA peaks give only 24 units out of 32. Yield was 84.6 % meaning that 168.92 mg was lost from the total. If 4/35 of LA was lost (88.6 % conversion), that equals 58.13 mg of LA, leaving 110.79 mg to GA. 110.79/381.02 equals to 9.3 units, when the difference was 8 units. Thus the NMR conversion results should match the simulation model output within 1.3/9.3, or 14.0 %. First simulation, model shown in Figure 10 with GA rate constant 3 order higher than LA, results are shown in Figure 23.

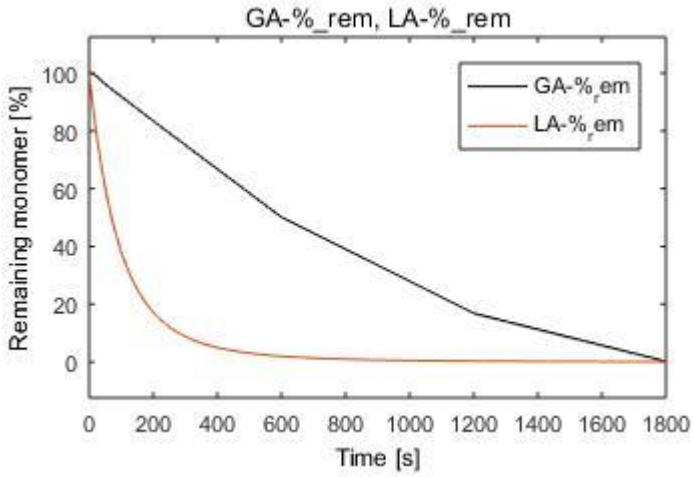


Figure 23. *Simulated polymerization of the product shown in Figure 22.*

Based on the GA conversion output, GA reacts right away after being fed in, so no monomers should be present. On the other hand, at 600 s only 2 % of lactide would be left, which probably is not the case, even though what was observed about solubilities and precipitation. So it might be possible that after PLA block has grown long enough, polymer might stay soluble and allow PGA block to grow next.

Problem with the lactide rate constant determination was that only yield was studied, not filtrate and without NMR, so if there was some unreacted PEG, that might be in the centrifuged product too, as well as monomers. Because LA was found to be very soluble in THF, solvent composition change may not change radically LA reaction rate. Thus LA rate constant was halved and so was done to the GA too. LA conversion curve

became realistic but no affect to GA, as expected. Because GA is not soluble in DCM, it's rate constant may vary a lot with the solvent composition and thus weighted average rate constant was taken to service. Modified GA subsystem and varying rate constant subsystem are shown in Figure 24.

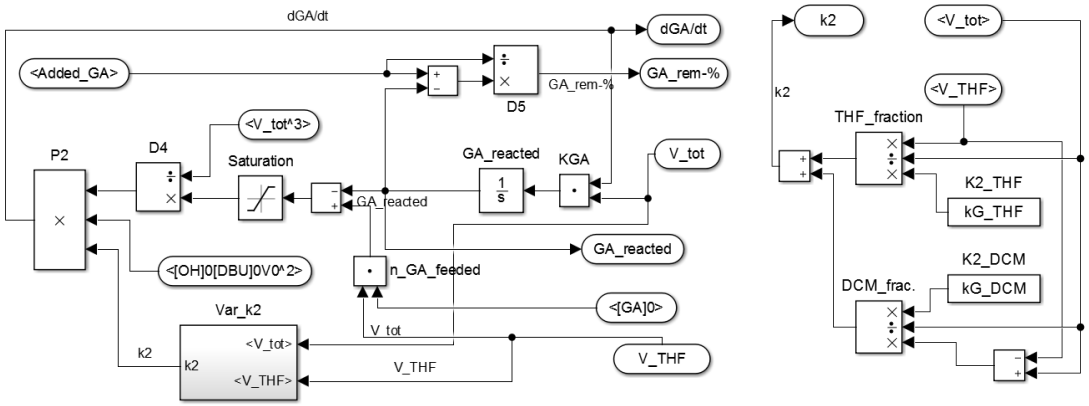


Figure 24. *Modified GA subsystem on the left with among solvent composition varying glycolide rate constant subsystem Var_k2 on the right.*

Simulation carried out with 3.66 as LA rate constant value, 3660 as GA rate constant value in THF and 0 in DCM is shown in Figure 25. Perhaps GA rate constant is not as high as reported or the monomer peak is mainly oligomers. Even when tested with an order lower rate constant, conversion was still almost 98 %.

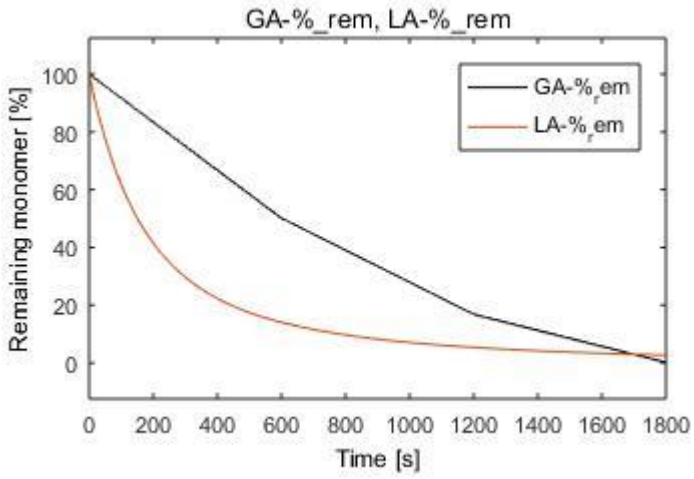


Figure 25. *First simulation results of polymerization of the product shown in Figure 22 with solvent composition varying GA rate constant.*

Model was thus tested next with the last manual feed trial product (otherwise excluded from this thesis), NMR shown in Figure 26, which was terminated at 20 min due to the excess precipitation and collected from filter paper.

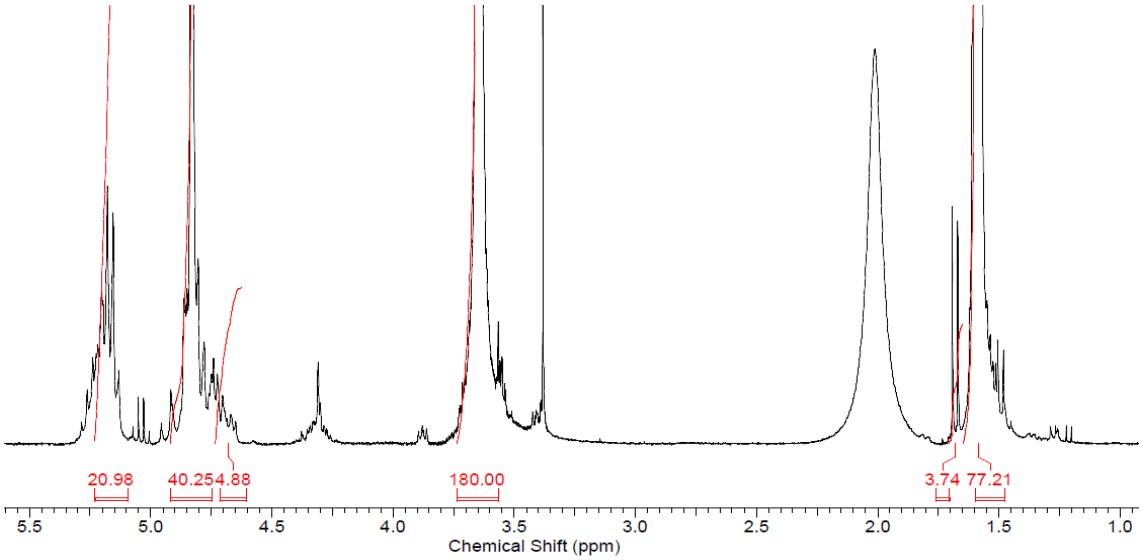


Figure 26. *NMR of early precipitated PEG-PLGA with GA in pure THF*

As seen from the integrals, average polymer formula is PEG_1-$PL_{11}G_{10}A$ and 11% of the glycolide did not polymerize. Originally 495.49 mg of LA, 248.36 mg of PEG and 348.12 mg of GA was used, all together 1091.97 mg and yield was 667 mg, or 61.1 %. Theoretically average polymer formula from starting materials, with 100 % yield, is PEG_1-$PL_{28}G_{25}A$. Carrying out calculations as with the previous sample, 425 mg was lost: 283 mg of LA (so conversion was 43 %), and product should contain 13 units of GA, so accuracy should lie within 2/13, or 15.4 %.

This time average drop size was found to be 10.24 µl and 14 drop rates were recorded. DCM was used 6 ml and THF 8 ml. Catalyst amount was 45 µl. In the first sample case simulation gave the same final LA conversion (88.6 %) with rate constant value 7.32/3.33, so that was employed in first simulation, with glycolide in THF 7320/3.33. Model predicted to latter one LA conversion of 67 % instead of 43 %. However, when polymerization rate of GA is above LA, polymer will become insoluble soon after, due to growing PGA block length and this phenomenon is excluded from the model.

Examination of conversion of mass, $\Delta n/\Delta t$, with GA rate constant in THF further decreased to 7320/20 (which means that GA rate constant would be only 170 times LA's rate constant), Figure 27 and Figure 28, reveals that according to this model 2:1 GA:LA growth occurred first time around 600 s both, which is in good agreement with observed mild precipitation around 9-10 min from start. Furthermore, when polymer head is already GA-rich when remaining GA go below LA, precipitation should be expected. Percentages were in quite good agreement with the NMR results at those points.

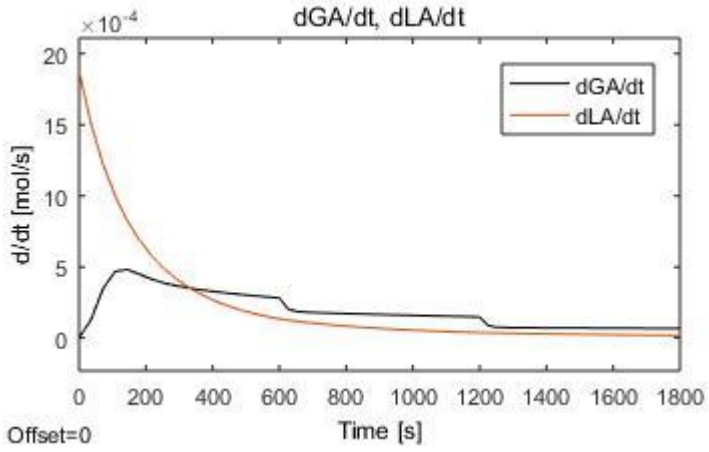


Figure 27. *Simulated conversions of PLA rich sample with GA solvent composition dependent rate constant and LA rate constant 7.32/3.33.*

Problem is that the model does not contain any part cross-linking reaction rate of one monomer species into reactivity and concentration of another monomer species, since this model has been created to simulate the GA feed rate needed for exactly 50:50, which means that interaction would be symmetric and constant throughout the polymerization, simply compensable in rate constant values. Apparently GA feed rate in the beginning, or [4], was not even close to what was needed to gain 50:50, making further study on these samples needless.

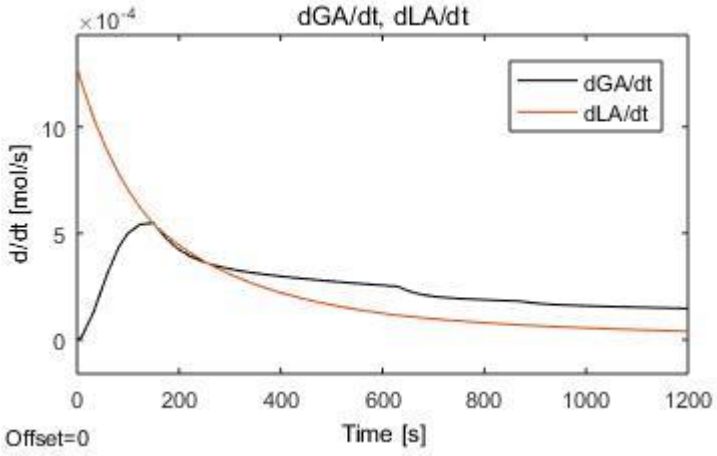


Figure 28. *Simulated conversions of almost 50:50 sample with GA solvent composition dependent rate constant and LA rate constant 7.32/3.33.*

5.3.2 Syntheses with Automatic Feed

Based on the solubility tests carried out according to 4.6.6 LA rate constant value in $CHCl_3$ was determined as described in 4.6.5. Solution for the synthesis was

- 363.85 mg (2.52 mmol) LA and
  85.65 mg (0.0418 mmol) PEG in 2.8 ml of DCM
- 5 mg of benzoic acid in 0.1 ml, 6 solutions

Catalyst amount was 36.3 µl, or 0.243 mmol.

NMR results are shown in Appendix E. Respective percentages of PLA of all LA, calculated from NMR peak areas, are shown in Table 16.

Table 16. *Reacted lactide share of the samples with respective reaction times*

Time [s]	450	900	1350	1800	2700	3600
Reacted [%]	53.0	71.9	73.9	74.0	85.5	92.8

In the NMR results broad peak appears in various positions, overlapping the 5 ppm range and an unknown multiplet overlaps 1.6 ppm range. For those reasons integrals are taken from different positions and half peaks. Average polymer formulas were calculated from equation (4) and as shown in section 5.1.2. *[DBU]* was 0.0867 M and *[PEG]* was 0.0149 M. Equation (13) graphical solution gives rate constant when natural logarithm is taken of $[LA]/[LA]_0$, divided by *[DBU]* and *[PEG]*, plotted against time and fitted to first order polynomial, is shown in Figure 29. Slope 0.5844 $l^2mol^{-2}s^{-1}$ presents the pseudo-first order rate constant. 900 s sample was rejected.

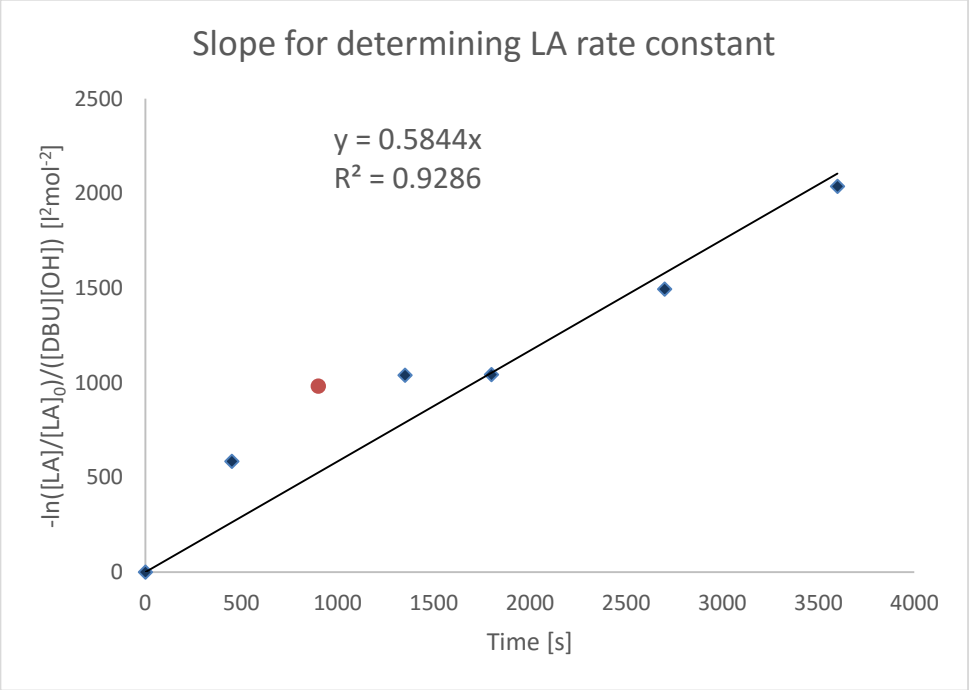


Figure 29. *LA rate constant determined by fitting the first order polynomial to the NMR results. Slope represents the rate constant.*

First simulations for GA feed rate demand vector were made for 200 mg of LA and 100 mg of PEG in 2.4 ml $CHCl_3$ and 160 mg of GA in 1.6 ml THF. DBU was used 32 µl in the model, shown in section 3.4, Figure 16, with solvent composition rate constant dependency presented earlier in the manual feed analysis of simulation model. Flow rate demand curve and sampled flow demand vector values are shown in Figure 30. First zero is cropped from pump flow demand vector.

Flow demand vector was then fed back from Matlab Workspace to the model and polymerization rates simulated, results are shown in Figure 31. All the outputs were checked and model was stable in every way, predicting 86 % conversion for LA and 88 % for GA.

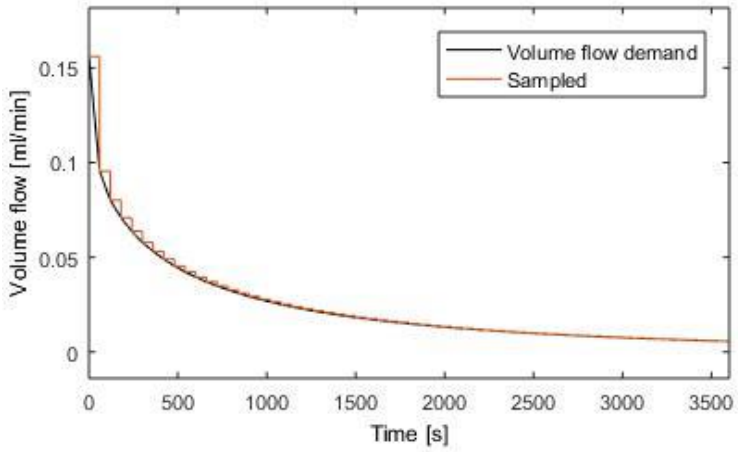


Figure 30. *Simulated flow demand vector for syringe pump needed to polymerize 50:50 PLGA in chloroform.*

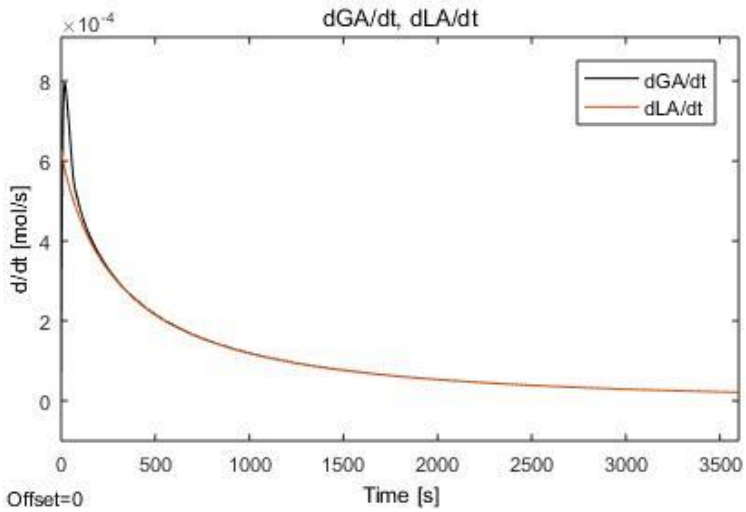


Figure 31. *Simulated polymerization rate in mol/s for GA and LA with syringe pump flow demand vector.*

Thus the first automatic feed run was designed and ready for action.

Solutions for the synthesis were

- 199.53 mg (1.38 mmol) of LA and
  100.24 mg (0.0489 mmol) of PEG in 2.4 ml $CHCl_3$
- 201.01 mg (1.73 mmol) of GA in 2.0 ml THF

Catalyst amount was 32 µl, or 0.214 mmol.

Polymerization was carried out as described in 4.6.4 and 4.6.9. Again, some, although very slight, discolouration appeared at around 9-10 min from start, otherwise reaction remained almost clear. At 50 min time it was clear that pump had stopped (noise from surroundings exceeds pump's own noise at low speeds), thus reaction was terminated, precipitated and filtered twice.

At termination, reaction mixture was rather viscous but turned back to fluid with 0.5 ml $CHCl_3$. Based on recorded volume fed, 1.26 ml GA in THF, or 1.09 mmol GA, was put in to the reaction at 40 min. Based on simulation, PLA formation should have continued to around 1.23 mmol of LA, meaning that expected average polymer formula would be, PEG_1-$PL_{25}G_{22}A$ – still almost 50:50. NMR results proved otherwise, integrals relative to PEG 180 value are shown in Figure 32.

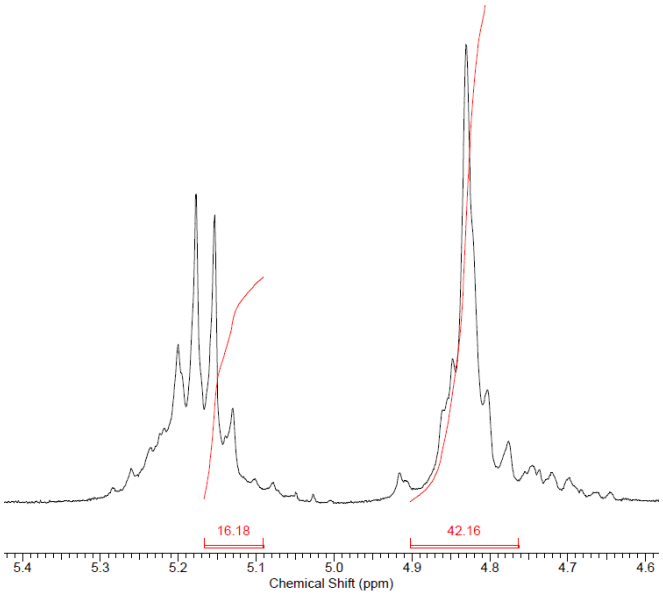


Figure 32. *NMR spectrum from PGA-PLA region, with peak areas integrated relative to 180 of PEG peak area.*

Average polymer formula was PEG_1-$PL_{16}G_{10}A$, meaning that glycolide's rate constant is not even nearly three order higher than lactide's in $CHCl_3$/THF. It also seems that lactide rate constant is affected by THF. Since labtime left was very limited, instead of cross-linking subsystem design, rate constants were simply multiplied with the polymer ratios, LA's by 16/26 and GA's by 10/26, in order to find better approximation for rate constant and thus flow demand curve for the final attempt.

Since model does not take precipitation into account, the final amount of reacted glycolide will not match. Final amount of lactide should be close, since discolouration remains modest throughout the process and only glycolide rich chains precipitate. Since first precipitation was observed at 9 min, that should be approximately the point where reacted amount curves cross. Finally, lactide rate constant value 0.5844 was used for both solvents, but the glycolide rate constant 584.4 value was needed to divide by 15 until precipitation criterion was fulfilled. Flow demand vector used in the synthesis was

used in the simulation too. Simulated reacted amounts and *dn/dt* for both monomers are shown in Figure 33 and Figure 34.

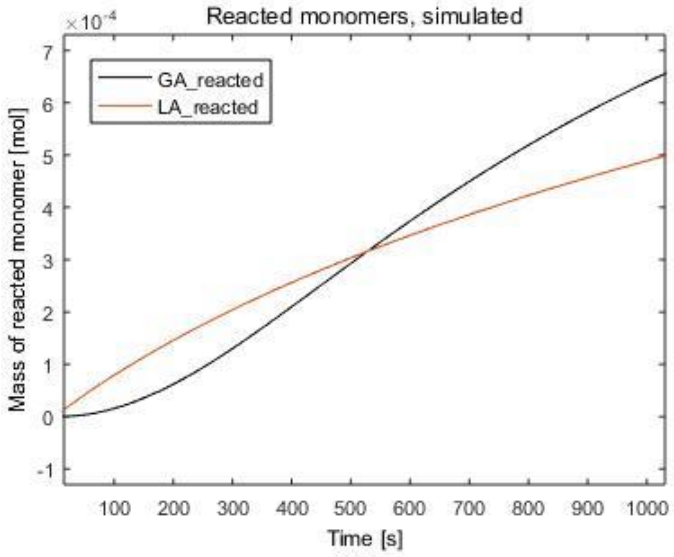


Figure 33. *Simulated amount of reacted monomers for GA and LA with LA rate constant value 0.5844 and GA rate constant value 584.4/15.*

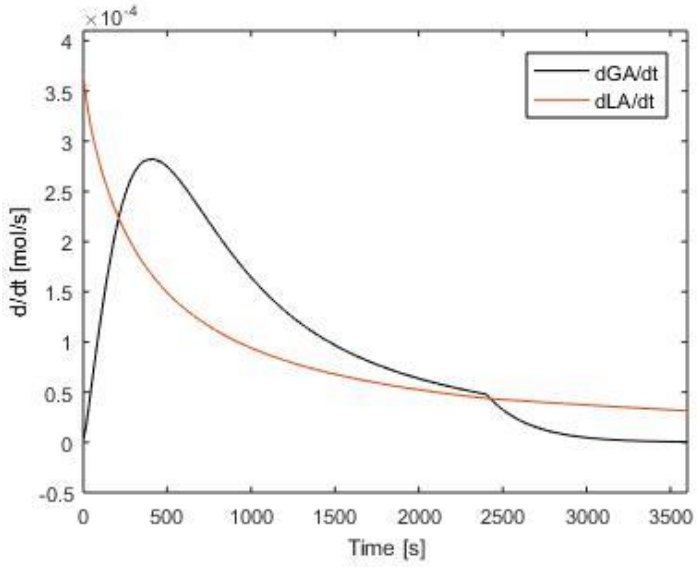


Figure 34. *Simulated conversion speeds for GA and LA with LA rate constant value 0.5844 and GA rate constant value 584.4/15.*

GA rate constant division by 16 took the first GA rich moment, assumed precipitation point, already over 10 min and 14 on the other hand too early. Anyway, accuracy of the model this simple and with so little verification measurement is hardly as good, but result suggests that in $CHCl_3$ GA rate constant is only 70 times LA rate constant.

Next step was to calculate target amounts of starting materials for 1000 mg batch. Reaction time was set to 60 min and based on lactide conversions observed, 61 % conversion was assumed possible with reasonable amount of catalyst. PEG_1-$PL_{28}G_{28}A$ still as target polymer formula, amount of monomers needed to be 46 times the amount

of PEG. Thus target masses of the starting materials, starting with 765 mg (5.31 mmol) of LA were: 616 mg of GA and 244 mg of PEG. Concentrations were kept the same as in first run, but DBU amount was chosen based on simulations. Probabilities for each monomer to react was set to 0.5, LA rate constant value to 0.5844 and GA rate constant value to 584.4/15. With 156 µl of DBU, simulated remaining monomers are shown in Figure 35 and conversion speeds in Figure 36.

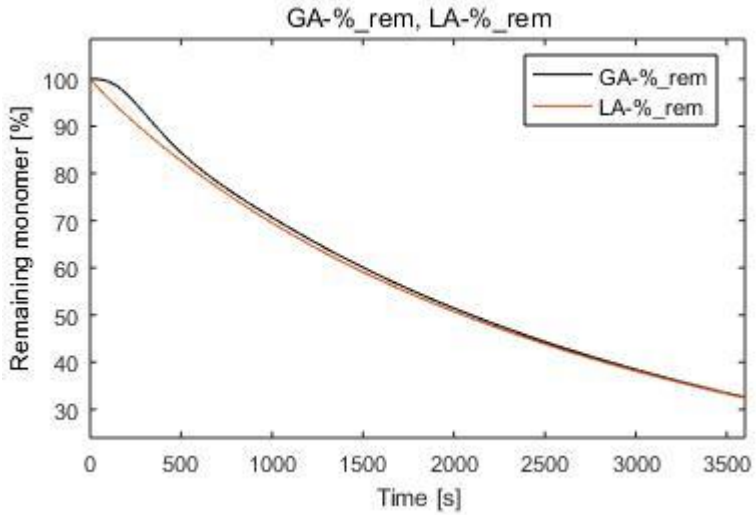


Figure 35. *Simulated amounts of remaining monomers, 1000 mg product mass target, 1 h reaction time.*

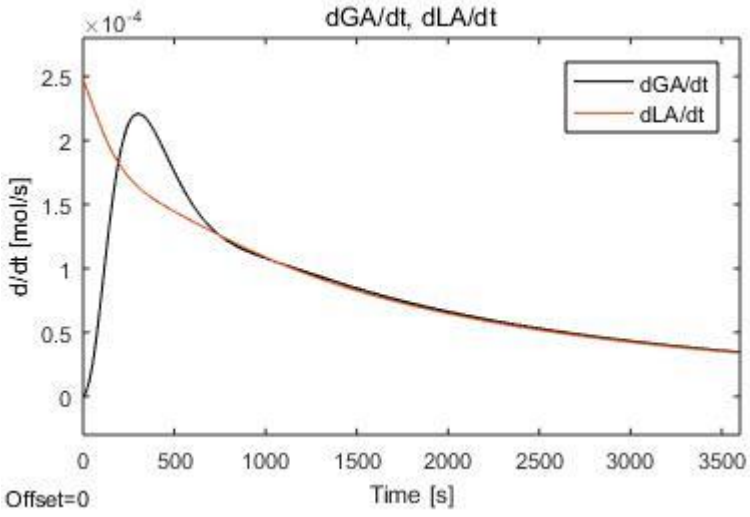


Figure 36. *Simulated conversion speeds, 1000 mg product mass target, 1 h reaction time.*

Simulated volume flow demand and sampled flow demand are shown in Figure 37.

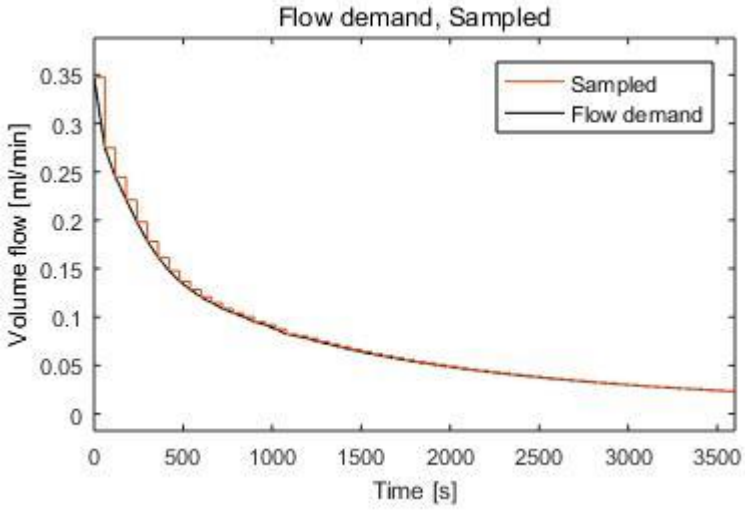


Figure 37. *Volume flow demand from simulation and sampled flow demand vector for pump protocol.*

Rate constants were as well defined as possible, so it was time to start the last run. Solutions for the synthesis were

- 770.13 mg (5.34 mmol) of LA and
  237.19 mg (0.116 mmol) of PEG in 9.18 ml $CHCl_3$
- 616.02 mg (5.31 mmol) of GA in 6.12 ml THF

Catalyst amount was 156 µl, or 1.04 mmol.

Polymerization was carried out as described in 4.6.4. At 8 min slight discolouration appeared again, which kept increasing. At 37 min viscosity was observed slightly elevated. 4.63 ml of GA in THF was injected to reaction mixture, so theoretical average polymer formula with 100 % yield would be PEG_1-$PL_{46}G_{36}A$ Reaction was terminated at 60 min, product was collected via centrifugation and dried in pre-weighted mini bottle in vacuum. Yield was 1006 mg, out of 1473.72 mg (466.04 mg GA, 4.63/6.12 share was fed in), which is 68 %.

Part of the batch was then precipitated in methanol. 237.42 mg of the sample was mainly dissolved in 2+2 ml $CHCl_3$, but still some of the product did not dissolve. Several minutes of vortexing and sonication were tried. Solution was cloudy and some particles were constantly on bottle walls. Cloudy solution was transferred with Pasteur pipette to methanol, but some product left back to the pipette walls. Additional 1 ml $CHCl_3$ was used to dissolve the remaining particles on the glass ware, but only some of them dissolved and were transferred to methanol too. Finally, those particles which did not dissolve from the walls, was decided to waste since they would not dissolve in future stages either.

Precipitation in methanol was filtered and washed product was gathered from filter paper. The filtrate was poured to pre-weighted petri dish and evaporated dry under fume hood. Both were finally dried in the vacuum. Dried purified product mass was

86.25 mg and filtrate mass was 97 mg, thus 183.25 mg was left, 77.2 % out of the original 237.42 mg, so a really remarkable share was lost mainly due to the insolubility. This solubility issue is not only detrimental for characterization, but will arise problems in future steps as well. Sample is not representative anymore since apparently the most PGA rich chains are lost. NMR will show how much each polymer blocks each sample contains: un-purified or raw, purified and filtrate.

Samples were dissolved to 1 ml $CDCl_3$. Purified polymer was weighted in 11.86 mg and with intensive, long sonication it finally formed very cloudy solution with no particles on the walls. 11.36 mg of un-purified polymer was dissolved similar way, easier, but solution was cloudy, just not as bad as purified. 18.15 mg of the filtrate dissolved easily. Amounts were chosen since if soluble, ^{13}C NMR might be possible. ^{1}H NMR spectrums of the samples are shown in Figure 38, Figure 39 and Figure 40.

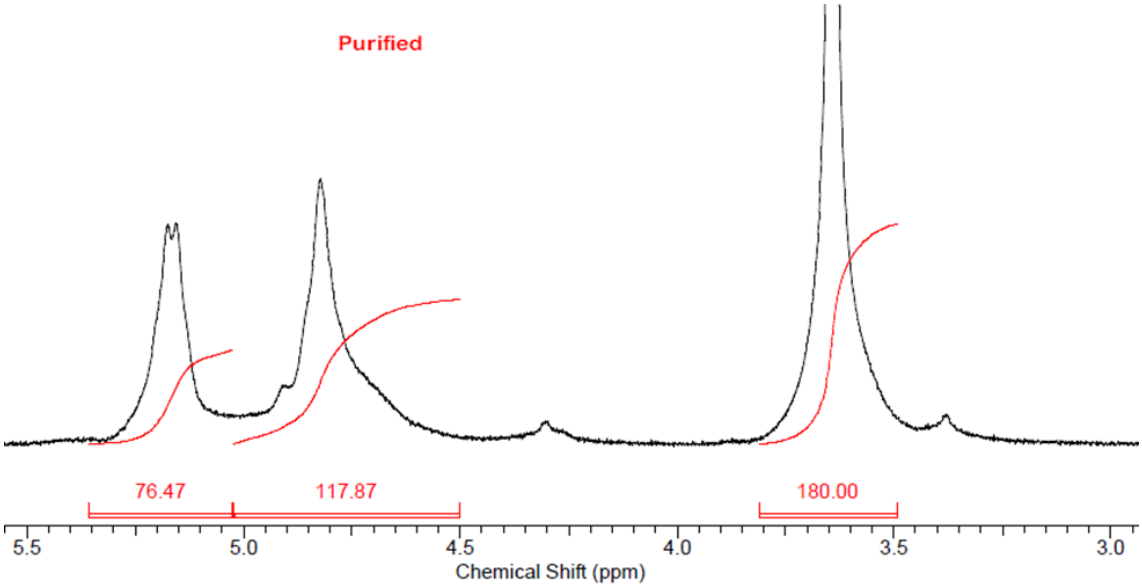


Figure 38. *NMR spectrum of PEG-PLGA purified in methanol. Syringe pump, second run.*

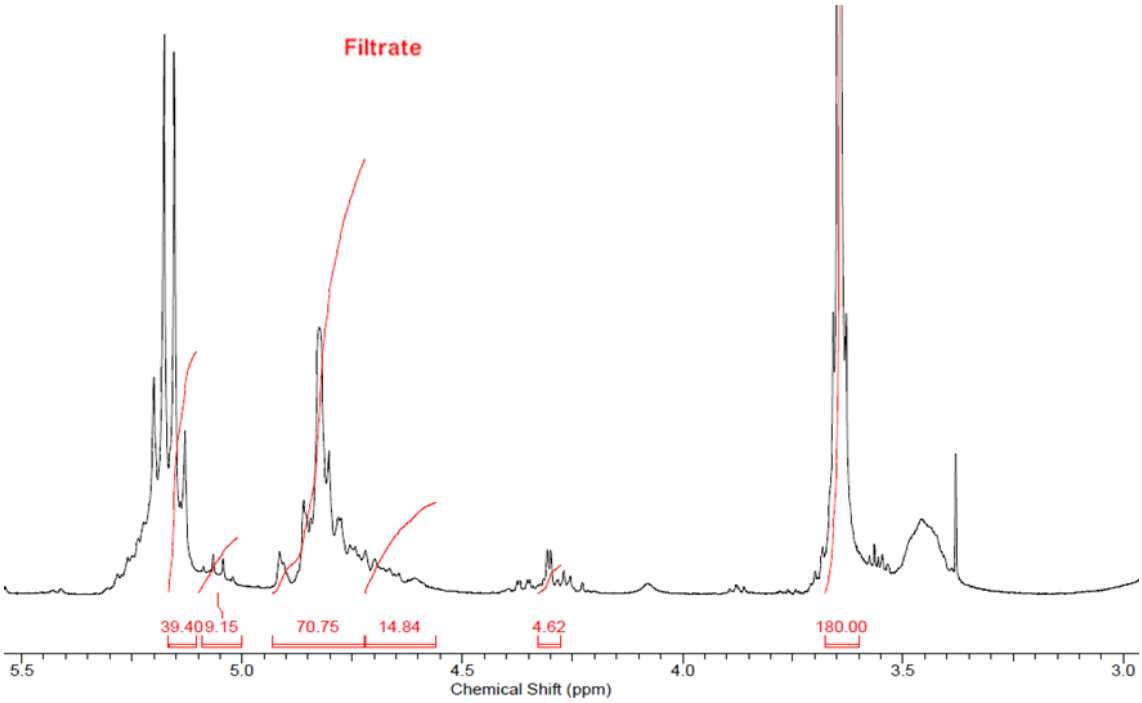


Figure 39. *NMR spectrum of filtrate of PEG-PLGA purified in methanol. Syringe pump, second run.*

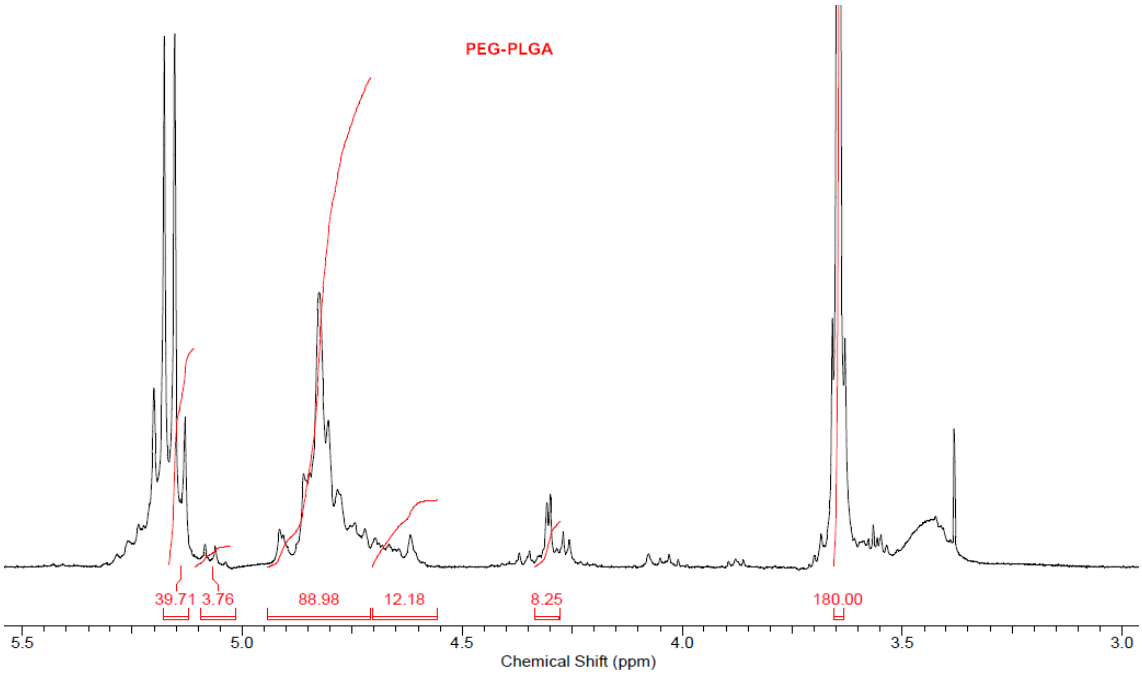


Figure 40. *NMR results of raw PEG-PLGA. Syringe pump, second run.*

In the purified sample solid particles caused peaks broadening, and major part of the sample had precipitated to bottom of the NMR tube during the measurement, but integrations should give a hint as it has been seen, methanol is effective in removing monomers and other impurities. Based on the integrals dissolved part of the sample and

particles floating in the range of NMR scope, purified polymer average formula is PEG_1-$PL_{38}G_{29}A$. In the filtrate impurities overlap especially LA and PLA CH_3 range, but peaks around 5 ppm give some estimate of the average polymer formula. Due to the obvious overlapping from left only right side of the PLA peak is integrated. Probably THF residues overlap PEG peak. Average formula of the filtrate, based on the integrals is PEG_1-$PL_{39}G_{17}A$ and LA:PLA ratio cannot be defined, GA:PGA ratio is 14.84: 70.75 (or 18 % of GA). Because 97 mg out of 237.42 mg is 41 %, actual GA monomer share in the raw sample should be 7 %. Average polymer formula for the raw sample, according to integrals, is PEG_1-$PL_{40}G_{22}A$ and LA:PLA ratio is 3.76: 39.71, or 8.6 % LA, GA:PGA ratio is 12.18:88.98, or 12 % GA.

So apparently 5.06-5.07 ppm peak is clearly partially overlapped with the same broad peak than 5.16 ppm peak, but around 10 % LA left in the raw sample, after 2-propanol precipitation and centrifugation seems fair assumption. GA share is fairly reliable, around 9 %. PLA share on the fractions just does not add up with the 68 % yield, since if GA lost is 14/36, or 181 mg, that leaves 286 mg to LA, which is 37 % while 6/46 is 13 %. First explanation is the accuracy of integration, or peaks. PEG peak may have too small value, but integration over the overlapping doublet gives polymer formulas that would be sticky, based on previous observations. Also some chains may have grown very glycolide rich in early stage, while still soluble in 2-propanol. And of course, the missing insoluble chains. On the other, chain length even in filtrate is longer than expected.

After the purified sample was taken out from NMR instrument, a lot of precipitation was found from the bottom of the tube. Liquid phase and some solid with it was poured out of the tube, rest was transferred to test tube with 2 ml of $CDCl_3$, test tube was sonicated and some of the solid dissolved. Content was sucked into a syringe and pushed through filter back to NMR tube in order to avoid peak broadening. NMR results, shown in Figure 41, reveal that average polymer formula was PEG_1-$PL_{44}G_{35}A$.

Clearly the PGA share increases with less and less soluble fractions and 22.8 % of the sample was lost, which did not even reach the characterization phase, because of this solubility issue. Purified sample is probably best representative one but PDI is not necessarily large if PDI of the PEG is small, since PLA amount does not vary much between different fractions. Average molecular mass for the filtrate was 9640 Da, for the raw material 10400 Da, for the purified material 10900 Da and for the re-dissolved sample 12400 Da. Purified sample was 57 % PLA, 43 % PGA, re-dissolved was 56:44 and with better suitable solvent 55:45 would have been reality. With solvents available more PGA rich fractions were not possible to obtain.

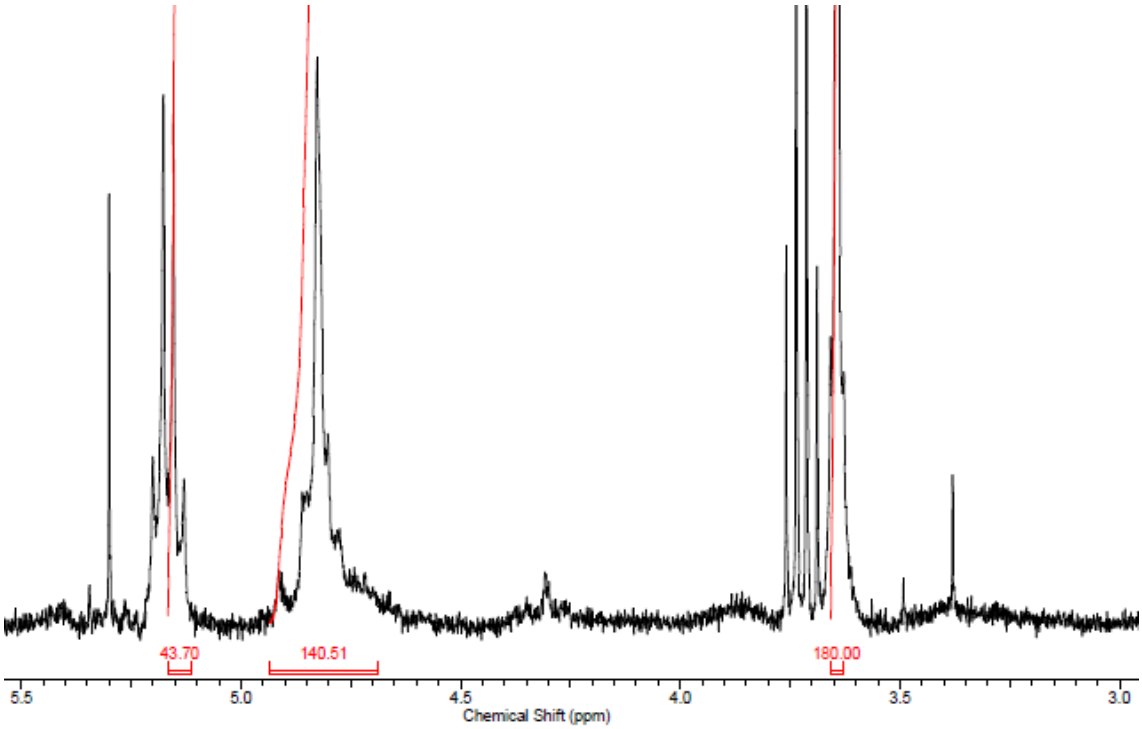


Figure 41. *NMR spectrum of the slightly soluble fraction, PEG-PLGA with syringe pump, second run.*

The simulation model was updated next for analysis purposes, since the polymer clearly is not 50:50, even though average polymer might be close to that. Cross-linking both monomers reactivities to another monomer concentration and rate constant may change simulation result significantly and must be thus checked. Because GA and LA are competing monomers, for equal rates probabilities to react must be equal, so condition $k_G \cdot [GA] = k_L \cdot [LA]$ must be fulfilled (rate constants are effective rate constant in the prevailing conditions). If only those conditions are taken into account when reaction occurs, probability that GA reacts, p(G), and p(L) respectively for LA are

$$P(G) = k_G \cdot [GA] / (k_G \cdot [GA] + k_L \cdot [LA]) \quad (25)$$

$$P(L) = k_L \cdot [LA] / (k_G \cdot [GA] + k_L \cdot [LA]) \quad (26)$$

Probabilities were added to the model shown in Figure 24, GA subsystem with equation (25) added is shown in Figure 42.

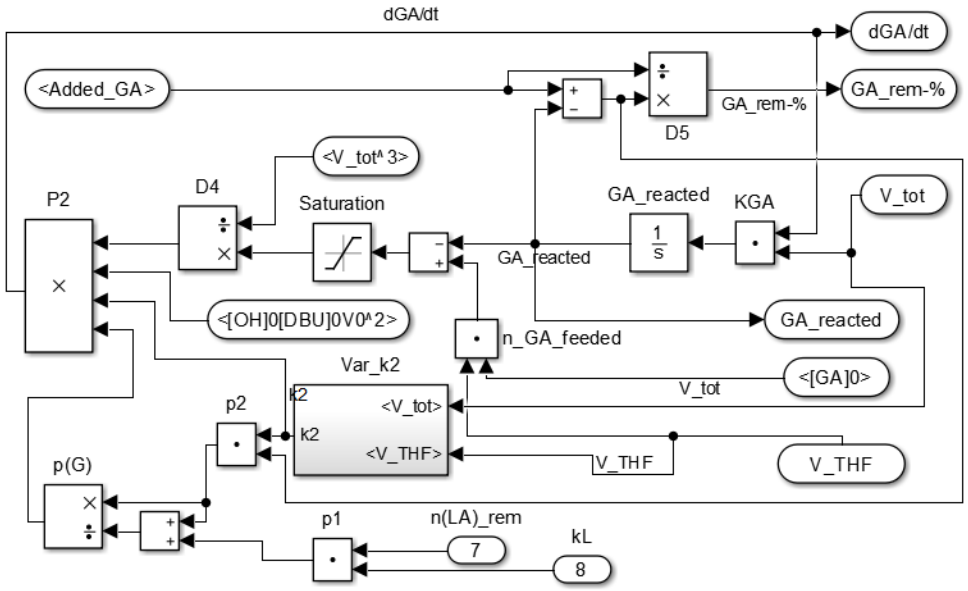


Figure 42. *Simulation model updated with the probabilities as cross-linkers between monomer species. GA subsystem in picture.*

To make results faster to interpret, automatic chain composition calculating subsystem, Figure 43, was created. Results were somewhat different: model predicted only 55 % conversion for LA and 76 % for GA. Reacted monomer curves crossed at 450 s, when precipitation was first observed at 8 min and chain was 7+7 monomers long that time, statistically having average 5 GA and 2 LA in last 7 units.

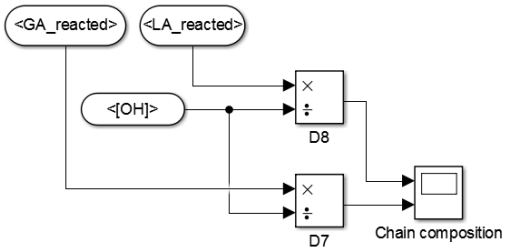


Figure 43. *Chain composition subsystem.*

GA was present app. 12.18/4 moles per one mole of PEG, which equals to 0.352 mmol or 40.9 mg and GA is not soluble in 2-propanol. Thus GA degree of conversion is app. 91 %, which equals to 33 units per chain. LA was originally 46 units and based on NMR there would be around 40 units in average, but yield was only 68 %. If the purified sample is closest to average, 4/33 precipitated early and washed away with 2-propanol. Based on the PGA results and discolouration observations, approximately 6-8 unit GA would take 2-3 units of LA with one PEG, so quite possibly 8 mol GA can take even 3 mol LA and 1 mol PEG from the yield. That is 1.5/33 of LA and 0.5/33 of PEG, 35.0 mg and 3.59 mg respectively with 90.6 mg GA (7/36 of GA), all together 129.2 mg. Rest of the missing yield, 467 mg – 129 mg, equal to 338 mg, cannot come from LA and losses in reaction vessel walls. If 38 units of LA is present in average

product, and reacted LA equals to 40 units. The only explanation possible is the obvious short chain solubility to 2-propanol and MeOH, but the exact chain composition limit is unknown.

The only things varied in the model were the rate constants. Doubling all gives approximately right conversions and from 200 to 450 *s* PGA increases 8 units from 2 while PLA increases 5 units from 9, which means quite possible precipitation. Chain composition is shown in Figure 44. Ratio of the rate constants of these monomers seems to actually be around 70.

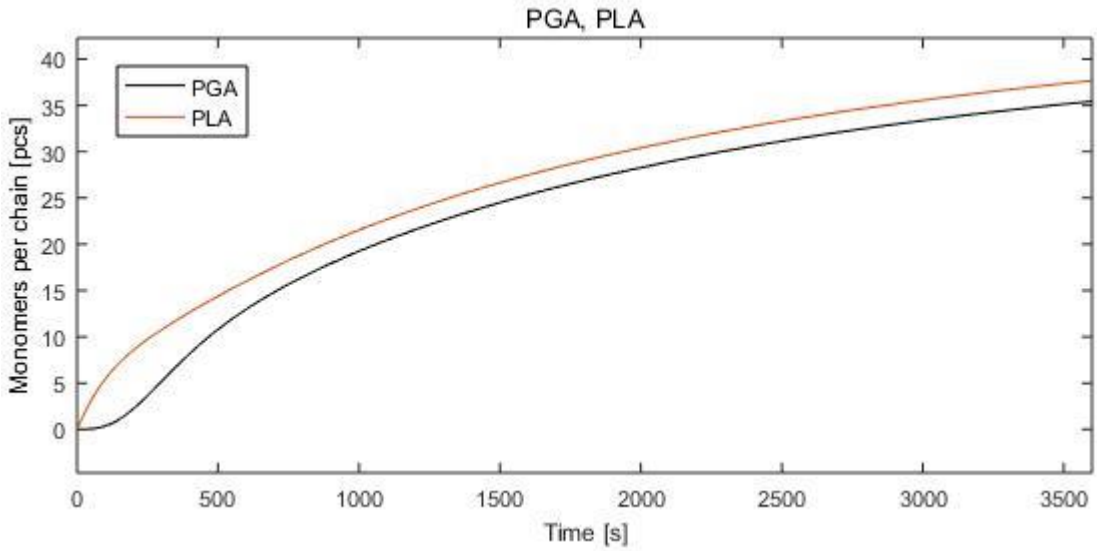


Figure 44. *Chain composition, simulated run 2 with cross-linking and doubled rate constants.*

Another possible reason for deviation from the first run is, because monomer concentrations were the same, that reaction is actually slightly higher order than one relative to DBU concentration.

DSC

DSC was measured according to 4.5.2. T_m was absent, as expected. T_g was found to be 18.68 ºC, Figure 46. Theoretically T_g of PEG_1-$PL_{38}G_{29}A$ should be around

$$T_g = \frac{2050 \cdot 233\text{K} + 38 \cdot 144 \cdot 333\text{K} + 29 \cdot 116 \cdot 308\text{K}}{2050 + 38 \cdot 144 + 29 \cdot 116} = 306\ \text{K}$$

Since PGA rich fractions are less soluble and measured T_g is less than 20 ºC, sample could not be more than PEG_1-$PL_{13}G_{29}A$. For comparison in the only 50:50 reference found, [4], T_gs were: theoretical 298 K / measured 280 K, theoretical 306 K / measured 289 K, with similar DSC.

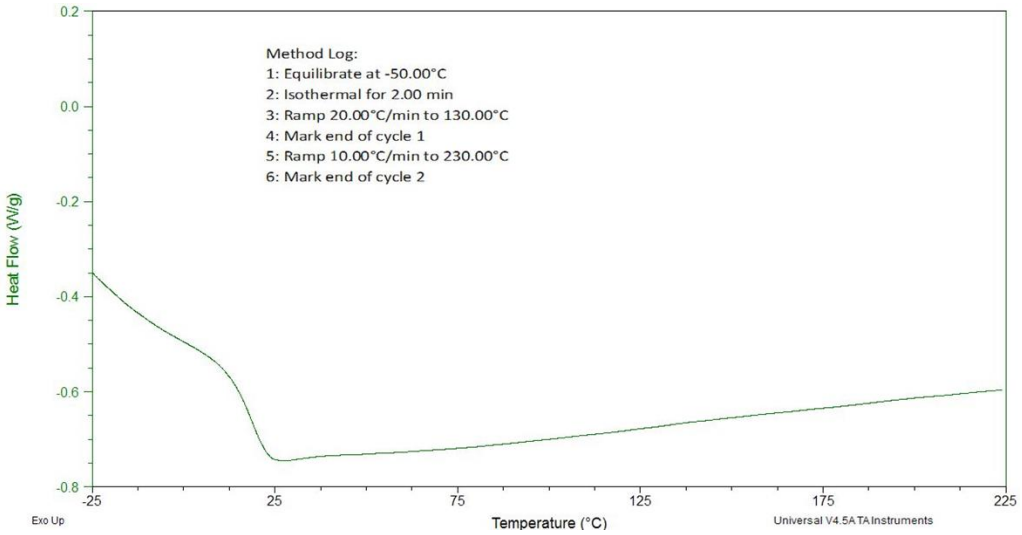


Figure 45. *DSC curve, PEG_1-$PL_{38}G_{29}A$ (purified)*

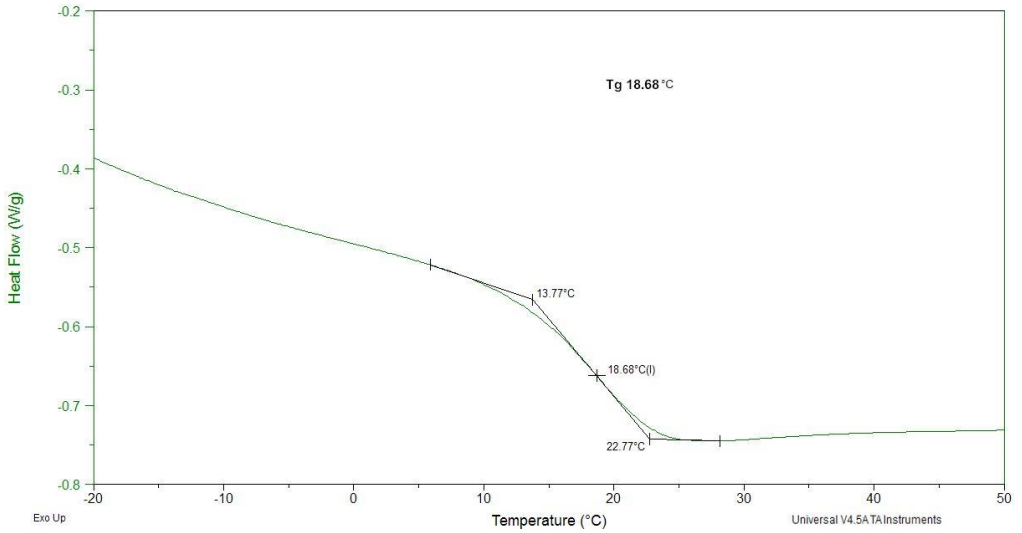


Figure 46. *Glass transition temperature of purified sample*

Based on DSC results here and in the reference, DSC and NMR results are in good agreement.

5.4 Syntheses of PEG-PLGA-LL

Because the manual feed PEG_1-$PL_{31}G_8A$ product seemed successful until NMR was measured, L-lysine incorporation was first attempted with that. *N*,*N*-di-Fmoc-L-lysine (Fmoc-LL) was incorporated to 250 mg of the product like described in 4.6.7. Total 5.5 ml of $CHCl_3$ was needed.

The amounts of a substances and masses needed was calculated from equation (4), Et_3N and $SOCl_2$ volumes were calculated from densities shown in Table 6. The amounts of a substances, masses, weighed masses and volumes of each compound are shown in Ta-

ble 17. Yield was supposed to tell molar mass of the polymer, which was 84.6 % of the 1096.9 mg and all the mass lost was assumed to come from 508.61 mg of LA. 15.4 % of the 1096.9 mg retracted leaves 337 mg, or 66.4 %, LA so PEG_1-$PL_{23.3}G_{32.5}A$ was assumed to be polymer formula, having thus average 9180 Da molar mass.

Yield was 195 mg out of 274 mg, 70.1 %. NMR results later revealed the polymer formula and the fact that the sample contained twice as much GA as PGA, which is roughly 20 % of the sample mass. At this point it seemed thus that this one-pot might even work.

Table 17. *Amounts, masses, weighted amounts and volumes of each compound in the Fmoc-LL addition.*

Compound	Amount [µmol]	Mass [mg]	Weighed [mg]	Volume [µl]
Polymer	27.2	250	249.64	ND
Fmoc-LL	40.8	24.13	24.12	ND
Et_3N	47.7	4.823	-	6.64
$SOCl_2$	47.7	5.670	-	3.48

Deprotection was supposed to be done in 20 % piperidine in chloroform solution, but since polymer solubility was tested and found that 195 mg needs approximately 16 ml of $CHCl_3$ (or even more DCM) and piperidine was only 5 ml available, another solution with the chemicals at hand was needed. Thus deprotection was carried out as described in 4.6.8.

Some of the polymer in the reaction vessel walls came out with 0.5 ml THF. Yield was 9.58 mg. Obviously something was wrong, single L-lysine hardly makes the polymer soluble in 2-propanol, so something has happened to it. NMR results of the protected and deprotected products with the Fmoc-LL spectrum are shown in Appendix G. All the spectrums have DMF residues, since 2.88 and 2.96 ppm peaks appear with 8.02 ppm peak.

Protected average polymer formula was PEG_1-$PL_{30}G_8A$, but as found from the spectrum, the deprotected average polymer formula seemed to be PEG_1-$PL_{35}G_8A$, but when DMF peaks integral 26.51 at 2.88 and 2.96 ppm and 5.03 broad peak integral at 4.82 ppm were compared to the 26.60 at 2.92 ppm in the spectrum of deprotected polymer, PGA integral should be rather 26.6 than 31.6, so average formula was rather PEG_1-$PL_{35}G_7A$ than PEG_1-$PL_{35}G_8A$. Perhaps thionyl chloride reacted with polymer's -OH group, but in any case, next round was realized with the author's original idea presented in the 3.2.1.

In the deprotected polymer spectrum THF residues from 1.8 ppm overlap lysine's γ-carbon's proton signal and due to 2-propanol residues visible at 1.22 ppm, β-carbon

CH_2- peaks integral would be arbitrary. In the protected polymer β-carbon CH_2- at 1.41-1.43 ppm integral value is 6.75 so there would be 3 lysines in every polymer – or more likely sample contains unreacted lysines.

For the next attempt purified polymer from second syringe pump run, PEG_1-$PL_{38}G_{29}A$, M=10900 g/mol, was weighed 97.80 mg. Amounts, masses, weighed amounts and volumes of reactants and reagent used in the process are shown in Table 18. Procedure presented in 3.2.1 was followed exactly.

Table 18. *Amounts, masses, weighed amounts and volumes of each compound in the Fmoc-LL addition.*

Compound	Amount [µmol]	Mass [mg]	Weighed [mg]	Volume [µl]
Polymer	8.97	97.80	249.64	ND
Fmoc-LL	9.97	5.89	24.12	ND
Et_3N	10.97	1.110	-	1.53
$SOCl_2$	10.97	1.305	-	0.80

Fmol-LL incorporation was realized as described in 4.6.7. Pattern used was $CHCl_3$, $CHCl_3$, $CHCl_3$, DMF, DMF, $CHCl_3$, DCM, $CHCl_3$, $CHCl_3$. Some of the polymer remained insoluble and was thus lost since the bottle was already half-full. After product was dried in vacuum, it had still a bad smell and yield was 79.09 mg out of 103.69 mg.

Fmoc deprotection was carried out as described in 4.6.8. Yield was zero.

6. DISCUSSION

Numerical and methodical results of different syntheses are discussed later in this section. Theoretical and experimental drop size were in the same order as measured ones. Designed nitrogen atmosphere implementation was successful and mandatory in order to succeed with reactions.

6.1 Polylactide

In the manual feed part of the PEG-PLGA synthesis, lactide conversion speed in DCM was determined based on yields and samples were collected via centrifugation. Rate constant value was found to be 5.77 and yield 88 % in 45 min. Later results revealed that this procedure may leave unreacted monomers too in the product, which caused increase in rate constant value. In the automatic feed part lactide rate constant value was found to be 0.5844 $CHCl_3$, when the rate constant was determined from raw samples with NMR after solvent evaporation. Degree of conversion was 93 % in 1 h, which is in good agreement with literature [4][15].

Even though rate constant determination was not accurate as based on yield, there is certainly difference between DCM and $CHCl_3$, because if the rate constant would be an order lower, no precipitation in manual feed part would have occurred, because short chains behave like having same density as 2-propanol (5.1.1). In the $CHCl_3$ the measured rate constant was in PLA synthesis 0.5844 $l^2mol^{-2}s^{-1}$ and in the final PLGA simulation it was found to be 1.2 $l^2mol^{-2}s^{-1}$. Conversions were high in both solvents. Final simulations gave very realistic results with rate constant 1.2 $l^2mol^{-2}s^{-1}$ in both solvents, which means that lactide rate constant does not significantly change with added THF. That result also implicates that LA polymerization rate is not affected by combating GA monomers which seems curious at first. Explanation might be very simple: since GA concentration is only a fraction of LA concentration, LA conversion depends mainly on probability of active chain end to meet LA monomer in a favourable angle

6.2 Polyglycolide

It was not even tried to polymerize PEG-PGA in the THF, since PEG solubility in THF was so limited (Table 15) and on the other hand, based on literature, PGA chains are not soluble in THF [48][57]. Thus PEG-PGA was synthesized in one-pot reaction with the final stage solvent mixture (THF/$CHCl_3$ 2/3) and 5.48 units long PGA block in

PEG-PGA was the average polymer obtained. When DBU was added, instant precipitation occurred and after a couple of seconds reaction mixture colour remained unchanged, which means, that the rate constant might 1000 times higher than LAs, as found previously [4]. Later studies on results, with reaction kinetics model employed, revealed that GA rate constant is significantly lower than reported in [4], approximately 170 times LA's rate constant in DCM. Visual observations made during the test (5.1.2) are not in contradiction with this result, because it is impossible to say exactly how many seconds precipitation continued or the exact moment when precipitation was fastest.

Apparently solvent mixtures need to be handled in a different manner when monomer is soluble in one, but not in another, component of the mixture, even though monomer is soluble in the mixture. Method proposed in this study was to use solvent composition dependent rate constant value, where rate constant is set to zero for the bad solvent fraction (5.3.2). Without solvent composition dependency GA rate constant value could not be set in a way that explains more than one result at the time. With solvent composition dependency one value fitted to all results studied, as it should, since rate constant should be constant.

When DCM was replaced with $CHCl_3$, GA rate constant decreased from 170 to approximately 70 times LA rate constant. This is probably a consequence of hydrogen bonding between solvent hydrogens and carbonyl oxygens, making thus GA less active in $CHCl_3$, which is curious since similar change was not found with LA.

6.3 Glycolide Feed Implementation and PEG:PLA:PGA

Manual feed results were all poor and when the syntheses were simulated afterwards, it became clear that GA rate constant value found from literature was far too high and there was no chance to synthesize 50:50 PLGA block using proposed feed rate profile. On the other hand, GA monomers were included to PGA peak integral in the only ^{1}H-NMR spectrum offered. The first PLGA synthesis with syringe pump was simulated with LA:GA rate constant ratio 1:1000 and resulting feed rate demand vector realized yielded to 2:1 LA:GA polymer (Figure 32), now in $CHCl_3$, instead of DCM.

GA rate constant in $CHCl_3$ was found to lie at around 1/15 of the reported (in DCM) value and approximately 70 times LA's rate constant. New rate constant value was then employed and simulated flow demand need was used in the syringe pump control. Product contained still more LA than GA, but was even longer than the target polymer – with very limited solubility. Simulation model was further tuned with to take into account other monomer's influence, in order to make it useful for other ratios than 50:50 too.

With manual feed the longest successful PEG-PLGA random copolymer chain obtained was PEG_1-$PL_{28}G_{14}A$ in comparison to the theoretical formula PEG_1-$PL_{38}G_{39}A$ (5.2.1). The most PGA rich polymer was PEG_1-$PL_{11}G_{10}A$ in comparison to theoretical formula PEG_1-$PL_{28}G_{25}A$. Only product that was not sticky and reproducibility within 10 % possible was PEG_1-$PL_{31}G_8A$ versus theoretical PEG_1-$PL_{35}G_{32}A$ (5.3.1). Shorter chain polymers could not be collected via filtration and centrifugation resulted to plenty of especially GA monomers in the yield. GA feed in the beginning was always too slow but there were still moments when the rate was too high, since early precipitation was always observed. The PEG_1-$PL_{28}G_{14}A$ was polymerized with Ø 0.7 mm needle and very dilute GA solution, which may refer to drop size significance in both ways, momentarily high total GA concentration and locally too high GA concentration. In all the manual feed implementations DCM was used as the PEG and LA solvent.

With automatic feed more concentrated GA solution and only THF was used with a Ø 0.4 mm needle instead of 1 ml pipette tip. The first product was PEG_1-$PL_{16}G_{10}A$ in comparison to theoretical PEG_1-$PL_{25}G_{22}A$ (p.55). Ratio of LA and GA rate constant values was found to be only 170:70 when solvent was changed from DCM to $CHCl_3$. In the following experiment, with updated model, resulting target feed rate vector was used in the second attempt. The obtained sample contained a lot of insoluble material and when the slightly soluble fraction was studied with NMR, PEG_1-$PL_{44}G_{35}A$ was found. Sample was purified by precipitation in methanol and filtrate was studied too: 22.8 % of the sample was lost, because it was not soluble in either $CHCl_3$ or DCM. The purified product was PEG_1-$PL_{38}G_{29}A$ (Figure 38) in comparison to the theoretical formula PEG_1-$PL_{46}G_{36}A$. The DSC results (p.63-65) were in good agreement with NMR, even though the NMR peaks were broadened and actual shapes were lost Figure 38).

Average polymer formulas, molar masses, corresponding theoretical formulas and GA feed method used are collected in the Table 19. It is quite clear that if not 50:50, at least even slightly more GA rich chain are insoluble in $CHCl_3$, which means that in order to polymerize 50:50, only very short chain polymers would be plausible, if any. It would be very interesting see the true NMR results of this latest product, but it was not possible because it did not dissolve to $CHCl_3$. It would also be very interesting to see NMR results for commercial 50:50 PLGA, since solubility may be quite different, if the product synthesized was truly random.

Table 19. *Manual and automatic feed results compared. m refers to manual, a to automatic, * to slightly soluble fraction.*

Product	Molar mass [g/mol]	Theoretical product	Method
PEG_1-$PL_{28}G_{14}A$	7700	PEG_1-$PL_{38}G_{39}A$	m
PEG_1-$PL_{11}G_{10}A$	4800	PEG_1-$PL_{28}G_{25}A$	m
PEG_1-$PL_{31}G_8A$	7400	PEG_1-$PL_{35}G_{35}A$	m
PEG_1-$PL_{38}G_{29}A$	10900	PEG_1-$PL_{46}G_{36}A$	a
PEG_1-$PL_{44}G_{35}A$*	12400	PEG_1-$PL_{46}G_{36}A$	a

Simulation model was first refined with different rate constants in the different solvents and finally further refined with actual simulated competing monomer concentrations and rate constants cross-linking between two subsystems. Reaction kinetics simulation was found to be excellent tool in synthesis development, especially when the system becomes mathematically complex, and results were excellent. Only two experimental trials were needed to get as close to the target ratio as possible with such limited solubility and it is quite probable, with the final refinements to the rate constants, that the pre-set target PEG_1-$PL_{28}G_{28}A$ polymer is only a couple of more experiments away. If it dissolves to $CHCl_3$, which unfortunately does not seem promising.

6.4 PEG-PLGA-LL

The final product synthesis failed mainly due to the solubility issue and the fact that over 80 % of the time available for experiments was lost to the manual feed trials. No time was left to optimize the deprotection time and reaction conditions, so whether decreasing the temperature and/or the reaction time would leave the polymer chain intact remained unknown.

PEG-PLGA-Fmoc-LL synthesis yield was 76 % with the product closest to target material was used and 70 % with the lactide rich short polymer, even though thionyl chloride was slightly yellowish and author was not aware, that it should have been distilled first, until the experimental part was over. Deprotection yield was with DBU and 24 h 19 %, with piperidine and 2 h 0 %. NMR results showed that Fmoc-LL addition was successful but quantitative results of deprotected product were not clear (Appendix G).

Piperidine seems too strong base, at least for probably quite random PLGA, since even those solid particles that were not soluble, did not survive the deprotection process. DBU did the job, as found from literature [64][61], but seems that it was also too violent to the polymer or deprotected amine groups attacked ester groups due to long reaction time.

In other articles [25][26], sent 2011 and 2014, carbobenzyloxy (CBZ) protected L-lysine was used to synthesize PEG-PLGA-PLL, which was then deprotected with HBr/Acetic acid. The only difference between the reaction schemes was the deprotection time, which was first a day and in later one 1 h. Since the two articles were written 3 years apart, sharing three common authors, they must have found this procedure functional and effective. If the L-lysine amine groups attack the polymer ester groups, acidic conditions would at least offer protons to attach to the amines faster and since bromide is better leaving group, ester bonds would not degrade. CBZ protection may also offer adequate steric hindrance to avoid lysine attacks during PLL polymerization and deprotection reaction time decrease to 1/24 may implicate that amine groups cause polymer degradation during longer exposure time. HBr concentration was not specified in either of the reports, nor in the report which earlier one referred to as the

polymerization procedure used [36], which makes a curious reader suspect that the HBr concentration might be critical for the success, since these are the only chemicals they do not specify, but Sigma Aldrich happens to have 33 w-% HBr in Acetic acid in stock [99].

7. SUMMARY AND CONCLUSIONS

Articles concerning PLGA 50:50 ROP in solution were searched with all the possible methods in order to make comparisons or find hints, but only one [4] was found, so according to the present knowledge of the author, the literature is scarce on this topic.

Adjusting glycolide feed manually was found impossible, but with the syringe pump, target polymer molar mass was obtained and probably target LA:GA ratio as well. Problem with the ratio was that polymers closer than 56:44 to 50:50 were practically insoluble in any solvent at hand. Since 50:50 is commonly known to be the limit, product characteristics may actually be more random than commercial ones, which could explain the solubility difference. But as long as solubility issue is not solved, some fractions will be lost.

Simulation model together with computer controlled syringe pump to implement glycolide feed was found very cost-effective and efficient method in synthesis development. Refined simulation model offered explanation and good correlation between the final product and simulation model, so continuing with this method seems very promising. Simulation model did not take precipitation into account and if the model would be constituted of distribution functions, it would not run anymore in laptop. But conversion speeds, chain composition and length could be compared to each other and to pre-set, among chain length and composition, sliding consecutive glycolide threshold value. Hitting the threshold would cause some pre-determined fraction of chains leave the reaction, causing simultaneously decrease in initiator concentration.

Synthesis with same solvent mixtures used for both monomers would be kinetically remarkably simpler and chloroform-tetrahydrofuran with equal shares works well as solvent for all the reactants and reagents used in this polymerization. If the other solvent would be dichloromethane instead of chloroform, slower initial glycolide feed rate would be needed and thus even 0.3 mm needle might work.

Because of the solubility issue, some fluorinated solvent should be tested, for example hexafluoroisopropanol, fluorobenzene or some chloro-fluoro compound like 1-Chloro-4-fluorobenzene might work as solvent in synthesis steps. In the L-lysine addition step CBZ protected amine would probably be the safe choice, but also the deprotection time needed in lower temperature with DBU should be tested. The thionyl chloride should also be distilled prior the use.

REFERENCES

[1] H. K. Makadia, S. J Siegel, Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier, Polymers 2011, 3, 1377-1397

[2] P. Gentile, V. Chiono, I. Carmagnola and P. V. Hatton, An Overview of Poly(lactic-co-glycolic) Acid (PLGA)-Based Biomaterials for Bone Tissue Engineering, International Journal of Molecular Sciences 2014, 15, 3640-3659

[3] K. Avgoustakis, A. Beletsi, Z. Panagi , P. Klepetsanis , A.G. Karydas , D.S. Ithakissios, PLGA–mPEG nanoparticles of cisplatin: in vitro nanoparticle degradation, in vitro drug release and in vivo drug residence in blood properties, Journal of Controlled Release 79 (2002), 123–135

[4] H. Qian, A. R. Wohl, J. T. Crow, C. W. Macosko and T. R. Hoye, Strategy for Control of "Random" Copolymerization of Lactide and Glycolide: Application to Synthesis of PEG-b-PLGA Block Polymers Having Narrow Dispersity, Macromolecules 2011, 44, 7132–7140

[5] K. Avgoustakis, Polylactic-Co-Glycolic Acid (PLGA), Encyclopedia of Biomaterials and Biomedical Engineering, Taylor & Francis, 2005. Available (accessed on 5.6.2016): http://www.pharmacy.upatras.gr/index.php/el/latest-news/doc_download/2415-paper-in-ebbe-encyclopedia

[6] C. D. C. Erbetta, R. J. Alves, J. M. Resende, R. F. de Souza Freitas, R. G. de Sousa, Synthesis and Characterization of Poly(D,L-Lactide-co-Glycolide) Copolymer, Journal of Biomaterials and Nanobiotechnology 2012, 3, 208-225

[7] PLA, PGA, and PLGA as biomaterials, by Colton Kenny, ref. 23.6.2016 http://webcache.googleusercontent.com/search?q=cache:http://openwetware.org/wiki/PLA,_PGA,_and_PLGA_as_biomaterials,_by_Colton_Kenny&gws_rd=cr&ei=N4V6V8z9MYWWsgH1pbP4DQ

[8] O. Dechy-Cabaret, B. Martin-Vaca, and D. Bourissou, Controlled Ring-Opening Polymerization of Lactide and Glycolide, Chemical Reviews 2004, Vol. 104, No. 12, 6147−6176

[9] C. Alemán, O. Bertran, K.N. Houk, et al., Thermodynamic and stereochemical aspects of the polymerizability of glycolide and lactide, Theoretical Chemistry Accounts (2012) 131: 1133. doi:10.1007/s00214-012-1133-y

[10] F. Suriano, O. Coulembier, J.s L. Hedrickb and P. Duboisa, Functionalized cyclic carbonates: from synthesis and metal-free catalysed ring-opening polymerization to applications, Polymer Chemistry 2011, 2, 528–533

[11] M. Myers, E. F. Connor, T. Glauser, A. Möck, G. Nyce, J. L. Hedrick, Phosphines: Nucleophilic Organic Catalysts for the Controlled Ring-Opening Polymerization of Lactides, Journal of Polymer Science: Part A: Polymer Chemistry, Vol. 40, 2002, 844–851

[12] J. Kadota, D. Pavlovic, J.-P. Desvergne, B. Bibal, F. Peruch and A. Deffieux, Ring-Opening Polymerization of L-Lactide Catalyzed by an Organocatalytic System Combining Acidic and Basic Sites, Macromolecules 2010, 43, 8874–8879

[13] A. Li, L. Lu, X. Li, L. He, C. Do, J. C. Garno and D. Zhang, Amidine-Mediated Zwitterionic Ring-Opening Polymerization of N-Alkyl N-Carboxyanhydride: Mechanism, Kinetics, and Architecture Elucidation, Macromolecules 2016, 49, 1163−1171

[14] S. Venkataraman, V. W. L. Ng, D. J. Coady, H. W. Horn, G. O. Jones, T. S. Fung, H. Sardon, R. M. Waymouth, J. L. Hedrick and Y. Y. Yang, A Simple and Facile Approach to Aliphatic N-Substituted Functional Eight-Membered Cyclic Carbonates and Their Organocatalytic Polymerization, Journal of the American Chemical Society 2015, 137, 13851−13860

[15] B. G. G. Lohmeijer, R. C. Pratt, F. Leibfarth, J. W. Logan, D. A. Long, A. P. Dove, F. Nederberg, J. Choi, C. Wade, R. M. Waymouth and J. L. Hedrick, Guanidine and Amidine Organocatalysts for Ring-Opening Polymerization of Cyclic Esters, Macromolecules 2006, 39, 8574-8583

[16] D. J. Heldebrant, P. G. Jessop, C. A. Thomas, C. A. Eckert and C. L. Liotta, The Reaction of 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) with Carbon Dioxide, The Journal of Organic Chemistry 2005, 70 (16), pp 5335–5338

[17] DBU SDS, Sigma Aldrich, Available (accessed on14.4.2016): http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=FI&language=fi&productNumber=139009&brand=ALDRICH&PageToGoToURL=http%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Faldrich%2F139009%3Flang%3Dfi ref. 14.4.2016

[18] K. Avgoustakis, A. Beletsi, Z. Panagi, P. Klepetsanis, E. Livaniou, G. Evangelatos , D.S. Ithakissios, Effect of copolymer composition on the physicochemical characteristics, in vitro stability, and biodistribution of PLGA–mPEG nanoparticles, International Journal of Pharmaceutics 259 (2003) 115–127

[19] H. Hart, L. E. Craine, D. J. Hart, C. M. Hadad, Organic Chemistry a Short Course, Houghton Mifflin Company, 2007, 577 p.

[20] R. Vivek, R. Thangam, V. NipunBabu, C. Rejeeth, S. Sivasubramanian, P. Gunasekaran, K. Muthuchelian and S. Kannan, Multifunctional HER2-Antibody Conjugated Polymeric Nanocarrier-Based Drug Delivery System for Multi-Drug-Resistant Breast Cancer Therapy, ACS Applied Materials & Interfaces 2014, 6, 6469−6480

[21] E. Cohen-Sela, M. Chorny, N. Koroukhov, H. D. Danenberg, G. Golomb, A new double emulsion solvent diffusion technique for encapsulating hydrophilic molecules in PLGA nanoparticles, Journal of Controlled Release, 133, 2009, 90–95

[22] G. Dördelmann, D. Kozlova, S. Karczewski, R. Lizio, S. Knauer and M. Epple, Calcium phosphate increases the encapsulation efficiency of hydrophilic drugs (proteins, nucleic acids) into poly(D,L-lactide-co-glycolide acid) nanoparticles for intracellular delivery, Journal of Materials Chemistry, 2014, 2, 7250

[23] Y. Li, Y. Pei, X. Zhang, Z. Gu, Z. Zhou, W. Yuan, J. Zhou, J. Zhu, X. Gao, PEGylated PLGA nanoparticles as protein carriers: synthesis, preparation and biodistribution in rats, Journal of Controlled Release 2001, 71, 203-211

[24] P. Zhou, X. Gao, T. An, C. Zhao, Y. Li, R. Li, R. Yang, Y. Wang, Lactosylated PLGA nanoparticles containing e-polylysine for the sustained release and liver-targeted delivery of the negatively charged proteins, International Journal of Pharmaceutics 478, 2015, 633–643

[25] J. Du, Y. Sun, Q. Shi, P. Liu, M. Zhu, C. Wang, L. Du and Y. Duan, Biodegradable Nanoparticles of mPEG-PLGA-PLL Triblock Copolymers as Novel Non-Viral Vectors for Improving siRNA Delivery and Gene Silencing, International Journal of Molecular Sciences 2012, 13, 516-533

[26] Z. He, Y. Sun, Q. Wang, M. Shen, M. Zhu, F. Li and Y. Duan, Degradation and Bio-Safety Evaluation of mPEG-PLGA-PLL Copolymer-Prepared Nanoparticles, The Journal of Physical Chemistry C 2015, 119, 3348−3362

[27] The Free Dictionare by Farlex, Available (accessed on 1.7.2016): http://medical-dictionary.thefreedictionary.com/biocompatible

[28] K. Masutani and Y. Kimura, Chapter 1: PLA Synthesis. From the Monomer to the Polymer, in Poly(lactic acid) Science and Technology: Processing, Properties, Additives and Applications, 2014, pp. 1-36. Available (accessed on 3.4.2016): http://pubs.rsc.org/en/content/chapterhtml/2014/bk9781849738798-00001?isbn=978-1-84973-879-8

[29] H. Tsuji, A. Mizuno, Y. Ikada, Properties and Morphology of Poly(L-lactide). III. Effects of Initial Crystallinity on Long-Term In Vitro Hydrolysis of High Molecu-

lar Weight Poly(L-lactide) Film in Phosphate Buffered Solution, Journal of Applied Polymer Science 2000, Vol. 77, 1452–1464

[30] Lactide and Glycolide Polymers in Sigma Aldrich web catalog. Available (accessed on 25.7.2016): http://www.sigmaaldrich.com/materials-science/material-science-products.html?TablePage=20202285

[31] K. M. Huh, Y. W. Cho, K. Park, PLGA-PEG Block Copolymers for Drug Formulations, Drug Development and Delivery 2003, Vol. 3 No. 5. Available (accessed on 5.6.2016): http://www.drug-dev.com/Main/Back-Issues/PLGAPEG-Block-Copolymers-for-Drug-Formulations-406.aspx

[32] F. dos Santos Peleias Jr, M. D. J., C. A. Zeituni, M. E. Chuery, M. Rostelato and P. F. Moreira Jr, Synthesis of PLGA using a C 3-symmetric Zr (IV) amine tris(phenolate) alkoxide initiator and the effects of gamma radiation on its properties, Polymer Bulletin 19.5.2016, 1-15.

[33] A. Orchel, K. Jelonek, J. Kasperczyk, P. Dobrzynski, A. Marcinkowski, E. Pamula, J. Orchel, I. Bielecki and A. Kulczycka, The influence of chain microstructure of biodegradable copolyesters obtained with low-toxic zirconium initiator to in vitro biocompatibility, BioMed Research International, Vol. 2013, Article ID 176946. Available (accessed on 17.8.2016): http://downloads.hindawi.com/journals/bmri/2013/176946.pdf

[34] P. Dobrzynski , J. Kasperczyk , H. Janeczek and M. Bero, Synthesis of Biodegradable Copolymers with the Use of Low Toxic Zirconium Compounds. 1. Copolymerization of Glycolide with l-Lactide Initiated by $Zr(Acac)_4$, Macromolecules 2001, 34 (18), pp 5090–5098

[35] Y. Li, M. Kroger, W. K. Liu, Endocytosis of PEGylated nanoparticles accompanied by structural and free energy changes of the grafted polyethylene glycol, Biomaterials 35 (2014), 8467-8478

[36] C. Deng, G. Rong, H. Tian, Z. Tang, X. Chen, X. Jing, Synthesis and characterization of poly(ethylene glycol)-b-poly (L-lactide)-b-poly(L-glutamic acid) triblock copolymer, Polymer 46 (2005), 653–659

[37] P. Dubois, O. Coulembier, J-M Raquez, Handbook of Ring-Opening Polymerization, John Wiley & Sons, 2009, ISBN: 978-3-527-31953-4, 408 p.

[38] J. P. Rao, K. E. Geckeler, Polymer nanoparticles: Preparation techniques and size-control parameters, Progress in Polymer Science 36 (2011), 887–913

[39] C. Cruje and D. B. Chithrani, Polyethylene Glycol Density and Length Affects Nanoparticle Uptake by Cancer Cells, Journal of Nanomedicine Research 2014,

Vol. 1, Issue 1. Available (accessed on 18.8.2016): http://medcraveonline.com/JNMR/JNMR-01-00006.pdf

[40] C. Wang, C.Liu, C.Wang, T. Hua, J. M. Obliosca, K. H. Lee, Y. Hwu, C. Yang, R. Liu, H. Lin, J. Je and G Margaritondo, Optimizing the size and surface properties of polyethylene glycol (PEG)–gold nanoparticles by intense x-ray irradiation, Journal Of Physics D: Applied Physics, 41 (2008). Available (accessed on 18.8.2016): http://www.ch.ntu.edu.tw/~rsliu/publications/2008-pdf%20-po-Hsiang/2008-pdf%20-po-Hsiang/14.pdf

[41] S. Hirsjärvi, S. Dufort, J. Gravier, I. Texier, Q. Yan, J. Bibette, L. Sancey, V.Josserand, C. Passirani, J-P Benoit, J-L Coll, Influence of size, surface coating and fine chemical composition on the in vitro reactivity and in vivo biodistribution of lipid nanocapsules versus lipid nanoemulsions in cancer models, Nanomedicine: Nanotechnology, Biology and Medicine 9 (2013), 375–387

[42] PEG-PLGA 2k-11.5k Product Sheet, Sigma Aldrich. Available (accessed on 6.7.2016): http://www.sigmaaldrich.com/catalog/product/aldrich/764760?lang=fi&region=FI 6.7.2016

[43] PEG-PLGA 5k-7k Product Sheet, Sigma Aldrich. Available (accessed on 6.7.2016): http://www.sigmaaldrich.com/catalog/product/aldrich/765139?lang=fi&region=FI

[44] F. Nederberg, E. F. Connor, M. Moller, T. Glauser and J. L. Hedrick, New Paradigms for Organic Catalysts: The First Organocatalytic Living Polymerization, Angewandte Chemie International Edition 2001, 40, 2712-2715

[45] K. A. M. Thakur, R. T. Kean, E. S. Hall, M. A. Doscotch and E. J. Munson, A Quantitative Method for Determination of Lactide Composition in Poly(lactide) Using 1H NMR, Analytical Chemistry 1997, 69 (21), 4303–4309

[46] Y. Yu, G. Storti, and M. Morbidelli, Kinetics of Ring-Opening Polymerization of L,L-Lactide, Industrial & Engineering Chemistry Research 2011, 50, 7927–7940

[47] H. R. Kricheldorf, I. Kreiser-Saunders, C. Boettcher, Polylactones. 31. Sn(II)octoate-initiated polymerization of L-lactide: a mechanistic study, Polymer 36 (1995), 1253-1259

[48] M. N. V. Ravi Kumar, Handbook of Polyester Drug Delivery System, CRC Press, 8.6.2016 – 738 p. Available (accessed on 9.6.2016): https://books.google.fi/books?id=5h9jDAAAQBAJ&pg=PA295&lpg=PA295&dq=PLGA+50:50+synthesis+solvent&source=bl&ots=ghjsCCfN16&sig=dc03hFFw

XjfRibeSVuF5Du4sffg&hl=fi&sa=X&ved=0ahUKEwjgps6twPDNAhVRKywKHUjhC-s4ChDoAQgjMAE#v=onepage&q=PLGA%2050%3A50%20synthesis%20solvent&f=false:

[49] S. Alimohammadi, R. Salehi, N. Amini and S. Davaran, Synthesis and Physico-chemical Characterization of Biodegradable PLGA-based Magnetic Nanoparticles Containing Amoxicilin, Bulletin of Korean Chemical Society 2012, Vol. 33, No. 10, 3225-3232

[50] M. Murayama, F. Sanda and T. Endo, Anionic Ring-Opening Polymerization of a Cyclic Carbonate Having a Norbornene Structure with Amine Initiators, Macromolecules 1998, 31 (3), 919–923

[51] H. R. Kricheldorf, S.-R. Lee and B. Weegen-Schulz, Macromolecular Chemistry and Physics 1996, 197, 1043-1054

[52] Benzoic Acid SDS Sigma Aldrich, Available (accessed on 30.7.2016): http://www.sigmaaldrich.com/MSDS/MSDS/PrintMSDSAction.do?name=msdspdf_1607212151553209 30.7.2016

[53] Oxidation of Ethylbenzene, measured Benzoic acid NMR spectrum. Available (accessed on 22.5.2016): http://www.gravitywaves.com/chemistry/CHE303L/OxidationArene_10.htm

[54] J. Clayden, N. Greeves, S. Warren, Organic Chemistry, Oxford University Press Inc., 2012, 1234 p.

[55] C. Thomas, F.Peruch and B. Bibal, Ring-opening polymerization of lactones using supramolecular organocatalysts under simple conditions, RSC Advances 2012, 2, 12851–12856

[56] A. Kapelski, Stereocontrolled, Ring-Opening Polymerization of Lactide Monomers by Lewis-Acidic Metal Complexes, Doctoral Thesis, Laboratories of Prof. Dr. Jun Okuda at the Institute of Inorganic Chemistry at RWTH Aachen University, Germany, 2009, 191 p.

[57] PLGA 5:95 Product Sheet, Sigma Aldrich. Available (accessed on 6.7.2016): http://www.sigmaaldrich.com/catalog/product/aldrich/790214?lang=fi&region=FI

[58] S. O. Ojwach and T. P. Zaca, Ring-Opening Polymerization of Lactides by (Pyrazol-1-ylmethyl)pyridine Zn(II) and Cu(II) Complexes: Kinetics, Mechanism and Tacticity Studies, South African Journal of Chemistry 2015, 68, 7–14

[59] C. Shi, D. Guo, K. Xiao, X. Wang, L. Wang & J. Luo, A drug-specific nanocarrier design for efficient anticancer therapy, NATURE COMMUNICATIONS 2015, 6, 7449

[60] D. Bhadra, S. Bhadra, N. K. Jain, Pegylated Lysine Based Copolymeric Dendritic Micelles For Solubilization And Delivery Of Artemether, Journal of Pharmaceutical Science (www.cspscanada.org) 2015, 8 (3), 467-482

[61] J. E. Sheppeck II, H. Kar and H. Hon, A convenient and scaleable procedure for removing the Fmoc group in solution, Tetrahedron Letters 41 (2000), 5329-5333

[62] K. D. Stigers, M. R. Koutroulis, M. De Chung and J. S. Nowic, Fmoc: A More Soluble Analogue of the 9-Fluorenylmethoxycarbonyl Protecting Group, Journal of Organic Chemistry 2000, 65, 3858-386

[63] S. Höck, R. Marti, R. Riedl, M. Simeunovic, Thermal Cleavage of the Fmoc Protection Group, CHIMIA International Journal for Chemistry 2010, Vol. 64, No. 3, 200-202(3)

[64] G. B. Fields, Methods for Removing the Fmoc Group, Available (accessed on 4.6.2016): http://www.renyi.hu/~stipsicz/skin/PepsynthProt/Ch_2.pdf

[65] Mechanism proposed by Tuija Alanko and verified by Docent Nuno Candeias

[66] Jari Rinkinen, Hydrauliikan ja koneautomatiikan perusteet, luentomateriaali, TTY, 1996

[67] Geoffrey Barnes, Ian Gentle, Interfacial Science: An Introduction, Oxford University Press, 2005, 325 p.

[68] THF (anhydrous) Product Sheet, Sigma Aldrich. Available (accessed on 15.7.2016): http://www.sigmaaldrich.com/catalog/product/sial/401757?lang=fi&region=FI

[69] Kinematic Viscosity of Tetrahydrofuran, DDBST GmbH. Available (accessed on 4.7.2016): http://www.ddbst.com/en/EED/PCP/VSK_C159.php

[70] Acetone Product Sheet, VWR. Available (accessed on 9.7.2016): https://us.vwr.com/store/product/4537832/acetone-j-t-baker

[71] Benzoic Acid Product Sheet, Sigma Aldrich. Available (accessed on 9.7.2016): http://www.sigmaaldrich.com/catalog/product/sial/242381?lang=fi&region=FI

[72] Chloroform (anhydrous) Product Sheet, Sigma Aldrich. Available (accessed on 9.7.2016): http://www.sigmaaldrich.com/catalog/product/sial/372978?lang=fi&region=FI

[73] MagniSolv™, deuterated solvents for NMR Product Brochure, Merck Millipore. Available (accessed on 9.7.2016): http://www.merckmillipore.com/INTERSHOP/web/WFS/Merck-FI-Site/en_US/-/EUR/ShowDocument-Pronet?id=14730

[74] DBU Product Sheet, Sigma Aldrich. Available (accessed on 15.7.2016): http://www.sigmaaldrich.com/catalog/product/aldrich/139009?lang=fi&region=FI

[75] Dichloromethane (anhydrous) Product Sheet, Sigma Aldrich. Available (accessed on 9.7.2016): http://www.sigmaaldrich.com/catalog/product/sial/270997?lang=fi&region=FI ref

[76] Lactide Product Sheet, Sigma Aldrich. Available (accessed on 15.7.2016): http://www.sigmaaldrich.com/catalog/product/aldrich/367044?lang=fi&region=FI&cm_sp=Insite-_-prodRecCold_xorders-_-prodRecCold2-1 ref. 15.7.2016

[77] Glycolide Product Sheet, Sigma Aldrich. Available (accessed on 9.7.2016): http://www.sigmaaldrich.com/catalog/product/sigma/g1796?lang=fi&region=FI

[78] Ethyl Acetate Product Sheet, Sigma Aldrich. Available (accessed on 9.7.2016): http://www.sigmaaldrich.com/catalog/product/sial/33211?lang=fi&region=FI

[79] Hexane Product Sheet, Sigma Aldrich. Available (accessed on 9.7.2016): http://www.sigmaaldrich.com/catalog/product/sial/208752?lang=fi&region=FI

[80] Available (accessed on 9.7.2016): https://fi.vwr.com/store/product/733881/methanol-for-analysis-emsure-acs-iso-reag-ph-eur-hdpe-plastic-bottle ref 9.7.2016

[81] *N*,*N*-Dimethylformamide (anhydrous) Product Sheet, Sigma Aldrich. Available (accessed on 9.7.2016): http://www.sigmaaldrich.com/catalog/product/sial/227056?lang=fi&region=FI

[82] Fmoc-Lys(Fmoc)-OH Product Sheet, Sigma Aldrich. Available (accessed on 9.7.2016): http://www.sigmaaldrich.com/catalog/product/aldrich/47317?lang=fi&region=FI

[83] Piperidine Product Sheet, Sigma Aldrich. Available (accessed on 9.7.2016): http://www.sigmaaldrich.com/catalog/product/sial/104094?lang=fi&region=FI

[84] Poly(ethylene glycol) methyl ether Product Sheet, Sigma Aldrich. Available (accessed on 9.7.2016): http://www.sigmaaldrich.com/catalog/substance/polyethyleneglycolmethylether12345900474411?lang=fi&region=FI

[85] Available (accessed on 9.7.2016): https://fi.vwr.com/store/product/732541/2-propanoli-technical ref 9.7.2016

[86] Thionyl chloride Product Sheet, Sigma Aldrich. Available (accessed on 15.7.2016): http://www.sigmaaldrich.com/catalog/product/sial/230464?lang=fi&region=FI

[87] Available (accessed on 9.7.2016): Product 28675.294 https://fi.vwr.com/store/product/766567/tolueeni-analar-normapur, ref. 15.7.2016

[88] Triethylamine Product Sheet, Sigma Aldrich. Available (accessed on 15.7.2016): http://www.sigmaaldrich.com/catalog/product/sial/471283?lang=fi&region=FI

[89] CRC Handbook of Chemistry and Physics, 96th ed., CRC Press, Taylor and Francis Group, 2015, Boca Raton, USA

[90] New Era syringe pumps. Available (accessed on 9.7.2016): http://www.syringepump.com/oem.php#aa ref. 26.6.2016

[91] Numatics R880G02A in Valves Direct. Available (accessed on 26.4.2016): http://www.valves-direct.com/product-item/p/numatics-r880g02a-g14-0-01-bar-series-r88-high-flow-precision-regulators-106817/

[92] A. Efimov, NMR Spectroscopy, lecture material 2016, TUT

[93] L-lysine NMR Spectrums, Biological Magnetic Resonance Data Bank. Available (accessed on 11.6.2016): http://bmrb.wisc.edu/metabolomics/mol_summary/show_data.php?molName=L_lysine&id=bmse000043

[94] H. E. Gottlieb, V. Kotlyar and A. Nudelman, NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities, Journal of Organic Chemistry, Vol. 62, No. 21, 1997

[95] G. W. Ehrenstein, G. Riedel, P. Trawiel, Thermal Analysis of Plastics: Theory and Practice, Carl Hanser Verlag GmbH & Co. KG, 2004, 399 p.

[96] C. A. van Blitterswijk, Tissue Engineering, Elsevier Academic Press, London, 2008, 760 p.

[97] PGA Product Sheet, Sigma Aldrich. Available (accessed on 18.8.2016): http://www.sigmaaldrich.com/catalog/product/aldrich/457620?lang=fi&region=FI

[98] P. Lan and L. Jia, Thermal Properties of Copoly(L-lactic acid/glycolic acid) by Direct Melt Polycondensation, Journal of Macromolecular Science, Part A : Pure and Applied Chemistry 2006, 43, 1887–1894

[99] Hydrobromic acid solution 33 wt. % in acetic acid, Product Sheet, Sigma Aldrich. Available (accessed on 15.7.2016): http://www.sigmaaldrich.com/catalog/product/fluka/18735?lang=fi&region=FI

[100] E. Valtanen, Koneenrakentajan taulukkokirja, 11. painos, Gummerus kirjapaino Oy, Jyväskylä 2000, 943 p.

APPENDIX A. NUMATICS MODEL R880G02A SPECIFICATIONS [91]

PIL numatics

PRECISION REGULATOR

G 1/4 to G 1/2

Series
R80-R82
R88

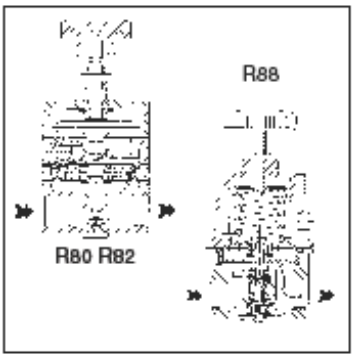
FEATURE

- *High precision and multi-stage pressure regulators*
- *Standard version R80, high relief version R82 and high flow version R88*

GENERAL / OPERATING PRINCIPLE

For R80-R82 series, the highest degree of regulation are achievable by reacting to downstream pressure fluctuations as small as 0,7 mbar. Action occurs as downstream pressure is piloted to the control chamber to act on a finely tuned stainless steel volume capsule.
A continuous bleed adjusts the pilot diaphragm causing appropriate movement of the valve.

The R88 Series pressure control regulator is designed for high flow and accurate pressure control utilizing a rolling diaphragm to insure a constant output pressure



SPECIFICATIONS	R80 - R82	R88
Flow rate (ANR)	460 l/min	1800 l/min (courbes p 2)
Relief capacity	R80 = 62 l/min ANR R82 = 305 l/min ANR	120 l/min ANR
Sensitivity	3 mbar	0,6 mbar
Pilot bleed rate	2,5 l/min	0,55 à 6,5 l/min
Maximum supply pressure	10 bar	17 bar
Filtered air	25 µm	25 µm
Max.oil concentration in air	1 mg/cm³	

CONSTRUCTION	
Body	Die cast zinc
Diaphragms	NBR
Knob	Phenolic plastic

CHOICE OF EQUIPMENT

R80 0G 02 F G

Precision regulator	type
standard	R80
High relief precision regulator	R82

Options	
–	Without option
G	With gauge
T	Tamperproof

Port size	
G1/4	02
G3/8	03

Pressure range	
D	0,1 à 2,8 bar
E	0,1 à 4 bar
F	0,1 à 8 bar

APPLICATIONS

- Air gauging, Gas mixing, Web tensioning, Roll loading, Air hoists

H

R88 0G 02 F G

Precision regulator	type
High flow precision regulator	R88

Options	
–	Without option
G	With gauge
M	Mounting brackets

Port size	
G1/4	02
G3/8	03
G1/2	04

Pressure range	
A	0 à 0,1 bar
B	0 à 1 bar
C	0 à 2 bar
E	0,05 à 4 bar
F	0,1 à 10 bar

APPLICATIONS

- Test equipment, Roll loading, Web tensioning, Actuators, Gas mixing, Test panels, Clutch and brake controls

Order exemple: **R800G02F**

This is a R80 Series Precision Regulator with high relief.
Port size is G 1/4. It has a pressure range from 8 bar

All leaflets are available on: **www.asconumatics.eu**

APPENDIX B. NITROGEN ATMOSPHERE DESIGN WITHOUT GLOVEBOX

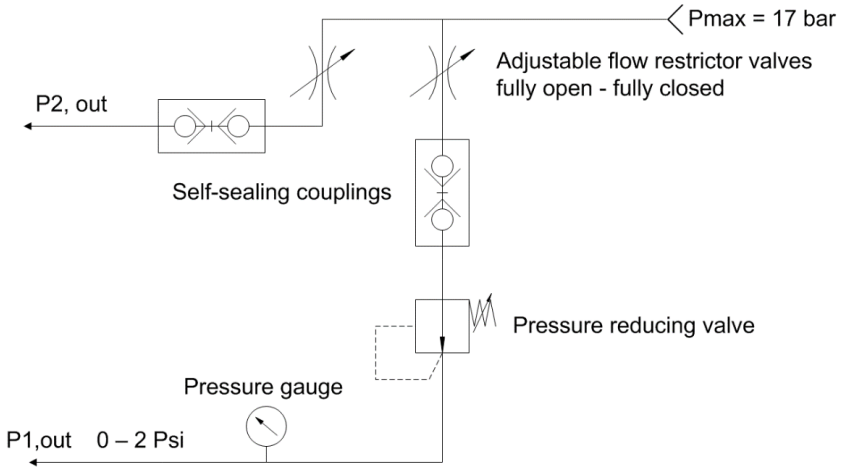
Inert atmosphere, in practice, requires that reaction is kept shielded with nitrogen or another inert gas, while adding catalyst or other reagents, release gaseous compound extracts and preventing air to enter into the reaction mixture. Ambient conditions with nitrogen atmosphere means ambient temperature and pressure. In this study ambient conditions were supposed to be kept with slow nitrogen flow though reaction, carrying gaseous extract from reaction mixture to fume hood, keeping pressure and temperature in reaction vessel equal to ambient pressure and temperature.

Pressure reducer valves mounted to nitrogen gas cylinders are most often designed to operate at range from a few bars to 15 bars while full cylinder pressure is 200 bar. Adjusting those valves to outlet port pressure setting less than 0.1 bar is virtually impossible: valve stem size is different order in pressure and volume flow ranges, also stem absolute
precision and clearance to the sleeve are greater than the total control range needed. One may succeed to set pressure for a moment right, but even smallest variations in flow, temperature or backpressure causes the stem to move just slightly to either close the cone or leave greater flow channel open.

When volatile solvents are present, it is essential to keep the nitrogen flow as low as possible, still having flow throughout the system to extract gaseous by-products. Nitrogen outlet needs to be a little a bit more restricted in comparison to inlet to ensure tiny overpressure in the reaction vessel. For example, 1 mm inlet with 0.8 mm outlet prevents air backflow to the reaction at outlet. Pressure difference order of magnitude through the reaction instrumentation is from a few to dozens pascals. For example, for a 2 m long pipe of 20 mm in diameter 2 mbar pressure loss requires around 100 ml/s flow [100, p. 454]. The flow rate commonly needed starts from bubble by bubble seen in bubble bottle: assuming that bubble diameter is 1 mm and rate is 10 bubbles per second, flow rate would be $5 \cdot 10^{-4}$ ml/s.

On the other hand, higher flow rates are frequently needed for shielding opened chemicals and starting materials, normally during the reaction when packing them back to storage. Thus the gas cylinder valve cannot be simply replaced with a low outlet pressure device or another nitrogen gas cylinder is needed, so a bypass manifold between the main valve and low pressure range pressure reducing valve is needed. Because the bypass flow is normally used with blow gun and leakages occur, it is convenient to equip bypass line with some kind of low cost restrictor, able to fully close, in order to save nitrogen. Pressure valve line is also convenient to equip with similar restrictor since the valve setting can thus be kept unchanged while cutting off the flow. Other basic equipment are quick couplings to both manifold outlets and pressure gauge.

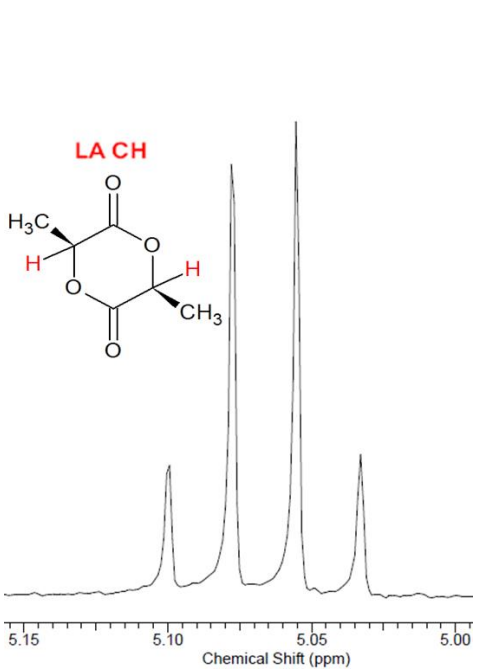
Taking all the details above under consideration, nitrogen control design yielded to schematic presented in 0. Experimental part of the study shall start with purchasing components and putting the system together.

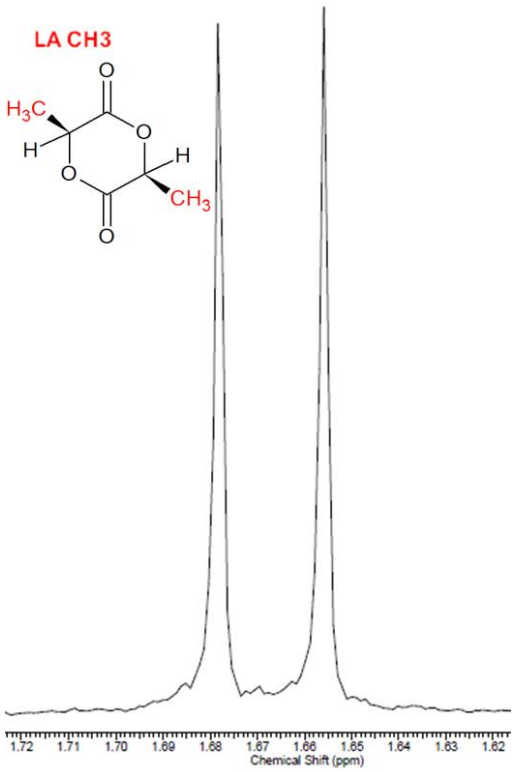


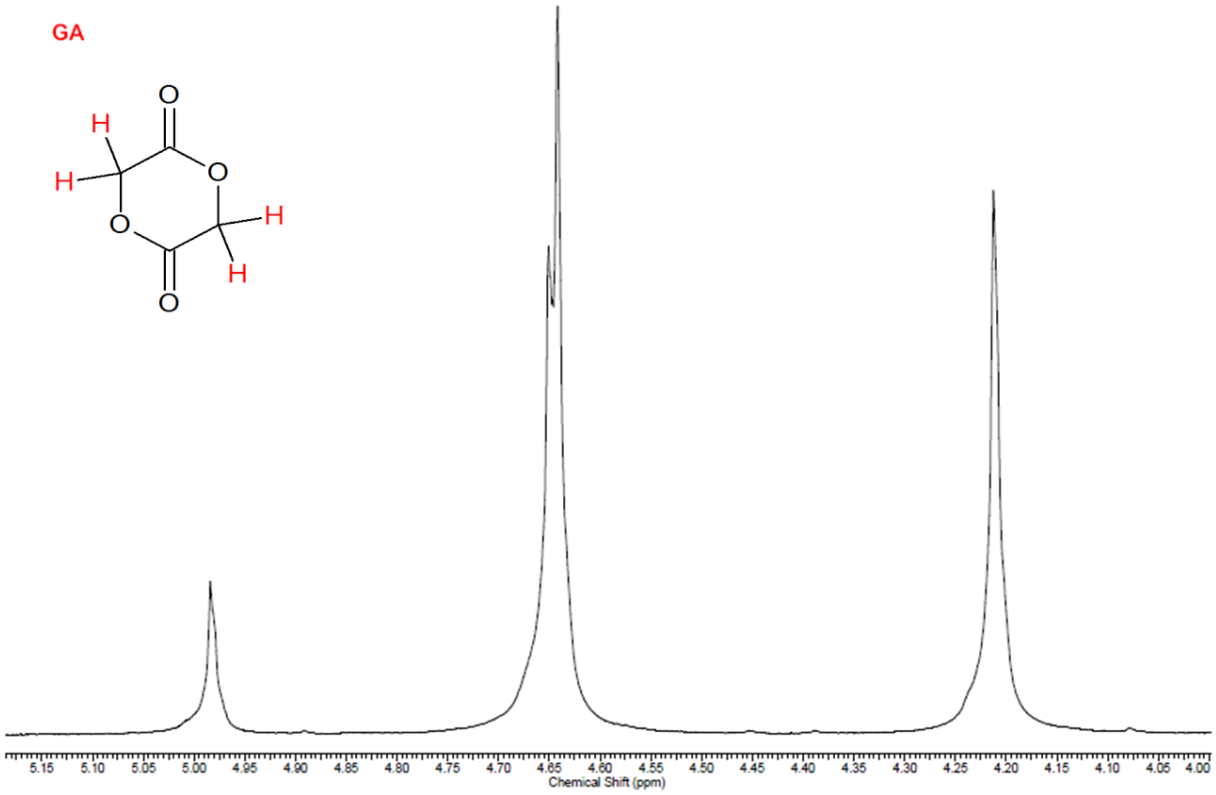
Schematic diagram of the pneumatics system designed, P1 and P2 refers to outlet port pressures, Pmax refers to highest allowed inlet port pressure.

APPENDIX C. MEASURED NMR REFERENCE SPECTRUMS

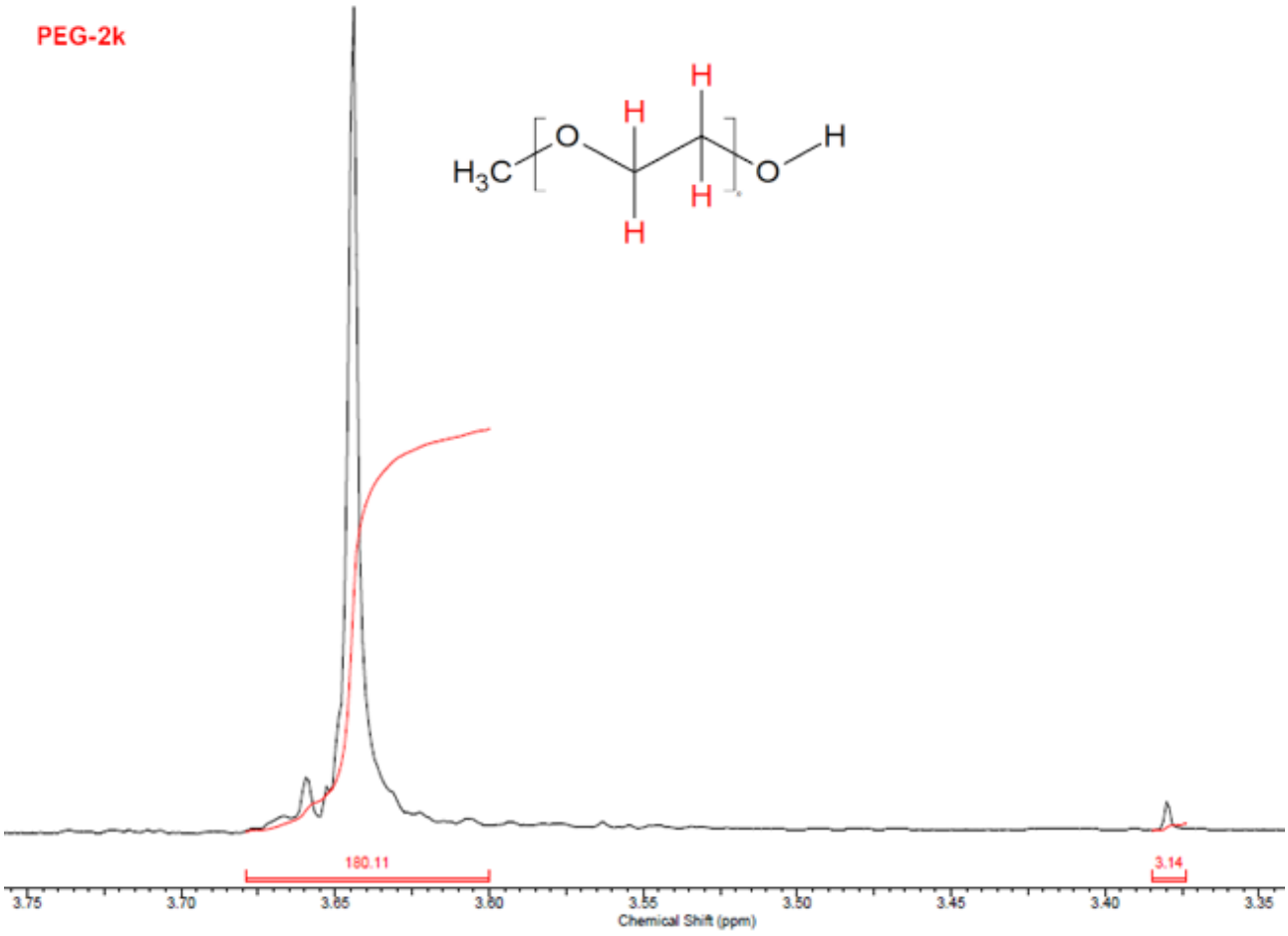
NMR reference spectrums were measured for monomers, initiator and protected lysine. Peaks and corresponding protons are shown in the LA, GA and PEG spectrums (PEG CH_3-O- at 3.37 ppm not indicated).

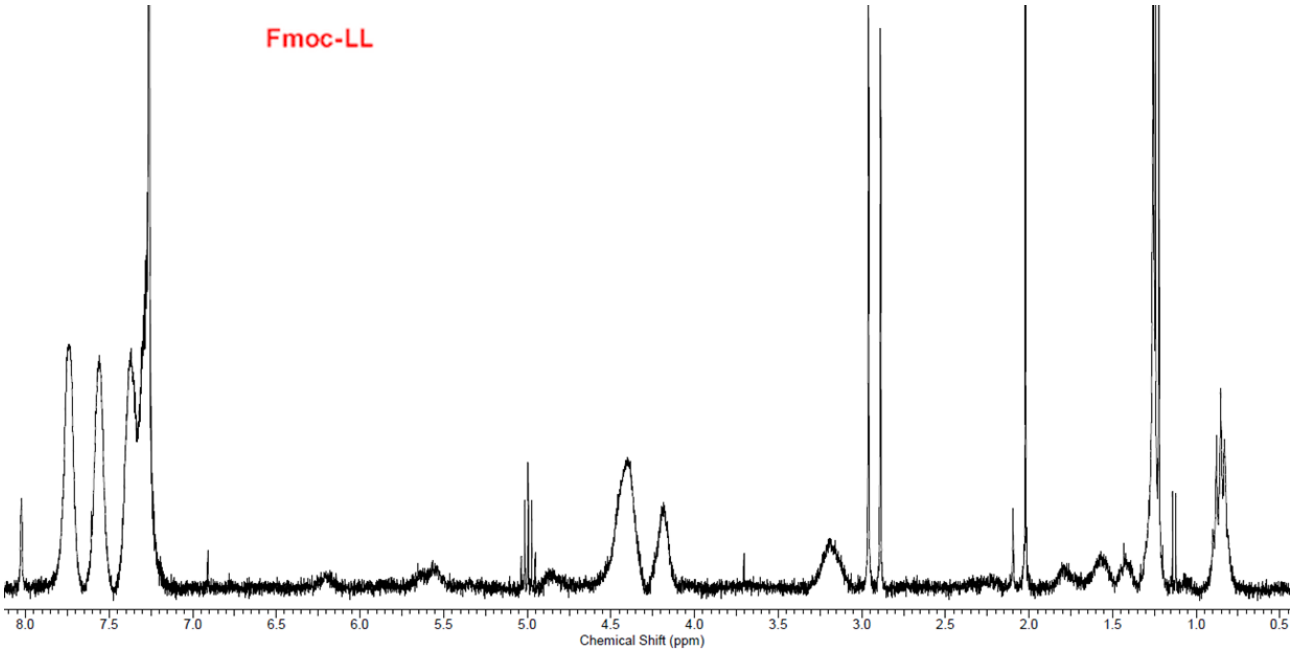






d


PEG-2k
H
H
H
H
H
H3C
O
O
H
180.11
3.14
3.75
3.70
3.65
3.60
3.55
3.50
3.45
3.40
3.35
Chemical Shift (ppm)


Fmoc-LL
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)

APPENDIX D: EVAPORATION CORRECTION FOR LACTIDE RATE CONSTANT DETERMINATION

Because evaporation rate depends on surface area and stirring rpm was all the way 300-350 *rpm*, surface area is approximately the spherical cap area ($2\pi rh$) if the thickness of the solution layer is constant. In this approximation evaporation rate would be linear between sampling times. Let *v* describe the evaporation rate, then

$$V_i = V_{0,i} - \frac{V_{0,i} + V_i}{2} = V_{0,i}(1 - v) \tag{D1}$$

where V_i denotes for the volume left at stage i and $V_{0,i}$ denotes volume initially in stage i. On the other hand, before and after sampling consecutive phases follow relation

$$V_{0,i+1} = V_i - V_{s,i} \tag{D2}$$

where $V_{s,i}$ denotes sample i volume. Thus V_3, the volume left at the end of phase three, at time four (end time of the reaction) is

$$\begin{aligned} V_3 &= V_{0,3}(1 - v) = (V_2 - V_{s,2})(1 - v) \\ &= ((V_{0,2}(1 - v) - V_{s,2})(1 - v) \\ &= ((V_1 - V_{s,1})(1 - v) - V_{s,2})(1 - v) \\ &= ((V_{0,0}(1 - v) - V_{s,1})(1 - v) - V_{s,2})(1 - v) \end{aligned} \tag{D3}$$

Texas Instruments TI-nSpire CX CAS symbolic calculator gives single solution

v = 0,15874 with multiplicity of 3.

Using equation (B1), computed sampling time volumes and average volumes for each time interval are shown in Table B1, with evaporation-correction constants $k^{i\prime}$, to multiply each concentration $[i]_0$ for each time interval calculated in average volume, calculated $k^{i\prime} = (V_{0,i} - \sum V_{s,i-1})/V_{ave,i}$.

Table B1. *Initial volumes in each sample time, average volumes between sample times and evaporation-correction constants.*

Time interval	**V_i [ml]**	**$V_{ave,i}$ [ml]**	**$k^{i\prime}$**
$t_{0,1}$	1.68252	1.84126	1.08621
$t_{1,2}$	0.94433	1.03343	1.39342
$t_{2,3}$	0.49999	0.54716	1.99210

Average rate constant could also be computed combining consecutive steps, which would be handy if the last sample carries superior accuracy. Combining all the three rate constant equations gives

$$\frac{[LA]_1}{k^{1'}[LA]_0} = e^{-k_L(k^{1'})^2[DBU][OH]t_1} \Leftrightarrow [LA]_1 = k^{1'}[LA]_0 e^{-k_L(k^{1'})^2[DBU][OH]t_1}$$

$$\frac{[LA]_2}{k^{2'}[LA]_1} = e^{-k_L(k^{2'})^2[DBU][OH]t_2} \Leftrightarrow [LA]_2 = k^{2'}[LA]_1 e^{-k_L(k^{2'})^2[DBU][OH]t_2}$$

$$\Leftrightarrow [LA]_2 = k^{2'}k^{1'}[LA]_0 e^{-k_L(k^{1'})^2[DBU][OH]t_1}\, e^{-k_L(k^{2'})^2[DBU][OH]t_2}$$

$$\frac{[LA]_3}{k^{3'}[LA]_2} = e^{-k_L(k^{3'})^2[DBU][OH]t_3} \Leftrightarrow [LA]_3 = k^{3'}[LA]_2 e^{-k_L(k^{3'})^2[DBU][OH]t_3}$$

$$\Leftrightarrow [LA]_3 = k^{3'}k^{2'}k^{1'}[LA]_0 e^{-k_L(k^{1'})^2[DBU][OH]t_1}\, e^{-k_L(k^{2'})^2[DBU][OH]t_2} e^{-k_L(k^{3'})^2[DBU][OH]t_3}$$

$$= k^{3'}k^{2'}k^{1'}[LA]_0 e^{-k_L[DBU][OH](t_1(k^{1'})^2+t_2(k^{2'})^2+t_3(k^{3'})^2)}$$

$$\Leftrightarrow ln\left(\frac{[LA]_3}{k^{3'}k^{2'}k^{1'}[LA]_0}\right) = -k_L[DBU][OH]t(t_1(k^{1'})^2 + t_2(k^{2'})^2 + t_3(k^{3'})^2)$$

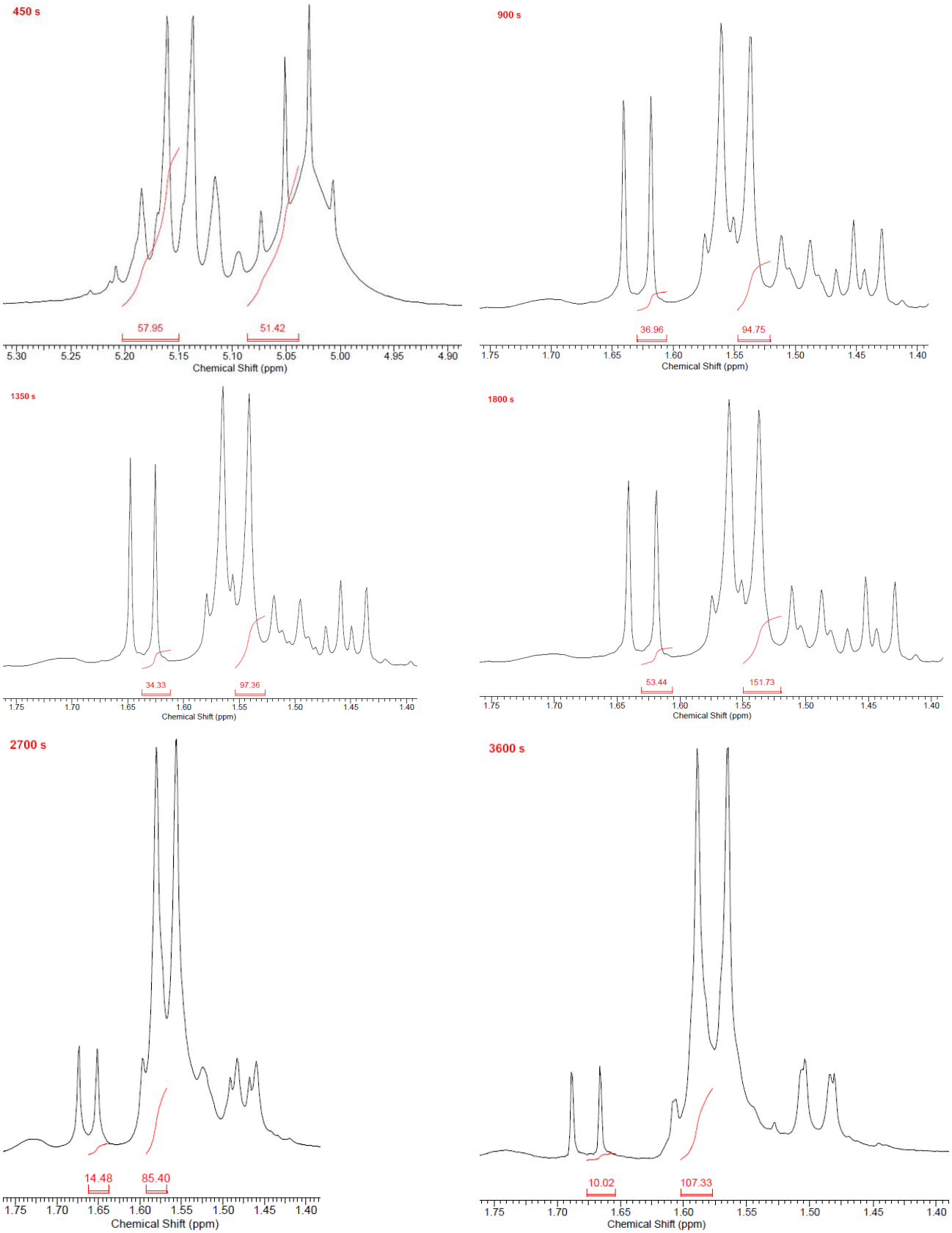
$$\Leftrightarrow k_L = -\frac{ln\left(\frac{[LA]_3}{k^{3'}k^{2'}k^{1'}[LA]_0}\right)}{[DBU][OH](t_1(k^{1'})^2 + t_2(k^{2'})^2 + t_3(k^{3'})^2)}$$

$$= -\frac{\ln\left(\frac{48.6\text{mM}}{1.99210\cdot 1.39342\cdot 1.08621\cdot 404.81\text{mM}}\right)}{15.045\ \text{mM}\cdot 6.8635\ \text{mM}\cdot(900\text{s}\cdot(1.08621)^2+780\text{s}\cdot(1.39342)^2+1020\text{s}\cdot(1.99210)^2)}$$

$$= 4.7124\ \frac{\text{l}^2}{\text{mol}^2\text{s}}$$

Value differs significantly from the time weighted average rate constant value 5.77, because this ignores intermediate samples and underlines last samples significance, so even though this is mathematically accurate, this does not apply here.

APPENDIX E. PLA RATE CONSTANT IN CHLOROFORM FROM NMR



NMR spectrums of PEG-PLA polymerization in chloroform with corresponding reaction times

APPENDIX F: PID CONTROLLER GAINS

PID controller gains that were used for the flow demand vector simulations in different phases of this study. Controller tuning needed depends on the dynamics of the system and is thus different with different rate constants. Controller tuning goal was simply match the conversion speeds as fast as possible, without overshoot.

First attempt	
P	100
I	1.2
D	0

Second attempt	
P	10
I	0
D	1300

Refined model	
P	20
I	0.2
D	500

APPENDIX G: PEG-PLGA-LL SYNTHESIS, NMR SPECTRUMS

Deprotected (red) and protected (green) polymer and protected L-lysine NMR spectrums. Deprotected in its correct position, protected at -0.1 ppm and L-lysine at -0.2 ppm.