Doctor of Philosophy Dissertation

Titled

Syntheses of Novel Biodegradable Materials from Biorenewable Resources through Nitroxide Mediated Polymerization; Green, Sustainable and Environmentally Benign Materials.

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Main supervisor : Professor Pierluigi Stipa (Università Politecnica delle Marche, UNIVPM)

Co-supervisor : Doctor Thomas Trimaille (Aix-Marseille Université, AMU**)**

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Prof. Pierluigi Stipa	Vernon Tebong Mbah

Certification

We, Professor Pierluigi Stipa, Dr. Didier Gigmes, Professor Mario Smet and Professor Jacques Lalevée, hereby certify that we have read this manuscript and recommend it for acceptance by the Università Politecnica delle Marche, a dissertation entitled syntheses of novel biodegradable materials from biorenewable resources through nitroxide mediated polymerization; green, sustainable and environmentally benign materials in fulfillment of a degree in Doctor of Philosophy "Doctor Europaeus" in Engineering at the Università Politecnica delle Marche, Italy.

Supervisor: Prof. Pierluigi Stipa

Referee: Dr. Didier Gigmes

Referee: Prof. Mario Smet

Referee: Prof. Jacques Lalevée

Declaration

I declare that the research work embodied in this dissertation entitled **syntheses of novel biodegradable materials from biorenewable resources through nitroxide mediated polymerization; green, sustainable and environmentally benign materials,** submitted for the degree of Doctor of Philosophy in engineering was carried out by me, at Università Politecnica delle Marche and Aix-Marseille Université, where references have been made to the work of others. I confirm that this work has not been previously or concurrently submitted for any other degree, diploma or qualification at the Università Politecnica delle Marche or Aix-Marseille Université.

Acknowledgements

I acknowledge the sponsorship and financing of this work by Ministero dell'Istruzione, dell' Università della Ricerca–MIUR, Italy, through Università Politecnica delle Marche (UNIVPM), Italy. Likewise, I give special thanks to my supervisor, professor Pierluigi Stipa who did not only act as my mentor but also a very good friend throughout my PhD program. Thanks to Dr. Roberto Cipolletti for his great support and Dr. Simona Sabatinni for the FTIR analyses and to all my team members at UNIVPM.

I equally acknowledge Aix-Marseille Université (AMU) for sponsoring part of this work and I give a huge thank you to Dr. Thomas Trimaille who supervised me during my time there. I appreciate the mentorship and hospitality offered to me by Dr. Didier Gigmes. Many thanks to my team members and others who supported me throughout my stay at AMU, especially Dr. Vincent Pertici whom I worked with closely and Dr. Marion Rollet who helped me with the SEC DMF analyses. I acknowledge the collaboration of all the other partners of UNIVPM that I worked with and are not mentioned here.

Many thanks to Dr. Giovanni Rafaiani at Università di Camerino and the team at Università di Bologna who collaborated with us.

To my father, Mr. Vincent Tebong Mbah and mother, Mrs. Mary Angwi Mbah, I cherish your unflinching support and encouragement during this period which I will always remember. Lastly, to Mr. Mike Miller, who also encouraged and supported me unwavering, I say thank you my very good friend.

Most importantly, I give an enormous gratitude to the almighty GOD who led me throughout this period as always.

List of Abbreviations

Atom transfer radical polymerization	ATRP
Biodegradable polymer	BDP
Bromoisobutyryl bromide	BIBB
Cellulose acetate	CA
Castor oil	CO
Dichloromethane	DCM
Electron paramagnetic resonance	EPR
Free radical polymerization	FRP
Free radical living polymerization	FRLP
Gel permeation chromatography	GPC
2-Hydroxyethyl acrylate	HEA
Polylactic acid	PLA
Polycaprolactone	PLC
Polyvinyl alcohol	PVA
Polyhydroxyalkanoate	PHA
Polyethylene glycol	PEG
Polyethylene glycol methacrylate	PEGMA
Poly L-alanine	PLAla
Poly(N-isopropylacrylamide)	PNIPAAm
Polystyrene	PS
Macroalkoxyamine	MA
2-(methacryloyloxy)ethyl oleate	MAEO
Methyl methacrylate	MMA
Nitroxide mediated polymerisation	NMP
N-isopropylacrylamide	NIPAm
Nuclear magnetic resonance	NMR

N,*N*,*N*′,*N*″,*N*″-Pentamethyldiethylenetriamine	PMDETA
Size exclusion chromatography	SEC
Ring opening polymerization	ROP
Triethylamine	TEA

Table of Contents

Abstract

We have depended on petroleum or fossil sources for raw materials to synthesize polymers in order to meet the high demand of polymeric materials for over a century. Notwithstanding, polymers derived from these sources (synthetic polymers) are toxic to the environment. They are also expensive and are scarce sometimes because their raw material sources are nonrenewable. Biodegradable polymers (BDP) derived from biorenewable resources on the other hand are ecofriendly, cost-effective and sustainable. The raw materials which are mainly from agricultural and forestry products and the shells of crustaceans are readily available, cheap and renewable. The final polymers derived from these raw materials are potential replacement to synthetic polymers.

As appealing as biodegradable polymers derived from biorenewable resources might be, there a number of challenges surrounding their synthesis and their properties sometimes do not meet the demands of certain applications. Some applications require very strong mechanical properties which may not be in possession of some biodegradable polymers derived from biorenewable resources. Copolymerization of these polymers with synthetic ones produces novel versatile polymeric materials which are biodegradable and possess robust mechanical properties suitable for different applications. Nitroxide mediated polymerization (NMP) is one of the most effective and efficient free radical living polymerization technique employed in chemical grafting of polymers. NMP as well as other free radical polymerization techniques can be used in tuning polymers into macromolecular architectures of different shapes, sizes and structures as opposed to conventional condensation polymerization. The advantage of NMP is that, it is a facile metal free process and therefore greener than other free radical techniques. This process is also viable for employment in the commercial production of polymers. In this light, the exploitation of the NMP technique for the production of biodegradable polymers from biorenewabe resources is essential and further research is required.

The first chapter discusses elaborately the objectives of replacing synthetic polymers from petroleum sources with biodegradable ones from biorenewable resources, underlining the main reasons for this work. It further states the development in the research and production of biodegradable polymers from biorenewable resources.

In the second chapter, the art of NMP and its role in the synthesis and development of biodegradable polymers from renewable resources was discussed. It also entails a brief discussion on nitroxides and alkoxyamines which are the most important species involved in the NMP process.

Chapter three constitutes the experimental synthesis of nitroxides, macro(alkoxyamines), monomers from biorenewable resources and graft copolymers. 2-phenyl-3-(phenylimino)-3H-indole 1-oxide (DPAIO) and its alkoxyamine were synthesized. N-tert-butyl-N-(1-diethyl phosphono-2,2-dimethylpropyl) nitroxide (SG1) nitroxide and its alkoxyamine, commercial BlocBuilder MA were mostly employed in the NMP process to produce the copolymers. The monomers derived from biorenewable resources included corn starch, cellulose, chitosan, castor oil, maleic acid, acrylamide and amino acids. Polystyrene (PS) and methyl methacrylate (MMA) are some synthetic polymers which were grafted onto the polymers from biorenewable resources. The copolymers in this chapter where synthesized through atom transfer radical addition (ATRA) and NMP.

The products synthesized in chapter three were characterized and the results discussed in chapter four. The characterization techniques used were near magnetic resonance (NMR), Fourier Transform Infra-red (FTIR), differential scanning calorimetry (DSC) and electron paramagnetic resonance (EPR). FTIR analyses confirmed the formation of several copolymers were formed but only cellulose acetate-g-poly (methyl methacrylate) (CA-g-PMMA) was further confirmed by NMR and DSC.

Chapter five is a summary of chapter one to four. It provides an elaborate argument on the replacement of synthetic polymers with biodegradable polymers from biorenewable resources.

Chapter six discusses a specific project carried out at Aix-Marseille Université, centre de la recherche scientifique (CNRS), institute chimie radicalaire (ICR). The aim of this project was to develop hydrogels for the treatment and recovery of ischemic stroke. A novel block-graft amphiphilic copolymer, polylactide-block-poly(N-isopropylamide-co-polyethylene glycol methacrylate) (PLA-b-P(NIPAAm-co-PEGMA)) was synthesized. 15wt% of the hydrogel formed by the polymer in phosphate buffer saline solution undergoes a sol-gel transition between 25°C and 37°C through micelle packing/rearrangement upon heating. The

synthesis of this biomaterial was based on the strategies of ring opening polymerization (ROP), intermolecular radical addition (IRA) and nitroxide mediated polymerization (NMP). Characterization of the copolymers was done by size exclusion chromatography (SEC), dynamic light scattering (DLS) and NMR. 15wt% of the hydrogel degrades after 48 h at 37°C by hydrolysis. The biomaterial also showed good mechanical properties since it did not shrink or break after heating at 50°C.

Key words: Biodegradable; Polymer; Nitroxide Mediated Polymerization, Biorenewable resources, cellulose, polylactide, poly(N-isopropylamide), SG1, BlocBuilder MA, hydrogel.

Riassunto

Con lo scopo di soddisfare l'elevata domanda di materiali polimerici con caratteristiche e proprieta molto varie, da oltre un secolo si è verificata una situazione di dipendenza da fonti di natura fossile (petrolio) per l'approvvigionamento di sostanze per la sintesi delle materie prime necessarie per la produzione di polimeri. Nonostante ciò, i polimeri derivati da queste fonti (polimeri sintetici) sono risultati dannosi per l'ambiente, introducendo un ulteriore costo per il loro smaltimento e, in quanto ottenuti da fonti non rinnovabili, spesso risultano scarseggiare. I polimeri biodegradabili (BDP), ottenuti da risorse biorinnovabili, sono invece ecologici, economici e sostenibili dal punto di vista ambientale. Le materie prime provengono infatti principalmente da fonti rinnovabili come prodotti agricoli, forestali e da materiale di scarto di origine animale (Es.: gusci di crostacei), risultando così facilmente disponibili e spesso più economici. I polimeri ottenuti da queste materie prime sono di conseguenza potenziali sostituti dei polimeri sintetici.

Per quanto comunque possano essere interessanti, i polimeri biodegradabili derivati da risorse biorinnovabili sono a volte caratterizzati da una serie di problematiche riguardo la loro sintesi; queste costituiscono al momento una serie di sfide, specialmente per quanto concerne le loro proprietà nei confronti delle loro possibili applicazioni. Alcune di quest'ultime infatti richiedono proprietà meccaniche molto spinte, che non necessariamente caratterizzano alcuni polimeri biodegradabili. Tuttavia, effettuando la copolimerizzazione di questi derivati con quelli di origine sintetica si è in grado di produrre nuovi materiali polimerici estremamente versatili caratterizzati da biodegradabilità, che possiedono allo stesso tempo le proprietà meccaniche richieste in diverse applicazioni. Nell'ambito della Polimerizzazione Radicalica Controllata (CRP), basata sull'uso di radicali liberi per la produzione dei cosiddetti "polimeri viventi", la Polimerizzazione Mediata da Nitrossidi (NMP) rappresenta una delle tecniche più efficaci e rispettosa dell'ambiente, consentendo di ottenere polimeri a innesto chimico. La NMP, come pure le altre tecniche di polimerizzazione radicaliche, può essere vantaggiosamente utilizzata per la produzione di polimeri con architetture macromolecolari di diverse forme, dimensioni e strutture, in maniera più efficiente rispetto la convenzionale

polimerizzazione per condensazione. In questo caso il vantaggio dell'uso della NMP risiede nel fatto di essere un processo che avviene a temperature decisamente più basse e senza l'uso di catalizzatori metallici, risultando così decisamente più ecocompatbile rispetto le tecniche classiche, e anche fattibile per un impiego commerciale nella produzione di polimeri. Sotto questa luce, l'uso della tecnica NMP per la produzione di polimeri biodegradabili da risorse biorinnovabili risulta particolarmente interessante, e necessita di ulteriori approfondimenti e ricerche.

Per quanto riguarda l'articolazione di questo lavoro di tesi, nel Primo Capitolo è stata svolta una discussone approfondita dei materiali di possibile impiego nell'ottica della sostituzione dei polimeri sintetici con quelli biodegradabili, sottolineando le ragioni principali di questo lavoro. Viene inoltre illustrato lo sviluppo nella ricerca e produzione di polimeri biodegradabili da risorse rinnovabili.

Nel Secondo Capitolo è stata descritta la metodica NMP e il suo ruolo nella sintesi e nello sviluppo di polimeri biodegradabili da risorse rinnovabili. A questo scopo, è stata anche riportata una breve discussione su nitrossidi e alcossilammine, che rappresentano le specie fondamentali che caratterizzano la NMP.

Il Terzo Capitolo riporta la sintesi sperimentale di nitrossidi, macro (alcossilammine), monomeri da risorse rinnovabili e copolimeri ad innesto. Sono stati sintetizzati il 2-fenil-3-(fenilimmino) -3H-indolo-1-ossido (DPAIO) e la sua alcossamina. Il nitrossido N-tert-butil-N- (1- dietilfosfono-2,2-dimetilpropil) (SG1) e la sua alcossiammina commerciale con Metil Acrilato (BlocBuilder) sono stati i derivati maggiormente impiegati nel processo NMP dei copolimeri. I materiali utilizzati originati da risorse biorinnovabili includono amido di mais, cellulosa, chitosano, olio di ricino, acido maleico e amminoacidi. Polistirene (PS) e metilmetacrilato (MMA) rappresentano invece alcuni dei polimeri sintetici che sono stati innestati sui polimeri da risorse biorinnovabili. I copolimeri sono stati quindi sintetizzati sia attraverso la NMP che la Polimerizzazione Radicalica con Trasferimento di Atomi (ATRP), realizzata per mezzo dell'aggiunta di opportuni agenti di trasferimento.

Nel Capitolo Quattro è stata riportata la caratterizzazione dei prodotti sintetizzati nel Terzo Capitolo insieme alla discussione dei risultati ottenuti. Le tecniche di caratterizzazione utilizzate sono rappresentate dalla Risonanza Magnetica Nucleare (NMR), dalla Spettroscopia Infrarossa a Trasformata di Fourier (FTIR), dalla Calorimetria a Scansione Differenziale (DSC) e dalla Risonanza Paramagnetica Elettronica (EPR). L'analisi condotta avvalendosi di queste tecniche ha permesso di confermare la formazione di numerosi copolimeri, fornendo allo stesso tempo i dati necessari per la loro caratterizzazione.

Il Capitolo Cinque riassume il lavoro svolto e i risultati ottenuti nel primo periodo della tesi, riportato nei capitoli precedenti, facendo il punto su quanto ottenuto per quanto riguarda il mio studio nei confronti dei copolimeri biodegradabili sintetizzati da risorse biorinnovabili.

Il Sesto Capitolo riporta invece i risultati ottenuti da un progetto specifico, realizzato nel periodo del mio dottorato, presso l'Université d'Aix-Marseille (AMU), nell'ambito dell'Institut de Chimie Radicalaire (ICR) associato al Centro Nazionale di Ricerca Scientifica (CNRS) a Marsiglia (Francia). Lo scopo di questo progetto è rappresentato dallo sviluppo di idrogel in vista di un loro possibile impiego nel trattamento e il recupero dell'ictus ischemico. In questo ambito è stato sintetizzato un nuovo copolimero anfifilico innestato a blocchi di acido polilattico-poli (N- isopropilammide-co-polietilenglicole metacrilato) [PLA-b-P (NIPAAm-co-PEGMA)]. Il 15% in peso dell'idrogel in questione è costituito dal polimero in soluzione salina in tampone fosfato, e in queste condizioni subisce una transizione sol-gel mediante impaccamento/riarrangiamento micellare se riscaldato tra i 25° C e i 37° C. La sintesi di questo biomateriale si basa sulle strategie di Polimerizzazione ad Apertura di Anello (ROP), di Addizione Radicalica Intermolecolare (IRA) e di Polimerizzazione radicalica Mediata da Nitrossidi (NMP). La caratterizzazione dei copolimeri ottenuti è stata effettuata mediante Cromatografia ad Esclusione Dimensionale (SEC), Diffrazione Dinamica della Luce (DLS) e NMR. L'idrogel ottenuto subisce degradazione dopo 48 ore a 37° C per idrolisi in misura del 15% in peso, mostrando però allo stesso tempo anche buone proprietà meccaniche con assenza di restringimento o frattura dopo riscaldamento a 50° C.

Parole chiave: Biodegradabile; Polimero; Polimerizzazione Mediata da Nitrossidi; Risorse Bioenergetiche; Cellulosa; Acido Polilattico; Poli (N-isopropilamide); SG1; BlocBuilder-MA; Idrogel.

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Chapter I. Introduction.

Polymers (plastics) make up more than 50% of materials used daily because they are cheap, light and can are easily processed. Furthermore, they are safe and convenient to use. They can be employed across a vast range of industries including packaging[1], pharmaceutical[2,3], food and agriculture, electronics[4], construction, automotive just to name a few. They can also be used in cultural heritage for the treatment of paintings and concrete. Advancement in technology and increase in global population are direct causes to the increase in demand and production of polymeric materials. However, most polymers used today are derived from petroleum or fossil sources and are often non-biodegradable[5] and non-compostable. In 2015, wide variety of petroleum-based synthetic polymers were estimated to be produced worldwide to the extent of approximately 140 million tons per year[6]. Although some of these conventional polymers are degradable[7], they may take several decades and produce toxins[8] in the process of

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[2] M. Gagliardi, 'A Poly(Ether-Ester) Copolymer for the Preparation of Nanocarriers with Improved Degradation and Drug Delivery Kinetics', *Materials Science and Engineering: C*, 59 (2016), 488–99.

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[4] Mihai Irimia-Vladu, '"Green" Electronics: Biodegradable and Biocompatible Materials and Devices for Sustainable Future', *Chem. Soc. Rev.*, 43.2 (2014), 588–610.

[5] Babak Ghanbarzadeh and Hadi Almasi, 'Biodegradable Polymers', in *Biodegradation - Life of Science*, ed. by Rolando Chamy (InTech, 2013).

[6] Sana Sheik, 'Biodegradation of Gamma Irradiated Low Density Polyethylene and Polypropylene by Endophytic Fungi', *International Biodeterioration & Biodegradation*, 105 (2015), 21–29.

[7] Jan P Eubeler, 'Biodegradation of Synthetic Polymers in the Aquatic Environment', 209.

[8] Pornpa Suriyamongkol, 'Biotechnological Approaches for the Production of Polyhydroxyalkanoates in Microorganisms and Plants — A Review', *Biotechnology Advances*, 25.2 (2007), 148–75.

degradation. I would define "biodegradable"[9] as the breakdown of a polymer into simple compounds such as oligomers, carbon dioxide, water, methane and mineral salts aerobically or anaerobically by microorganisms. Meanwhile "composting" is the decomposition of organic matter in the presence of a bacteria, air, carbon and water into a humus-like material known as compost[10]. Compost is generally used as manure for the growth of plants. The majority of polymer materials synthesized from raw materials derived from petroleum sources end up in landfills or buried in the soil[11], marine environment and are sometimes littered around residential areas after use and therefore cause environmental pollution and occupy space. In 2004, plastic made up 7.4% of a municipal solid waste collection in Western Europe only. Studies have shown that millions of micro-plastics are found in large water bodies and are detrimental to marine life. This also put human life at risk since we consume organisms for aquatic media such as fish and other seafoods. Conventional techniques such incineration employed as a means of getting rid of these polymeric materials turn out to instead augment the level of atmospheric pollution through the production of toxic gases. In fact, this activity produces a large number of gas that promote global warming. Recycling polymeric materials has been an effective solution to the management of polymer waste. Nonetheless, it is a laborious process (involves plastic removal, separation, washing, grinding before obtaining to final product), expensive, and not very efficient because significant parts of the materials are not accounted for after recycling. Another issue with depending on petroleum as a major source for obtaining polymeric materials is their non-renewability. This means they may deplete over time which might lead to future shortage of polymeric materials needed for consumption. Besides, the process of extraction of raw materials from petroleum sources usually require heavy equipment which are very expensive causing the finished polymers to be expensive as well. The heavy equipment used in the extraction process also penetrate and drill holes into

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[9] Dr Rolf-Joachim Mller, '12 Biodegradability of Polymers: Regulations and Methods for Testing', 10.

[10] M. Sánchez-García, 'Biochar Accelerates Organic Matter Degradation and Enhances N Mineralisation during Composting of Poultry Manure without a Relevant Impact on Gas Emissions', *Bioresource Technology*, 192 (2015), 272–79.

[11] Ioanna Kyrikou and Demetres Briassoulis, 'Biodegradation of Agricultural Plastic Films: A Critical Review', *Journal of Polymers and the Environment*, 15.2 (2007), 125–50.

rocks deep down into the earth leaving them fractured. Sometimes chemicals are being injected at high pressure into subterranean rocks and bore holes so as to force existing fissures and extract oil or gas. This activity known as fracking may lead to future disasters such as earth quake.

Through research, an environmentally benign, sustainable and economically viable solution has been found in the syntheses and production of polymeric materials. It has been established that polymers can be derived from biorenewable resources. Novel polymers made from biorenewable resources are found to be inherently biocompatible, biodegradable and compostable[12]. These biorenewable resources include agricultural products[13] such as potato[14], cassava, corn, wheat from which starch and PLA can be extracted. Likewise, cellulose, which is the most abundant polymer on the surface of the earth can be obtained from forestry products such as wood. Chitosan, which is another biorenewable resource for polymeric materials is obtained from the shells of crustaceans. Vegetable or fatty oils[15,16] are extracted from seeds while amino acids are from proteins. Since these materials are subjected to microbial action, their products are likely to be broken down by microorganisms either in the presence or absence of oxygen to produce methane, carbon dioxide, water, mineral salts and other biomasses. This is a cheap, sustainable and eco-friendly cycle which may be beneficial to companies

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[13] Tillman U. Gerngross, 'Can Biotechnology Move Us toward a Sustainable Society?', *Nature Biotechnology*, 17.6 (1999), 541–44.

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economically and also to the environment. It is a potential lasting solution to environmental pollution caused by polymer waste.

As promising and fascinating as polymers derived from biorenewable resources in solving the environmental crisis might look, they have some drawbacks. Polymers/monomers from biorenewable resources (bio-based) are difficult to modify chemically to suit certain applications as opposed to their synthetic counterparts. Although the properties of some polymers derived from biorenewable resources are comparable to the ones derived from petroleum sources, the magnificent mechanical properties of the latter are still needed. Therefore, grafting polymers[17,18] derived from biorenewable resource with synthetic ones is seen as a very pragmatic solution. This way, the emergent materials are copolymers which are versatile, biodegradable and possess the required properties suitable for various applications.

The techniques used in the synthesis of novel polymers play a very crucial role in their architectural design to suit their purpose or application. Conventional techniques such as condensation polymerization which have been employed over many decades cannot be controlled. On the other hand, free radical polymerization[19,20] (FRP) which is one of the most widely employed modern technique today is a controlled polymerization process. Some FRP involve the use of living species such as nitroxides and alkoxyamines. Polymeric materials can

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[18] Nevin Çankaya, 'Synthesis of Graft Copolymers onto Starch and Its Semiconducting Properties', *Results in Physics*, 6 (2016), 538–42.

[19] Alan Aguirre-Soto, 'Visible-Light Organic Photocatalysis for Latent Radical-Initiated Polymerization via $2e^{-}/1H^{+}$ Transfers: Initiation with Parallels to Photosynthesis', *Journal of the American Chemical Society*, 136.20 (2014), 7418–27.

[20] Mingyi Tang, 'Polyacrylamide Grafting of Modified Graphene Oxides by in Situ Free Radical Polymerization', *Materials Research Bulletin*, 60 (2014), 576–83.

be engineered through free radical living polymerization[21,22] (FRLP) to obtain tailor-made architectures of new materials with different structures, shapes and sizes. Despite the tremendous potential of FRLP, its economic and environmental factors limit its industrial use. Among the FRLP techniques, nitroxide mediated polymerization (NMP) is a well-controlled[23] metal free process and it is closing the gap between the laboratory and industrial use, hence NMP is highly explored[24]. All the factors discussed above are essential and it is compelling to addressed them in order to elevate this research.

In this study, NMP was employed as the main technique to synthesis biodegradable polymers from a wide range of biorenewable raw materials. The main goal was to synthesize as many copolymers as possible and investigate their biodegradability and other characteristic properties i.e. complete **the green polymer cycle**. The NMP process including the synthesis of nitroxides and alkoxyamines initiators is also discussed. It is worth noting that amongst the nitroxides/alkoxyamines employed in this work, commercial SG1/BlocBuilder[25] was the most efficient. The last chapter (6) talks about a project carried out at Aix-Marseille Université on the development of hydrogels for the treatment and recovery of ischemic stroke.

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[21] Jin-Ying Yuan, Cai-Yuan Pan, and Ben Zhong Tang, '"Living" Free Radical Ring-Opening Polymerization of 5,6-Benzo-2-Methylene-1,3-Dioxepane Using the Atom Transfer Radical Polymerization Method', *Macromolecules*, 34.2 (2001), 211–14.

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[24] Valentin Poirier, 'One-Pot Synthesis of Lactide-Styrene Diblock Copolymers via Catalytic Immortal Ring-Opening Polymerization of Lactide and Nitroxide-Mediated Polymerization of Styrene', *ChemSusChem*, 3.5 (2010), 579–90.

[25] Muriel Lansalot, 'A Step Towards High-Molecular-Weight Living/Controlled Polystyrene Using SG1-Mediated Polymerization', *Macromolecular Reaction Engineering*, 4.6–7 (2010), 403–14.

1.1. State of the Art.

Biodegradable polymers first introduced in the 1980s represent a group of materials which can be easily broken into nontoxic products by microbial or enzymatic action. They may also be compostable. Most bio-based (biopolymers)[26] polymers are biodegradable. It is worth noting that polyethylene (PE) and nylon 11 (NY11) can be produced from biomass or renewable resources, however they are non-biodegradable. Acetyl cellulose (AcC) is either biodegradable or non-biodegradable, depending on the degree of acetylation. Despite the fact that polycaprolactone (PCL) and poly (butylene succinate) (PBS) are petroleum based, they can be degraded by microorganisms. It is worth mentioning that water, heat, light and other factors can also cause the degradation some polymeric materials. A substantial amount of progressive work has been done on the synthesis and investigation of biodegradable polymers from biorenewable resources[27]. Statistics show that about 4000 publications and 1000 patents have been documented on bio-based polymers[28,29] from 1993 to 2012. Following the trend in fig.2, I am estimating that about 7000 publications and 2500 patents will be documented by the end of this year. Likewise, the production of BDP and bio-based polymers increased from 2015 to 2016 and is estimated to increase drastically from 2017 to 2022. However, I can only assume that the drop-in production of BDP and bio-based polymers in 2017 is due to political reasons (fig.3). BDP can be produced through fermentation of microorganisms, chemical synthesis or by chemical modification of from natural products. This document will focus more on chemical

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[26] Jane G Tirrell and David A Tirrell, 'Synthesis of Biopolymers: Proteins, Polyesters, Polysaccharides and Polynucleotides', *Current Opinion in Solid State and Materials Science*, 1.3 (1996), 407–11.

[27] Alessandro Gandini, 'Progress of Polymers from Renewable Resources: Furans, Vegetable Oils, and Polysaccharides', *Chemical Reviews*, 116.3 (2016), 1637–69.

[28] Ramesh P Babu, Kevin O'Connor, and Ramakrishna Seeram, 'Current Progress on Bio-Based Polymers and Their Future Trends', *Progress in Biomaterials*, 2.1 (2013), 8.

[29] Meryem Koruyucu, 'Synthesis, Characterization and Polymerization of Novel Sugars Based on Methacrylate', *Iranian Polymer Journal*, 25.5 (2016), 455–63.

synthesis and chemical modified biodegradable polymers because of the ease with which the process can be controlled.

The green polymer cycle (fig.1) is **my personal design** demonstrating how increasing the activities surrounding BDP from biorenewable resources would tremendously change the status quo of polymeric materials towards a more benign and beneficial environment, and at the same time positively influence the economics in the polymer industry. My personal suggestion is that organizations or companies which cannot operate on all the steps involved in the cycle should collaborate with others which operate on the steps they lack. A practical 50% scale operation on the green polymer cycle by all the players involved in the polymer industry would bring a colossal economic and environmental profit to the entire globe.

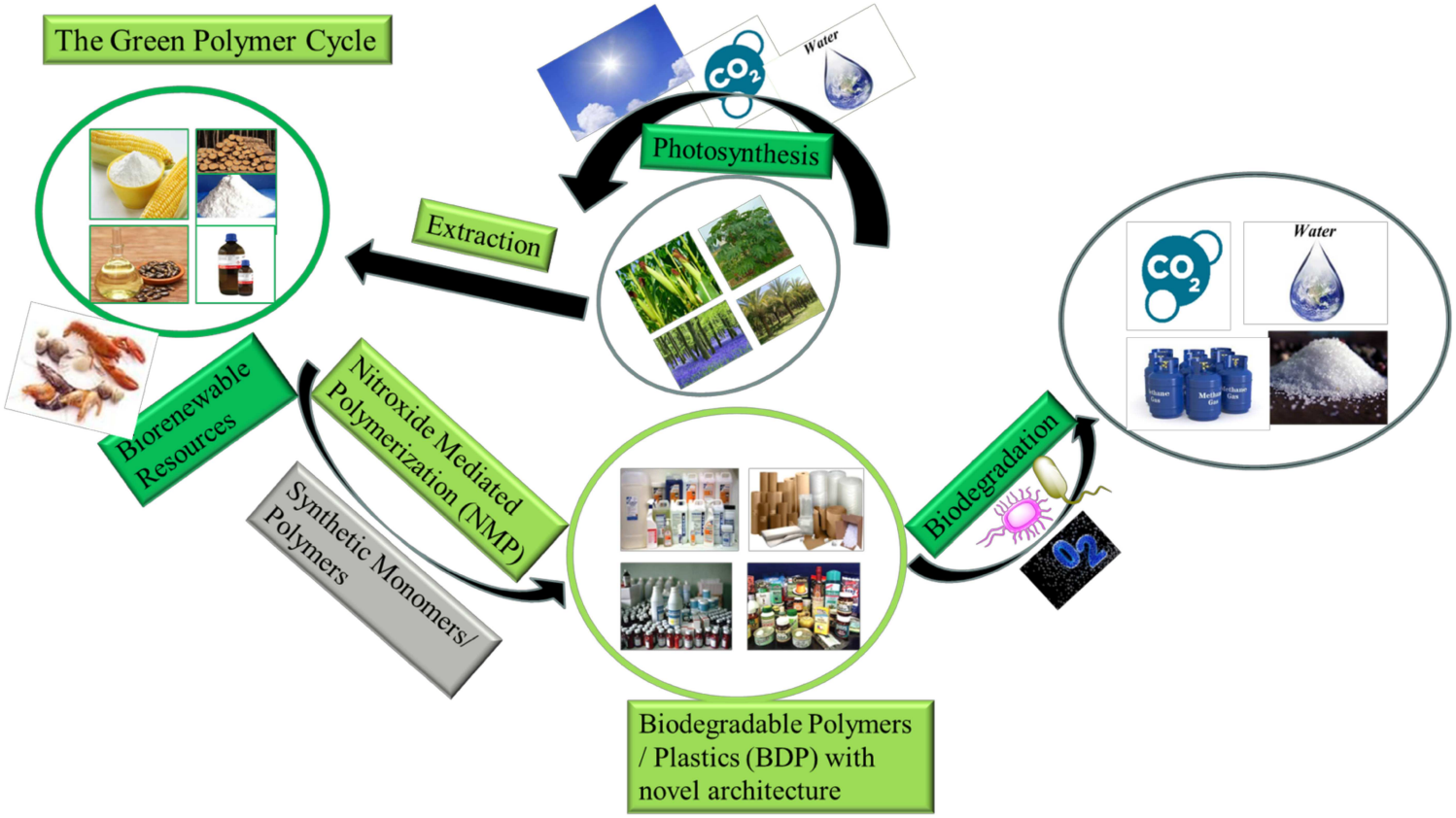

Fig. 1. The Green Polymer Cycle.

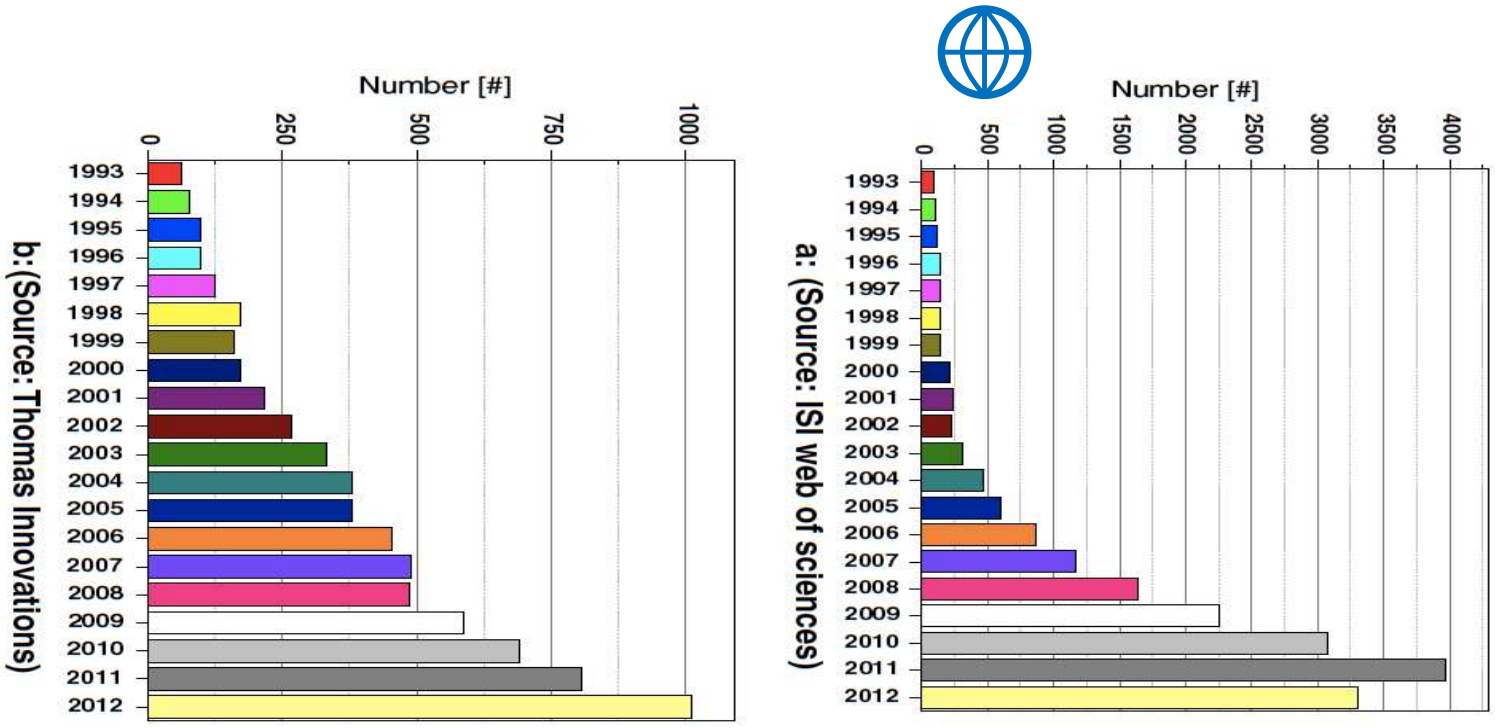

Fig. 2. Citations trend of (a) publications and (b) patents on bio-based polymers in recent years. Babu et al. Progress in Biomaterials 2013, 2:8.

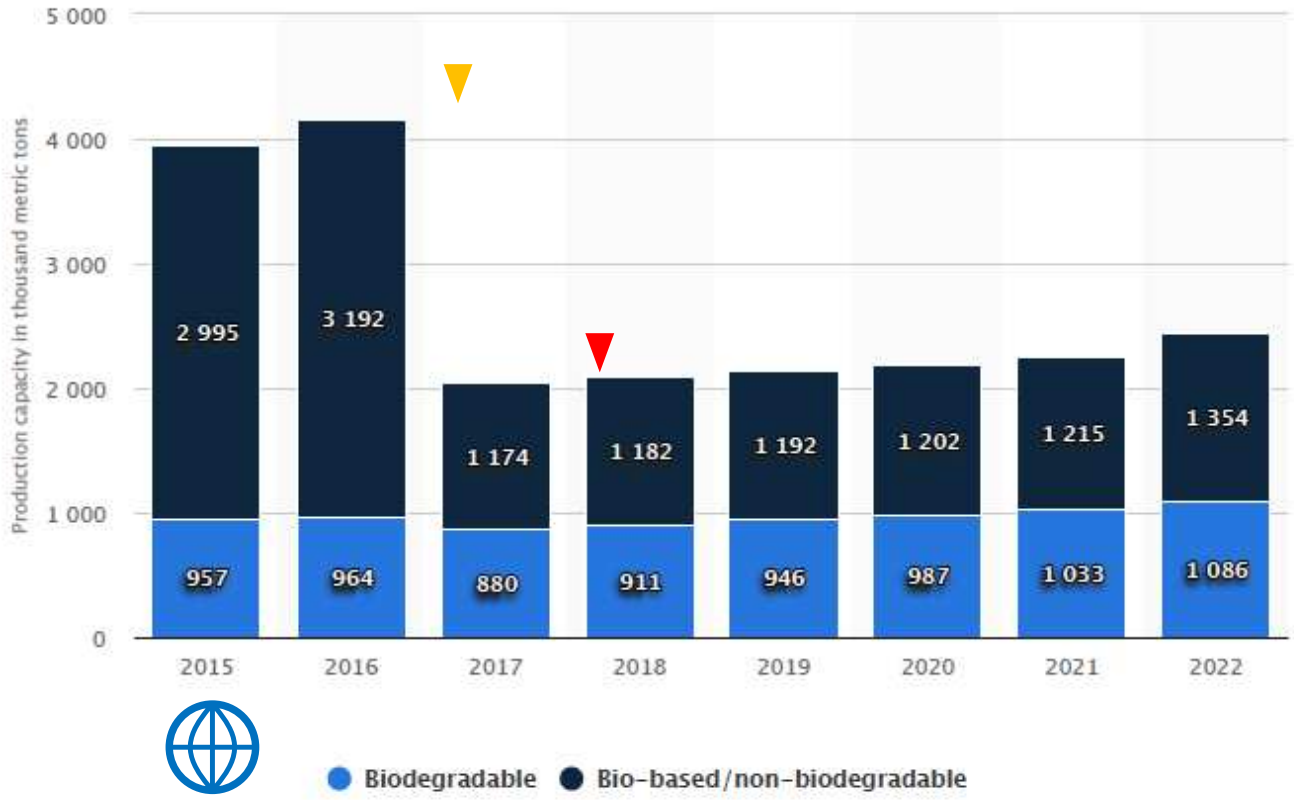

Fig. 3. Production capacity of bioplastics from 2015 to 2022. © Statista 2018.

1.2. Chemically modified Biodegradable Polymers.

Biodegradable polymers can be synthesized chemically, physically or by the use of microorganisms (biosynthesis)[30,31] such as bacteria and fungi. Natural polymers[32,33,34] can also be chemically modified with either polymers/monomers from biorenewable resources or synthetic ones. The chemical modification of polymers/monomers from biorenewable resources is advantageous because the process can be controlled to obtain macromolecular architectures to suit particular applications.

1.3. Biodegradable polymers from biorenewable resources.

1.3.1. Cellulose.

Cellulose is the most abundant biopolymer on earth constituting of plant cell walls and containing more than half of the organic carbon on earth. Herbivores largely depend on this biorenewable polymer as food source because their digestive tracts contain microbes that produce cellulose-hydrolyzing cellulases; various cellulase types are also synthesized by

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[30] Udo Conrad, 'Polymers from Plants to Develop Biodegradable Plastics', *Trends in Plant Science*, 10.11 (2005), 511–12.

[31] Y Poirier, 'Production of New Polymeric Compounds in Plants', *Current Opinion in Biotechnology*, 10.2 (1999), 181–85.

[32] Jürgen Scheller and Udo Conrad, 'Plant-Based Material, Protein and Biodegradable Plastic', *Current Opinion in Plant Biology*, 8.2 (2005), 188–96.

[33] C. V. Stevens, 'Polymeric Surfactants Based on Inulin, a Polysaccharide Extracted from Chicory. 1. Synthesis and Interfacial Properties', *Biomacromolecules*, 2.4 (2001), 1256–59.

[34] Brian P. Mooney, 'The Second Green Revolution? Production of Plant-Based Biodegradable Plastics', *Biochemical Journal*, 418.2 (2009), 219–32.

fungi[35,36]. The aerobic biodegradation of organic polymeric test materials is investigated in aquatic test systems based on respirometry and the evolution of carbon dioxide[37,38]. A good number of new possibilities for polymeric materials can be offered by cellulose-based materials since these significant materials have only be narrowly exploited.

1.3.2. Cellulosic biocomposites

Biocomposites[39] which are new generation material of fiber reinforced can be obtained by embedding natural cellulose containing fibres in a biodegradable polymeric matrix[40]. Biocomposites are derived fully from biorenewable resources as opposed to traditional compounds which consist of very stable components very difficult to decompose. This provides a higher chance of their convenient removal after use i.e. biodegradation, composting or carbon

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[35] N. Kolarova and J. Augustín, 'Production of Polysaccharide Hydrolases in the GenusRhizopus', *Folia Microbiologica*, 46.3 (2001), 223–26.

[36] E. Gomes, T. Iembo, and R. Da Silva, 'Production, Characterization and Properties of Polysaccharide Depolymerizing Enzymes from a Strain Of Curvularia Inaequalis', *Folia Microbiologica*, 46.4 (2001), 303–8

[37] U Pagga, J Boelens, and B De Wilde, 'Determination of tee aerobic biodegradability of polymeric material in a laboratory controlled composting test', 13.

[38] Udo Pagga, 'Determination of the Aerobic Biodegradability of Polymeric Material in Aquatic Batch Tests', *Chemosphere*, 42.3 (2001), 319–31.

[39] A.S. Herrmann, J. Nickel, and U. Riedel, 'Construction Materials Based upon Biologically Renewable Resources—from Components to Finished Parts', *Polymer Degradation and Stability*, 59.1–3 (1998), 251–61.

[40] Navin Chand, R. K. Tiwary, and P. K. Rohatgi, 'Bibliography Resource Structure Properties of Natural Cellulosic Fibres ? An Annotated Bibliography', *Journal of Materials Science*, 23.2 (1988), 381–87.

dioxide neutral combustion[41]. An extremely important overview was published with 250 references centered around the use of renewable raw materials for production of cheap cellulose-based biocomposites[42]. The approaches of natural fibre alteration for production of reinforced plastic composites were also reviewed and analogized[43]. Among more than 400 papers dedicated to the development of biocomposites, the most cited is an experimental paper[44] which is dedicated to the suitable modification of natural fibres with silan or stearic acid to decrease moisture absorption. The authors have shown that this property influences both mechanical strength and specific density. Mohanty et al. (2000) have demonstrated that a colossal amount of research work is being done to combine pristine materials containing cellulose with materials such as glass, metals, plastics, inorganics, and synthetic fibres to produce novel materials that are tailored for end-use requirements. A wide distribution of such cost-effective materials and high-performance composites on the market was attained for wood and similar lignocellulosic biocomposites[45]. Since 1998, Dow Polyurethanes produces a new generation of biocomposite wood-replacement panels made from wheat straw and Dow's polyurethane resin. Phenix Biocomposites is an important vendor of Environ® biocomposite panels which are a decorative interior material that respects the environment by using recycled paper products, a soy-based resin system, and color additives. In Europe, the research is coordinated by the Scientific Centre of Excellence which is self-financing and staffed by an

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[41] U. Riedel and J. Nickel, 'Konstruktionswerkstoffe aus nachwachsenden Rohstoffen (BioVerbunde)', *Materialwissenschaft und Werkstofftechnik*, 32.5 (2001), 493–98

[42] A. K. Mohanty, M. Misra, and G. Hinrichsen, 'Biofibres, Biodegradable Polymers and Biocomposites: An Overview', *Macromolecular Materials and Engineering*, 276–277.1 (2000), 1–24.

[43] Jayamol George, M. S. Sreekala, and Sabu Thomas, 'A Review on Interface Modification and Characterization of Natural Fiber Reinforced Plastic Composites', *Polymer Engineering & Science*, 41.9 (2001), 1471–85.

[44] A. K. Bledzki, S. Reihmane, and J. Gassan, 'Properties and Modification Methods for Vegetable Fibers for Natural Fiber Composites', *Journal of Applied Polymer Science*, 59.8 (1996), 1329–36.

[45] C. A. S. Hill, 'Wood-Plastic Composites: Strategies for Compatibilising the Phases.', *Journal of the Institute of Wood Science*, 15.3 (2000), 140–46.

interdisciplinary team of wood, polymer and material scientists, biologists, chemists and physicists, with many years of experience of research into industrial utilization of wood, plant fibres and plant polymers. Under the name Fasal®, Austel markets injection molding granules, a thermoplastic granulated material which contains about 50 % wood, such as waste wood or weak wood in chip form or as sawdust. A similar product is sold by the German company LignoPol under the name Lignopol®, which is based on composites comprising lignin and natural fibres plus additives. A sustainable thermoplastic material made solely from lignin and cellulose, mainly used for injection molded wood applications is produced and developed by Tecnaro GmbH, with a current capacity of 300 tons annual output. It is marketed under the trade name Arboform®. The Treeplast® product made from wood chips (50 %), with crushed corn and natural resin only is absolutely natural, renewable and biodegradable. This project is made of six companies funded by the European Commission.

1.3.3. Cellulose acetate.

Cellulose acetate is a member of the cellulose ester family. It is a thermoplastic with an amorphous structure. Incorporating acetyl groups into cellulose produces a tough cellulose acetate material. It is particularly suitable to make non-flammable and other coating materials with high melting point, toughness, clarity, and good resistance to UV light, chemicals, oils, and greases. A review published scrutinized possible cellulose ester applications with peculiarity on biodegradable plastics, composites, laminates, optical films, and separation membranes[46]. While cellulose monoacetate films required 10–12 days for extensive degradation, most of 80 % cellulose diacetate films were able to degrade in 4–5 days[47] in the most cited paper delineating the mechanism of aerobic biodegradation of cellulose acetates. After comparing several test methods, it was demonstrated that cellulose acetate can be

[46] Kevin J Edgar, 'Advances in Cellulose Ester Performance and Application', *Progress in Polymer Science*, 26.9 (2001), 1605–88.

[47] Charles M. Buchanan, Robert M. Gardner, and Ronald J. Komarek, 'Aerobic Biodegradation of Cellulose Acetate', *Journal of Applied Polymer Science*, 47.10 (1993), 1709–19.

degraded anaerobically to methane[48]. It was also discovered that the bacteria isolated from soil preferentially degrade the cellulose part of the cellulose acetate molecule under aerobic conditions[49]. Among the three leading producers of cellulose acetate in the United States, Celanese controls approximately 46 % of production capacity, Eastman owns approximately 44 %, and Primester holds the remaining 10 %.

1.3.4. Starch.

Starch is a polysaccharide with major plant storage form of glucose consisting of amylose and amylopectin. The glucose units in amylose are 1,4-α-D-linked together in straight chains, whereas the glucose chains in amylopectin are highly branched. Biodegradable polymer production from starch can be done principally in three ways. Employing low amount starch to prepare starch composites[50,51] with other plastics to improve the biodegradability of traditional oil-based polymer materials is one way. Another mode is to prepare starch composites consisting of more than half the mass of starch in the content. This type of material exhibits mechanical properties similar to conventional plastics but at a lower price[52,53] and is known as

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[48] Stefan Gartiser, Mathis Wallrabenstein, and Gabi Stiene, 'Assessment of Several Test Methods for the Determination of the Anaerobic Biodegradability of Polymers', 15.

[49] Kiyofumi Sakai, 'Biodegradation of Cellulose Acetate by *Neisseria Sicca*', *Bioscience, Biotechnology, and Biochemistry*, 60.10 (1996), 1617–22.

[50] R. A. De Graaf and L. P. B. M. Janssen, 'The Production of a New Partially Biodegradable Starch Plastic by Reactive Extrusion', *Polymer Engineering & Science*, 40.9 (2000), 2086–94.

[51] Materials Program (Matthew Weinberg) Energy, 'Biopolymers: Making Materials Nature's Way', 86.

[52] Catia Bastioli, 'Biodegradable Materials - Present Situation and Future Perspectives', *Macromolecular Symposia*, 135.1 (1998), 193–204.

[53] Catia Bastioli, 'Global Status of the Production of Biobased Packaging Materials', *Starch - Stärke*, 53.8 (2001), 351.

plastified starch. The extrusion process of mixtures of granular starch in the presence of soy protein[54] , glycerol, alginate, lignin, humic substances, urea, sucrose, ammonium chloride, as plasticizers[55] is also a means of preparing starch biodegradable polymers. The hydrophilic nature of starch makes them difficult to mix and form homogenous products with synthetic plastics, thus they are incompatible. Chemical blending of starch with synthetic polymers is seen a solution to surmount the problem of incompatibility. This allows for a reduction in the price of synthetic plastics and increases the biodegradability of the final product[56,57,58,59].

[54] 'Processibility and Preperties of Biodegradable Plastics Made from Agricultural Biopolymers.Pdf'.

[55] Gregory M Glenn and Julie Hsu, 'Compression-Formed Starch-Based Plastic', *Industrial Crops and Products*, 7.1 (1997), 37–44.

[56] Anthony Burgess-Cassler, Syed H Imam, and J Michael Gould, 'High-Molecular-Weight Amylase Activities from Bacteria Degrading Starch-Plastic Films', 3.

[57] S H Imam, 'The Use of cp/mas r3c-nmr For Evaluating Starch Degradation in Injection Molded Starch-Plastic Composites', 4.

[58] R. P. Wool, 'Biodegradation Dynamics of Polymer-Starch Composites', *Journal of Applied Polymer Science*, 77.8 (2000), 1643–57.

[59] Vincent T. Breslin and R. Lawrence Swanson, 'Deterioration of Starch-Plastic Composites in the Environment', *Air & Waste*, 43.3 (1993), 325–35.

A series of papers[60,61,62,63] described a technique for the preparation of starch composites with different copolymers. The conventional synthesis of starch composites by copolymerization with maize starch using KMnO4– citric acid as a redox initiator system was used. Wool et al. (2000) studied the kinetics of biodegradation of polyethylene–starch composites detecting the microbial invasion with scanning electron microscopy. It is worth mentioning that the disintegration of starch plastic blends derived from petroleum products is not the same as biodegradation (Burgesscassler et al. (1991) and Imam et al. (1993)). In an experiment, the scanning electron microscopy[64] was used to monitor the biodegradation of starch–plastic films prepared as a starch composite with PHA. Aquino et al. (2001) described the pattern of starch degradation by thermophilic fungi at a temperature typical for fast composting. Novon initially using the trade name Ecostar® to commercialize PLA-plastic starch blends is currently marketing a new generation of modified starch blends with auto-oxidants under the trade name ECO-3®. This is a product that contains 10–20 % of starch and is recommended as an additive to conventional plastics. Samples of low-density polyethylene film containing 20 % ECO-3® demonstrated a 95 % reduction in elongation after being buried in soil for 18 months. Novon International and Novon Polymer China is now the successful producer and vendor of other new starch composites under the trade names Poly- Novon® and Degra-Novon®. Meanwhile in Europe, the most important vendor of starch-based additives for

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[60] A. Hebeish, E. El-Alfy, and A. Bayazeed, 'Synthesis of Vinyl Polymer-Starch Composites to Serve as Size Base Materials', *Starch - Stärke*, 40.5 (1988), 191–96.

[61] A. Hebeish, M. R. El-Zairy, 'Poly(Acrylic Acid) Starch Composite as a Substitute for Sodium Alginate in Printing Cotton Fabrics with Reactive Dyes', *Starch - Stärke*, 43.3 (1991), 98–102.

[62] A. Hebeish, M. H. El-Rafie, A. Higazy, and M. A. Ramadan, 'Poly(Acrylic Acid)-Starch Composites. A Key for Improving Sizeability and Desizeability of Starch from Cotton Textiles', *Starch - Stärke*, 44.3 (1992), 101–7.

[63] A. Hebeish, M. H. El-Rafie, A. Higazy, and M. Ramadan, 'Synthesis, Characterization and Properties of Polyacrylamide-Starch Composites', *Starch - Starke*, 48.5 (1996), 175–79.

[64] Luis V. Lopez-Llorca and Maria F. Colom Valiente, 'Study of Biodegradation of Starch-Plastic Films in Soil Using Scanning Electron Microscopy', *Micron*, 24.5 (1993), 457–63.

production biodegradable polymers with a low content of starch is the multinational Amylum Group.

Starch composites with medium amount of starch (≈40–60 %) are called plastified starch materials. Although they exhibit mechanical properties similar to conventional plastics such as polypropylene, and are generally resistant to oils and alcohols, they degrade when exposed to hot water. They are completely biodegradable and compostable, and are a good replacement for traditional plastics in food service, food packaging, personal health care, etc. Usually, they are basically derived from corn starch (40–60 %) and the rest is performance-enhancing additives and other biodegradable materials. In biologically active environments such as compost facilities and wastewater treatments systems, they display degradation characteristics similar to leaves, wood chips and paper. Traditional plastic processes used for starch composite treatment are mostly blow or injection molding, extrusion and thermoforming. The results of the biodegradation of plastic films containing corn starch (40 % dry mass) in combination with polyethylene studied[65] proved that up to about 40 % of starch in the starch-plastic films disappeared after a 60-days exposure. Novaton which was created at the end of the 80s as a research and development company is the largest supplier of plastified starch materials in Europe and presently investing in the field of biodegradable thermoplastic materials as core business activity, marketed worldwide with the trademark Mater-Bi® (Bastiolli 1998). Mater-Bi® products are thermoplastic materials, mainly derived from corn, wheat and potato starch. A recent merger between Novamont and Goodyear (the largest tire manufacture) has discovered a technology that makes it possible to produce an environmentally friendly and biodegradable tire. In order to target applications such as personal hygiene and disposable medical products, Agri-Tech Industries commercialized a material that contains up to 50 % (W/W) starch. Vegemat®, a bioplastic produced by the French company Vivadur contains 40–50 % starch, 30–40 % proteins, 5–10 % lipids, and 5–10 % natural additives. This product is obtained by transforming corn without separation or purification of constituent parts in the absence of petroplastics. Vegemat® biodegrades usually in 8 weeks.

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[65] S. H. Imam, 'Fate of Starch-Containing Plastic Films Exposed in Aquatic Habitats', *Current Microbiology*, 25.1 (1992), 1–8.

Thermoplastic starch is a bioplastic with a very high amount of starch (>90 %) obtained from biorenewable resources. They can be processed by traditional techniques for plastics. Coloring and flame-retardant additives are possible applications of this type of material. This type of material can degrade completely within 5 days in aqueous aerobic testing and in 45 days in controlled compost or can also decompose in water. Some final products of starch which are commercialized are starch-based tubes, degradable compost bags, and agricultural mulch films. Most conventional plastic are processed through blowing and injection molding, extrusion and thermoforming. In the more than 90 research papers that were dedicated to thermoplastic starch during the last two decades, approximately half of the research data was addressed to optimization of the extrusion process. The second large group of research papers was focused on biodegradation of thermoplastic starch. Starch-polycaprolactone blended thermoplastic was used by Shin et al. to analyze the relationship between its mechanical properties and biodegradability. The results confirmed that the degree of biodegradation of starch composites is proportional to starch content and the material degrades in 6 weeks during composting. Martin et al. (2001) reported similar results for mechanical properties of multilayer films based on thermoplastic wheat starch. Biotec GmbH with Bioplast® and Novon International with Novon® are among the big players in the marketing of biodegradable polymer with a high amount of starch. Bioplast® is manufactured by compounding and melting starch and other completely biodegradable ingredients and then processed to granules.

A completely biodegradable and compostable material that can replace polystyrene foam as biodegradable packaging material known as foam starch can be synthesized from 100% starch. It also can be pressed into starch-based sheets for thin-walled products, such as trays, disposable dishes, etc. This material exhibits antistatic, insulating and shock-absorbing properties. (Glenn and Orts 2001) reported that foam starch can be produce by environmentally blowing into a foam material or through compression-explosion process using steam. Miladinov and Hanna (2001) described an alternative process that permitted the control of the final product properties by the temperature and ethanol additive to acetylated starch foam. The porous structure[66] of starch foam plays an important role in disposing of food packaging and

[66] R.L Shogren, 'Structure and Morphology of Baked Starch Foams', *Polymer*, 39.25 (1998), 6649–55.

articles after use by composting. A composting[67] test was used to demonstrate the degradation of foam starch within a few weeks without accumulation of residues. It should be noted that a broad spectrum of microorganisms is competent to degrade foamed starch products. It was demonstrated that all bacteria isolated from the digestive tract of different mammals fully degraded starch substrates[68] indicating that the materials based on foamed starch may also be biodegraded in standard wastewater cleaning plants. Among the few producers of foam starch, National Starch & Chemical produces and sell in 20 countries a loose-fill packaging material under the trade name Eco-foam®. Norel Unisource (USA) produces foamed starch loose-fill under the trade name Envirofill®. Biotec GmbH with Biopur®, Novon Polymers (China) and SudStarke (Denmark) are also some important producers and vendors of foam starch materials.

1.3.5. Chitin and Chitosan.

Chitin (poly-N-acetyl-D-glucosamine, 2-acetamido-2-deoxy-1,4-β-D-glucan) is amongst the three most abundant polysaccharides in nature. Chitosan is derived from deacytelated chitin. This is done by washing and putting chitin through boiling lye to remove acetate from the molecule. After which the product is hydrolysis and the resulting chitosan is washed, dried, ground, weighed and packed for sale. The lactic fermentation of shrimp shells described in detail[69] as an alternative to the three stages of the classical process. Other details can be found in a recent comprehensive review[70] and in the handbook edited by Muzzarelli and Peter (1997). Chitin is a natural and soluble product. The shells of crustaceans and cell walls of fungi are the main sources of chitin. It is readily biocompatible, environmentally benign and

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[67] Karl F. Tiefenbacher, 'Starch-Based Foamed Materials—Use and Degradation Properties', *Journal of Macromolecular Science, Part A*, 30.9–10 (1993), 727–31.

[68] J Simonek, 'Chitinolytic Bacteria of the Mammal Digestive Tract', 3.

[69] M. S. Rao, J. Muñoz, and W. F. Stevens, 'Critical Factors in Chitin Production by Fermentation of Shrimp Biowaste', *Applied Microbiology and Biotechnology*, 54.6 (2000), 808–13.

[70] Tricomed Sa, 'Marcin H. Struszczyk', 2002, 10.

can be degraded by microorganisms. Several writers,[71,72] were able to determine its's ability to degrade in Actinomycetes living in soil (Hanzlíková and Jandera 1993) and in other bacterial species[73] . A great deal of information can be obtained from the literature concerning the degradation of chitin or production of chitin-degrading enzymes as summarized by Felse and Panda (2000). Boyer, in 1994 measured the potential rates of chitin degradation and its mineralization and concluded that under aerobic conditions in water environment 88–93 % of particulate chitin was transformed to carbon dioxide within a few days. A comprehensive review[74] was published on biodegradation and bioactivity of chitin and its derivatives. Chitin and chitosan are applied (Kumar 2000) in the textile industry, in medicine (Singla and Chawla 2001) and in food production[75] as a result of the book published by Goosen (1997), dedicated to the application of chitin and chitosan. Kumar et al. (2002) reviewed the present state of art in a summary of 418 references regarding the synthetic methods and characterization of nanoparticles, the suitability of polymeric systems for various drugs, drug loading and drug release properties of various systems such as nanoparticles, hydrogels, microspheres, films, membranes, tablets, etc. Chitosan-based plastics[76] applied in tissue engineering were

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[71] Yoshikane Itoh, 'Functional Analysis of the Chitin-Binding Domain of a Family 19 Chitinase from *Streptomyces Griseus* HUT6037: Substrate-Binding Affinity and *Cis* -Dominant Increase of Antifungal Function', *Bioscience, Biotechnology, and Biochemistry*, 66.5 (2002), 1084–92.

[72] Hildgund Schrempf, 'Recognition and Degradation of Chitin by Streptomycetes', 5.

[73] M. Hashimoto, 'Expression and Characterization of the Chitin-Binding Domain of Chitinase A1 from Bacillus Circulans WL-12', *Journal of Bacteriology*, 182.11 (2000), 3045–54.

[74] Tiicomed Sa, 'MARCIN H. STRUSZCZYK', 2002, 11.

[75] You-Jin Jeon, Fereidoon Shahidi, and Se-Kwon Kim, 'Preparation of Chitin and Chitosan Oligomers and their Applications in Physiological Functional Foods', *Food Reviews International*, 16.2 (2000), 159–76.

[76] S Raghunadh Acharyulu, T Gomathi, and P N Sudha, 'Physico-Chemical Characterization of Cross-linked Chitosan-Polyacrylonitrile Polymer Blends', 2013, 10.

delineated[77] .Furthermore, Jenkins and Hudson (2001) evaluated the recent state of art (178 references) in the blending chitin or chitosan to prepare biodegradable polymers. In the USA Biopolymer Engineering with ChitoPure® is one of the world's first manufacturers of chitosan products. They compete in trade on the Atlantic coast with Primex, Iceland (ChitoClear®) and Groupe RT (Canada) with Kitomer®. Key suppliers of chitin in the Pacific region are Kate International (India), Sonat Co. (Russia) and Taizhou Candorly Sea Biochemical & Health Products Co., Dalian Xindie Chitin Co., both from China. Meanwhile, in Europe, the largest trader of chitin and chitosan is Sigma Chemicals.

1.3.6. Neutral polysaccharides.

Gellan gum is a newly commercially developed microbial polysaccharide. It is an extracellular polysaccharide produced by Sphingomonas sp. (Pollock 1993). Its chemical composition is a tetrasaccharide unit, viz. D-glucose, D-glucuronic acid, D-glucose and L-rham- nose. In the presence of divalent cations, gellan gum forms a transparent gel which is heat-resistant. Kasapis (1995), concisely reported that it can be processed into a material unsuitable for food processing based on this character. Nonetheless, some new applications[78] make use of the excellent properties of glean gum for the production of biodegradable plastics[79]. An elaboration on how enzymes assimilate and depolymerize gellan gum in a bacterium[80] has been done. Kelco Biopolymers (Division of Merck) developed and produced gellan gum under

[77] D W Hutmacher, J C H Goh, and S H Teoh, 'An Introduction to Biodegradable Materials for Tissue Engineering Applications', 30 (2001), 9.

[78] R M Banik, B Kanari, and S N Upadhyay, 'Exopolysaccharide of the Gellan Family: Prospects and Potential', 8.

[79] B. Manna, A. Gambhir, and P. Ghosh, 'Production and Rheological Characteristics of the Microbial Polysaccharide Gellan', *Letters in Applied Microbiology*, 23.3 (2008), 141–45.

[80] Wataru Hashimoto, 'Enzymatic and Genetic Bases on Assimilation, Depolymerization, and Transport of Heteropolysaccharides in Bacteria', *Journal of Bioscience and Bioengineering*, 87.2 (1999), 123–36.

the trade names Kelcogel® and Gelrite®. This polymer has applications in the food industry as a gelling agent in frostings, glazes, icings, jams and media for tissue cell cultures.

Pullulan is a polysaccharide of α-D-glucose that is a copolymer of maltotriose (1,4-bonding) and isomaltose (1,6- bonding) with an additional 1,3-branching. It has adhesive properties and can be used as Laminarin and curdlan. Laminarin (1,3-β-D-glucan) is a polysaccharide which can be extracted from cell-wall of many microorganisms such as bacteria, fungi or algae (Augustin 1998). Mostly, it is obtained from the cell walls of baker's yeast, which is a by-product in the production of alcoholic beverages (Osumi 1998). Nfiura et al. (1996) described the biodegradation of 1,3-β-D-glucan in vivo and found that it varied from 1 to 24 hours influenced by the molar mass of the polymer. Among the vendors of chemicals, ABAC GmbH (Switzerland) and Biopolymer Engineering (USA), are the largest suppliers of medical grade yeast glucan used in stimulating the immune system. A patent written by Karinen and Bergelin (1993) on the preparation of glucan-enriched alimentary fibres, which are currently a very crucial product on the market of cholesterol-controlling functional foods (Cho and Dreher 2001). Recently, Nurture Inc. (USA) patented[81] the technique of glucan film preparation. Takeda Chemical Industries Ltd. (Japan) transformed the production of curdlan to an industrial scale in 1989, and only then was curdlan approved and commercialized for food usage in Korea, Taiwan, and Japan. In 1996, Pureglucan®, the trade name of curdlan, was launched in the US market as a formulation aid, processing aid, stabilizer, and thickener or texture modifier for food use[82] . Pureglucan® is known to be non-toxic and non-carcinogenic. Presently, curdlan is mostly used in the food industry. However, recent development in the field made possible its use for the production of special biodegradable plastic for medical applications.

Other neutral polysaccharides were observed to fully biodegrade in three days. It is safe to say that the use of microbial polysaccharides in the food, pharmaceutical and chemical industries has increased steadily during the past decade. There are natural gelling polysaccharides suitable for new biomedical applications (Murano 2000). A frequently cited

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[81] Richard C Potter and Seeley Lake, '(54) Method for Concentrating B-glucan', 10.

[82] E.J.F. Spicer, E.I. Goldenthal, and T. Ikeda, 'A Toxicological Assessment of Curdlan', *Food and Chemical Toxicology*, 37.4 (1999), 455–79.

survey on research activities in Europe in the field of saccharide polymers of microbial origin[83] has been published. The most important neutral polysaccharides produced by bacteria are xanthan, dextran and gellan. Commercially significant fungal polysaccharides are pullulan and glucan. Xanthan, a natural biopolymer produced by the culture fermentation of Xanthomonas campestris, is mostly applied as a gelling agent in food and pharmaceutical industry. Dextran is the generic name given to a large family of microbial polysaccharides. It is produced by fermentation or enzymic conversion of the feedstock sucrose by Leuconostoc mesenteroides[84]. Saccharomyces cerevisiae, Lactobacillus plantarum, and Lactobacillus sanfrancisco (Anonymous 2000d), which are currently used in food processing[85] . However, both dextran and xanthan have not yet been commercially developed for industrial production of biodegradable polymers.

1.3.7. Soy-based plastics.

The raw, hulled soy beans contain, depending upon variety, approximately 18 % oil, 38 % protein, 30 % saccharides, and 14 % moisture and ash. The saccharide component is normally about 15 % of soluble saccharides and 15 % of starch. Since 1940, when Henry Ford presented in public the strength of a car body made of a soybean-based material, this material is taken as highly prospective for production of soybean– formaldehyde-based thermoset composite from

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[83] Vittorio Crescenzi, 'Microbial Polysaccharides of Applied Interest: Ongoing Research Activities in Europe', *Biotechnology Progress*, 11.3 (1995), 251–59.

[84] R S Karthikeyan, S K Rakshit, and A Baradarajan, 'Optimization of Batch Fermentation Conditions for Dextran Production', *Bioprocess Engineering*, 1996, 5.

[85] D.V. Zasypkin, E.E. Braudo, and V.B. Tolstoguzov, 'Multicomponent Biopolymer Gels', *Food Hydrocolloids*, 11.2 (1997), 159–70.

renewable resources. Some papers[86,87] reported on the comeback of soy-based plastic in the USA. An investigation was done on aerobic degradation of protein–starch plastics[88] and the data summarized. In another publication of an extensive study on soy protein films during composting[89]; it was found that the samples degraded with 50 % mass loss in about 10 d and with up to 95 % mass loss in 30 d. There are four main ways how to prepare soy-protein plastic materials. Current research and development is focused on commercializing of the following soy products: (1) soy–phenol–resorcinol–formaldehyde; (2) an improved waterproof product to replace phenol–formaldehyde (Rhim and Weller 2000); (3) a foaming glue for plywood (Park and Hettiarachchy 1999; Lodha and Netravali 2002); (4) an improved water- resistant product to replace urea–formaldehyde materials (Otaigbe et al. 1999). The mechanical properties and morphology of soy-plastic materials[90] were described. Soy plastics can possess significantly higher Young's module (4.4 GPa) than those of petrochemical plastics and show good potential as an alternative for replacing petrochemical, nonbiodegradable plastics for engineering applications. An innovative approach to improve the resistance of soy protein plastics to water

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[86] Inke Paetau, Chin-Zue Chen, and Jay-lin Jane, 'Biodegradable Plastic Made from Soybean Products. 1. Effect of Preparation and Processing on Mechanical Properties and Water Absorption', *Industrial & Engineering Chemistry Research*, 33.7 (1994a), 1821–27.

[87] PAETAU I., CHEN C.Z., JANE J.: Biodegradable plastic made from soybean products. Part II. Effect of cross-linking and incorporation of cellulose on the mechanical properties and water absorption. J.Environ.Biodegrad.Polym. 2, 211–217 (1994b).

[88] K. E. Spence, 'Soil and Marine Biodegradation of Protein—Starch Plastics', in *Hydrogels and Biodegradable Polymers for Bioapplications*, ed. by Raphael M. Ottenbrite, Samuel J. Huang, and Kinam Park (Washington, DC: American Chemical Society, 1996), DCXXVII, 149–58.

[89] S. K. Park, N. S. Hettiarachchy, and L. Were, 'Degradation Behavior of Soy Protein−Wheat Gluten Films in Simulated Soil Conditions', *Journal of Agricultural and Food Chemistry*, 48.7 (2000), 3027–31.

[90] H.-J. Sue, S. Wang, and J.-L. Jane, 'Morphology and Mechanical Behaviour of Engineering Soy Plastics', *Polymer*, 38.20 (1997), 5035–40.

by polyphosphate fillers[91] was studied and published. When compared with starch biocomposites the commercialization of soy-protein plastics is limited to the USA. A commercial production facility for soy-based adhesives was started-up by Heartland Resource Technologies (Iowa). Urethane Soy System Company (Illinois) is now producing soy-based polyols for rigid and flexible urethane foams under the name SoyOyl®. Dow Chemical developed soy-based biodegradable[92] material BioBalance® which is used in carpet backings.

1.3.8. Polylactic acid.

Polylactic acid (PLA) is largely produced and currently sold at 1 USD/kg by Chronopol, which currently operates a 1000 ton per year PLA facility in Colorado. Cargill Dow LLC, the global leader in commercialization of PLA, and Mitsui Chemicals, a leading Japanese chemical company, have announced collaboration on the further business development of PLA – a polymer made from annually renewable resources, and have signed an agreement to accelerate the market development for PLA in the Pacific zone. PLA[93] production and use are well documented in the literature. Generally, PLA is based on lactic acid that uses dextrose from corn or sugar beet as a feedstock for fermentation. Lactic acid is the basic monomer for PLA production. The improvement of continuous lactic acid fermentation[94] is a big challenge for

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[91] Joshua U. Otaigbe, Daniel O. Adams, 'Bioabsorbable Soy Protein Plastic Composites: Effect of Polyphosphate Fillers on Water Absorption and Mechanical Properties', 1997, 10.

[92] Shida Miao, 'A Novel Vegetable Oil–Lactate Hybrid Monomer for Synthesis of High- T_g Polyurethanes', *Journal of Polymer Science Part A: Polymer Chemistry*, 48.1 (2010), 243–50.

[93] I. Scholl, 'Allergen-Loaded Biodegradable Poly(d,l-Lactic-Co-Glycolic) Acid Nanoparticles down-Regulate an Ongoing Th2 Response in the BALB/c Mouse Model', *Clinical Experimental Allergy*, 34.2 (2004), 315–21.

[94] K. Melzoch, 'Lactic Acid Production in a Continuous Culture Using Lignocellulosic Hydrolysate as a Substrate. Identification of a Physiological Model', *Folia Microbiologica*, 41.2 (1996), 211–15.

bioengineers as documented[95]. Furthermore, the biotechnological aspects of PLA production[96] was recently summarized. The primary biodegradability[97,98] of PLA was investigated using hydrolysis tests at various composting temperatures and pH. It was demonstrated that composting is a useful method for PLA biodegradation. This is in agreement with the results obtained from the studies of the biotic degradation of PLA oligomers[99] at 25 and 58 °C in an aquatic aerobic biodegradation test for six months.

1.3.9. Polycaprolactone.

Polycaprolactone (PCL) is a biodegradable thermoplastic polymer obtained by chemical synthesis from crude oil. Solvay Group, the world's largest producer of PCL (Capa®), prepares its monomer using a unique process, which utilizes in the first step a high-strength aqueous hydrogen peroxide agent (87 %, W/W) and acetic acid to produce peracetic acid. This is used in the second step to oxidize cyclohexanone by a Bayer Villiger reaction to caprolactone. The polymer is produced by a ring opening[100] and addition polymerization reaction rather than by the condensation polymerization reaction normally used for polyester production. This

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[95] Sunhoon Kwon, 'High-Rate Continuous Production of Lactic Acid ByLactobacillus Rhamnosus in a Two-Stage Membrane Cell-Recycle Bioreactor', *Biotechnology and Bioengineering*, 73.1 (2001), 25–34.

[96] R. E. Drumright, P. R. Gruber, and D. E. Henton, 'Polylactic Acid Technology', *Advanced Materials*, 12.23 (2000), 1841–46.

[97] Kimura Toshinori, 'Hydrolysis Characteristics of Biodegradable Plastic (Poly Lactic Acid).', *NIPPON SHOKUHIN KAGAKU KOGAKU KAISHI*, 49.9 (2002), 598–604.

[98] James M Anderson and Matthew S Shive, 'Biodegradation and Biocompatibility of PLA and PLGA Microspheres', *Advanced Drug Delivery Reviews*, 1997, 20.

[99] S. Karjomaa, 'Microbial Degradation of Poly-(l-Lactic Acid) Oligomers', *Polymer Degradation and Stability*, 59.1–3 (1998), 333–36.

[100] Yingying Ren, 'Boric Acid as Biocatalyst for Living Ring-Opening Polymerization of ε -Caprolactone', *Polymer*, 78 (2015), 51–58.

specialized Solvay process yields a high-purity, highly reactive caprolactone monomer, which is ideally suited for consistent polymer production. PCL has good water, oil, solvent, and chlorine resistance. It has a low mp (58–60 °C) and low viscosity and is easy to process. PCL is used mainly in thermoplastic polyurethanes, resins for surface coatings, adhesives and synthetic leather and fabrics. It also serves to make stiffeners for shoes and orthopedic splints, and fully biodegradable compostable bags, sutures, and fibres. The low melting point makes the material suited for composting as a means of disposal, due to the temperature during composting which routinely exceeds 60 °C. Degradation time is very short. In Sweden there has been an attempt to produce PCL bags, but they degraded before reaching the customers. The biodegradation of PCL at 50 °C by a thermotolerant Aspergillus sp.[101] was also described. The material was degraded and assimilated after 6 days and degradation products were identified as succinic, butyric, valeric and caproic acids. Although the polymer is now being prepared from fossil resources, it may also be prepared from renewable resources via chemical treatment of saccharides. In the first step, the saccharides could be converted to ethanol and acetic acid by fermentation. In the second step, ethanol can be converted to cyclo- hexanone by use of chromic acid. Naturally, such product is expensive and is therefore mixed with large amounts of other natural materials to obtain a good biodegradable material at a low price. In a recently summarized paper, three different strategies that can be used for preparing starch–PCL blends with desired mechanical parameters[102] were elaborated. The used natural chitin[103] as blend material (up to 60 %, W/W) to design a novel thermo-resistant plastic material has also been investigated. An evaluation on the thermal dependence of material strength and water resistance of PCL–soy protein[104] isolate blends with up to 50 % PCL (SPI–PCL) alone or with

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[101] James G Sanchez, Akio Tsuchii, and Yutaka Tokiwa, 'Degradation of Polycaprolactone at 50 ◦C by a Thermotolerant Aspergillus Sp.5.

[102] U S Ishiaku, 'Mechanical Properties and Enzymic Degradation of Thermoplastic and Granular Sago Starch ®lled Poly(e-Caprolactone)', *European Polymer Journal*, 2002, 9.

[103] Anle Yang, Renjie Wu, and Pinfang Zhu, 'Thermal Analysis and Miscibility of Chitin/Polycaprolactone Blends', *Journal of Applied Polymer Science*, 81.13 (2001), 3117–23.

[104] Zhikai Zhong and Xiuzhi S Sun, 'Properties of Soy Protein Isolate/Polycaprolactone Blends Compatibilized by Methylene Diphenyl Diisocyanateq', 2001, 9.

the addition of 0.5, 1, 2 and 5 % methyl- enebiphenyl-4,4´-diisocyanate has been reported. Zhang et al. (2000) observed an increase in biodegradation of cellulose derivatives such as cellulose acetate, ethylcellulose and hydroxyethylcellulose acetate when PCL was used as a blending material. Nitz et al. (2001) studied the properties of PCL composites with wood and lignin. The addition of maleinanhydride improved mechanical properties of PCL blend material during reactive extrusion. Due to its low melting point, biocompatibility and high biodegradability, PCL has an important bio- medical use. Moreover, the application of PCL fibers for incorporation into a PCL matrix for craniofacial bone repair[105] was clearly described. Hattori et al. (2001) pointed out that PCL was successfully proven as a new and inexpensive technique for bone fixation. The PCL fibre was fixed tightly by melting and the strength of this thread was higher than that of a stainless-steel wire with the same cross-sectional area. Shen et al. (2000) described the results of the application of PCL-biodegradable composites for regulation of drug release in an organism. The current state of art of preparation and characterization of composites based on biodegradable polymers[106] for "in vivo" application has been summarized making it easy to understand.

1.3.10. Polyvinyl alcohol.

PVA(polyvinyl alcohol) exists only as a polymer, monomer has not yet been isolated. It is manufactured from vinyl acetate monomer in a multistep process when the monomer is polymerized into polyvinyl acetate and then hydrolyzed to PVA. The most suitable renewable base material is ethanol. Over 400 Gg (i.e. 4 × 105 ton) of PVA are produced each year, in many grades, for use in diverse applications. Other details can be found in a comprehensive monograph of Finch (1992) that provides a critical and authoritative review of 20 years of research and developments of the PVA technology. PVA combines high tensile strength with ease of film formation and shows excellent adhesive and bonding characteristics. It may be

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[105] J. E. Gough, 'Synthesis, Degradation, Andin Vitro Cell Responses of Sodium Phosphate Glasses for Craniofacial Bone Repair', *Journal of Biomedical Materials Research*, 59.3 (2002), 481–89.

[106] L Calandrelli, 'Preparation and Characterization of Composites Based on Biodegradable Polymers for ªin Vivoº Application', 2000, 7.

partially hydrolyzed and thus have better adhesion to hydrophobic surfaces, its water resistance being increased with increasing hydrolysis. The super-hydrolyzed grades of PVA should be used when maximum water resistance and humidity resistance are desired. PVA has a wide use in production of adhesives or paper coatings, ceramics, in reprography and photography, in medicine and biotechnology and in manufacturing of biodegradable polymer films. The biodegradability of PVA[107] in the nature has been extensively studied and the mechanism of metabolic degradation of PVA by microorganisms[108] was also described. Usually, PVA biodegrades in microbial active environments within 5–6 weeks. In a publication, 73 % of PVA[109] was found to be degraded by fungus Phanerochaete chrysosporium in a water environment within 5 d. When compared with other BDP, the market of PVA is huge. According to the web server www.chemindustry.com there are many suppliers of PVA as is apparent from the following list of PVA trade names: Akwa Tears, Alcotex, Alvyl, Aracet, Cipoviol, Celvol, Elvanol, Ethenol, Gelvatol, Gohsenol, Ivalon, Kuralon, Kurare, Lemol, Liquifilm, Mowiol, Polydesis, Polysizer, Polyvinol, Polyviol, Poval, Resis- toflex, Rhodoviol, Sno Tears, Solvar, Sumitex, Vibatex, Vinacol, Vinalak, Vinarol, Vinarole, Vinavilol, Vinol, Vinylon. However, the list is not yet complete because new and high production capacities are now opening in the Republic of Korea, India and Southeast Asia. Consumption of PVA in the United States, Western Europe and Japan increased at an overall rate of almost 2 % annually between 1992 and 2000.

Many microorganisms overproduce polyesters and neutral polysaccharides if they have access to a carbon source. Both groups of compounds are easy biodegradable and they can be

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[107] Masayuki Shimao, 'Biodegradation of Plastics', *Current Opinion in Biotechnology*, 12.3 (2001), 242–47.

[108] Fusako Kawai, 'Breakdown of Plastics and Polymers by Microorganisms', in *Microbial and Eznymatic Bioproducts*, by M. Hiroto and others (Berlin, Heidelberg: Springer Berlin Heidelberg, 1995), LII, 151–94.

[109] Mei-Hua Huang, Yang-Ping Shih, and Shiu-Mei Liu, 'Biodegradation of Polyvinyl Alcohol by *phanerochaete chrysosporium* after Pretreatment with Fenton's reagent', *Journal of Environmental Science and Health, Part A*, 37.1 (2002), 29–41.

produced from renewable resources. Polyesters, which are more important for the production of BDP, consist of simple carbon chain monomers. About 90 polyesters are identified as intracellular storage compounds performing different properties[110].

1.3.11. Polyesters.

Polyhydroxyalkanoates (PHA) are linear polyesters produced in nature by bacterial fermentation[111,112,113,114] from renewable natural sources such as sugar or lipids (Anderson and Dawes 1990). Large chemical companies such as Imperial Chemical Industries developed ways to produce PHA[115]. In the case of PHA, however, the bacterium Ralstonia eutropha converts sugar directly into plastic[116]. Usually, PHA naturally accumulates within the

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[110] D. Jendrossek, A. Schirmer, and H. G. Schlegel, 'Biodegradation of Polyhydroxyalkanoic Acids', *Applied Microbiology and Biotechnology*, 46.5–6 (1996), 451–63.

[111] Tomoji Katoh, 'Dynamics and Modeling on Fermentative Production of Poly (i-Hydroxybutyric Acid) from Sugars via Lactate by a Mixed Culture of Lactobacillus Delbrueckii and Alcaligenes Eutrophus', *Journal of Biotechnology*, 1999, 22.

[112] Y. Ke, 'Synthetic Routes to Degradable Copolymers Deriving from the Biosynthesized Polyhydroxyalkanoates: A Mini Review', *Express Polymer Letters*, 10.1 (2016), 36–53.

[113] K Sudesh, H Abe, and Y Doi, 'Synthesis, Structure and Properties of Polyhydroxyalkanoates: Biological Polyesters', *Progress in Polymer Science*, 25.10 (2000), 1503–55.

[114] V. Mittendorf, 'Synthesis of Medium-Chain-Length Polyhydroxyalkanoates in Arabidopsis Thaliana Using Intermediates of Peroxisomal Fatty Acid -Oxidation', *Proceedings of the National Academy of Sciences*, 95.23 (1998), 13397–402.

[115] Christophe M. Thomas, 'Stereocontrolled Ring-Opening Polymerization of Cyclic Esters: Synthesis of New Polyester Microstructures', *Chem. Soc. Rev.*, 39.1 (2010), 165–73.

[116] Jian Yu, 'Kinetics Modeling of Inhibition and Utilization of Mixed Volatile Fatty Acids in the Formation of Polyhydroxyalkanoates by Ralstonia Eutropha', *Process Biochemistry*, 37.7 (2002), 731–38.

microbes as granules that can constitute up to 90 % of a single cell mass. Recently, Jian et al. (2002) described the kinetics of PHA granule formation in a batch submersed culture. In the same year, an extensive quantitative description of process kinetic to a mixed culture in a fed-batch process[117,118,119].

The PHA plastics have a wide range of industrially useful properties[120] which will allow them to be used in both performance and commodity applications. At one end of the property range, the plastics are semi-crystalline with properties similar to polypropylene. At the other end of the range, the PHA are elastomeric – similar to natural rubber. The PHA plastics can be extruded into films, molded or coated onto other substrates, using conventional processing equipment. In addition, the PHA can be prepared either in a latex form, or as dry powders ready for melt processing. Typical PHA products are expected to encompass everyday items such as packaging materials, lawn and leaf bags, disposable diapers, fast food service ware, single-use medical devices, paints, as well as performance materials, which take advantage of superior properties inherent to the PHA.

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[117] Masayuki Tohyama, 'Modeling of the Mixed Culture and Periodic Control for PHB Production', *Biochemical Engineering Journal*, 10.3 (2002), 157–73.

[118] Guocheng C. Du, 'Feeding Strategy of Propionic Acid for Production of Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) with Ralstonia Eutropha', *Biochemical Engineering Journal*, 8.2 (2001), 103–10.

[119] Guocheng Du, 'Continuous Production of Poly-3-Hydroxybutyrate by Ralstonia Eutropha in a Two-Stage Culture System', *Journal of Biotechnology*, 88.1 (2001), 59–65.

[120] Sang Yup Lee, 'Plastic Bacteria? Progress and Prospects for Polyhydroxyalkanoate Production in Bacteria', *Trends in Biotechnology*, 14.11 (1996), 431–38.

Poly-3-hydroxybutyrate (PHB)[121,122] is an energy reserve polyester naturally accumulated by a wide variety of microorganisms; it is accumulated amongst others (e.g., Azotobacter chroococcum[123], Bacillus mycoides[124], by the bacteria Ralstonia eutropha (Alcaligenes eutrophus) in storage granules[125]. In a recent report, the progress in application of auto-retransgenic plants for the synthesis of PHB[126] was summed up. PHB-like copolymer called PHBV [poly(hydroxybutyrate–valerate)] is less stiff and tougher and is used as packaging material. Since April 1996, Monsanto Company has the sole rights for the production of PHBV under the trade name BiopolTM (Anonymous 2000c). PHA and PHB can be either thermoplastic or elastomeric materials, with melting points ranging from 40 to 180 °C. The most common type of PHA is PHB; it has properties similar to those of polypropylene. However, it is stiffer and more brittle. It is biocompatible and therefore can be implanted in the body without causing inflammations. The producer claims that it is nontoxic. The current price is very high compared to other oil-derived polymers. It was discovered that making 1 kg of PHB from genetically modified corn plants would require about 300 % more energy than that needed to manufacture an equal amount of fossil fuel-based polyethylene[127]. Thus, the benefit

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[121] Magdalena Wróbel, Jacek Zebrowski, and Jan Szopa, 'Polyhydroxybutyrate Synthesis in Transgenic Flax', *Journal of Biotechnology*, 107.1 (2004), 41–54.

[122] Yves Poirier, 'Synthesis of High-Molecular-Weight Poly([R]-(-)-3-Hydroxybutyrate) in Transgen=c Arabidopsis Thaliana Plant Cells', 6.

[123] J. Parshad, 'Poly-3-Hydroxybutyrate Production ByAzotobacter Chroococcum', *Folia Microbiologica*, 46.4 (2001), 315–20.

[124] P. S. Thakur, 'Growth-Associated Production of Poly-3-Hydroxybutyrate ByBacillus Mycoides', *Folia Microbiologica*, 46.6 (2001), 488–94.

[125] Y Poirier, 'Production of New Polymeric Compounds in Plants', *Current Opinion in Biotechnology*, 10.2 (1999), 181–85.

[126] Maria N. Somleva, 'Production of Polyhydroxybutyrate in Switchgrass, a Value-Added Co-Product in an Important Lignocellulosic Biomass Crop', *Plant Biotechnology Journal*, 6.7 (2008), 663–78.

[127] Tillman U. Gerngross, 'Can Biotechnology Move Us toward a Sustainable Society?', *Nature Biotechnology*, 17.6 (1999), 541–44.

of using corn instead of oil as a raw material could not offset this substantially higher energy demand. On the other hand, Choi and Lee (1997) demonstrated that the economic feasibility of PHB fermentation is comparable with petrochemical processes when cheap or waste substrates are used (Wong and Lee 1998; Yu and Si 2001) and unconventional microorganisms applied[128,129]. Recently, Du et al. (2001a,b) described a sophisticated two-stage culture process that has a chance to improve the economical effectiveness of the fermentation process. The PHA and PHB are biodegraded in microbially active environments within 5–6 weeks (Shimao 2001). The action of some enzymes produced by microorganisms degrades plastic material, which is then absorbed through the cell wall and metabolized. It is normally broken down to carbon dioxide and water when degraded under aerobic conditions. In the absence of oxygen, the degradation is faster, and methane is also produced. In a report summarizing and comparing the biomedical applications of polyesters[130], the relation of BDP for development of implant/medical devices, sutures, bone plates, joint replacements, ligaments, vascular grafts, intramolecular lenses, etc was established.

[128] O. Kofroiova and L Pta, 'Poly(3-Hydroxybutyrate) Granules of Bacillusmegaterium', 2.

[129] S. Pal, A. Manna, and A. K. Paul, 'Nutritional and Cultural Conditions for Production of Poly-3-Hydroxybutyric Acid ByAzotobacter Chroococcum', *Folia Microbiologica*, 43.2 (1998), 177–81.

[130] S. Ramakrishna, 'Biomedical Applications of Polymer-Composite Materials: A Review', *Composites Science and Technology*, 61.9 (2001), 1189–1224.

Chapter 2. Nitroxides, alkoxyamines and nitroxide mediated polymerization.

2.1. Nitroxides

Nitroxides[131], which are also known as nitroxyl radicals, are N,N-disubstituted NO radicals with a delocalized unpaired electron shared between the atoms of nitrogen and oxygen. Nitroxides possess a unique structure which makes them stable. The effectiveness of nitroxide radicals as mediators of well-controlled radical polymerization can be attributed to their stability and at the same time the radicals can be very persistent in a reaction mixture. The persistent radical effect (PRE) is responsible for the living character in polymers synthesized through NMP and gives room for further modification of polymer. Nitroxide structures[132] are mostly represented with the radical the oxygen, however, there exists another resonance structure which provides more explanation to their stability in which the radical is on the nitrogen, which is double-bonded to the oxygen. Besides nitroxides used in NMP usually contain bulky, sterically hindering groups in the R1 and R2 positions. A substantial steric bulk of these groups entirely prevents radical coupling in the N-centered resonance form, as much as greatly reducing it in the O-centered form. In the absence of resonance provided by allyl or aromatic groups α to the N, these bulky groups can contribute to the stability of the nitroxide[133]. The result is a decrease stability of the nitroxide, probably due to the fact that they offer less sterically hindered sites for radical coupling to take place. The resulting inactivity of the radical makes hemolytic cleavage of the alkoxyamine quite fast in more sterically hindered species.

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[131] Ludger Tebben and Armido Studer, 'Nitroxides: Applications in Synthesis and in Polymer Chemistry', *Angewandte Chemie International Edition*, 50.22 (2011), 5034–68.

[132] Paola Astolfi, 'Indolinic Nitroxides: Evaluation of Their Potential as Universal Control Agents for Nitroxide Mediated Polymerization', *Polymer Chemistry*, 4.13 (2013), 3694-704.

[133] William H. Daly, 'Recent Developments in Cellulose Grafting Chemistry Utilizing Barton Ester Intermediates and Nitroxide Mediation', *Macromolecular Symposia*, 174.1 (2001), 155–64.

DPAIO, SG1 and TEMPO[134,135] are some known stable and functional nitroxides which have been used extensively in polymer syntheses.

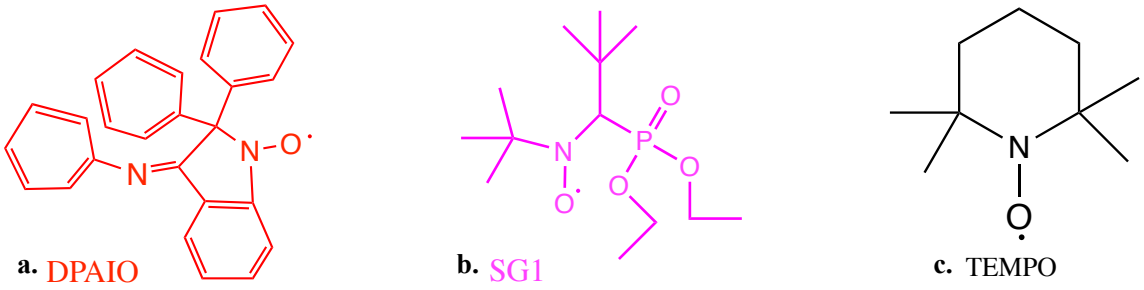


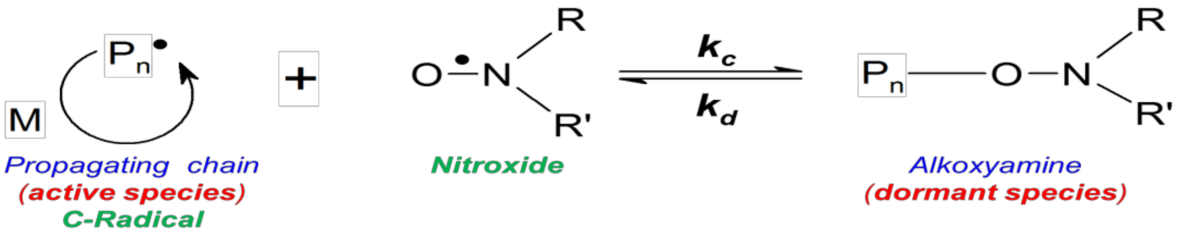
Fig. 4. The chemical structures of DPAIO (a), SG1 (b) and TEMPO (c) nitroxides.

[134] J. Lokaj and P. Holler, 'Nitroxide-Mediated Homopolymerization and Copolymerization of 2-Vinylpyridine with Styrene', *Journal of Applied Polymer Science*, 80.11 (2001), 2024–30.

[135] Rifat Jabbar, 'Nitroxide-Mediated Synthesis of Styrenic-Based Segmented and Tapered Block Copolymers Using Poly(Lactide)-Functionalized TEMPO Macromediators', *Journal of Applied Polymer Science*, 109.5 (2008), 3185–95.

2.2. Alkoxyamines

Alkoxyamines[136,137] are alcohols bonded to a secondary amine by an N-O single bond. They are derived from nitroxides and have demonstrated to be highly functional precursors of C-centered radicals in synthesis and also in polymer chemistry. They can be synthesized using the nucleophilic substitution of the hydroxylamine anion[138] on the corresponding alkyl halide, the Meisenheimer rearrangement of allyl or benzyl amine N-oxides, or the reaction between an oxoammonium salt with an olefin. The majority of the reaction consists in the in-situ generation of an alkyl radical followed by its trapping by a nitroxide. An alkoxyamine can be further defined as molecule



Scheme1. Typical radical process of alkoxyamine formation.

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[136] Gérard Audran, Paul Brémond, and Sylvain. R. A. Marque, 'Labile Alkoxyamines: Past, Present, and Future', *Chem. Commun.*, 50.59 (2014), 7921–28.

[137] Hasan Palandoken, 'A Facile Synthesis of (Tert-Alkoxy)Amines', *Tetrahedron Letters*, 46.39 (2005), 6667–69.

[138] S. Coseri, 'Mild and Selective Oxidation of Cellulose Fibers in the Presence of *N* - Hydroxyphthalimide', *Biomacromolecules*, 10.8 (2009), 2294–99.

which undergoes reversibe thermal homolysis[139] to produce an initiating radical and a persistent nitroxide[140,141]. However, pre-functionalizing alkoxyamines[142,143] induce important structural changes when compared to the parent molecules. In particular, all pre-functionalized SG1-based alkoxyamines[144] employed for (bio)conjugation so far have amide functionalities whereas the parent BlocBuilder and AMA alkoxyamines have a carboxylic acid group. Since the dissociation rate constant (kd) of the alkoxyamine is governed by the structure of the alkyl moiety, with a combination of polar, steric and stabilization effects, such structural modifications are likely to impact their dissociation. This is of high significance since, for a given monomer and nitroxide, the kd value determines the degree of control over the polymerization. Therefore, pre-functionalization of alkoxyamines could alter their controlling ability compared to that of their non-functionalized counterparts.

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[139] Gérard Audran, 'Chemically Triggered C–ON Bond Homolysis in Alkoxyamines. 6. Effect of the Counteranion', *The Journal of Organic Chemistry*, 78.15 (2013), 7754–57.

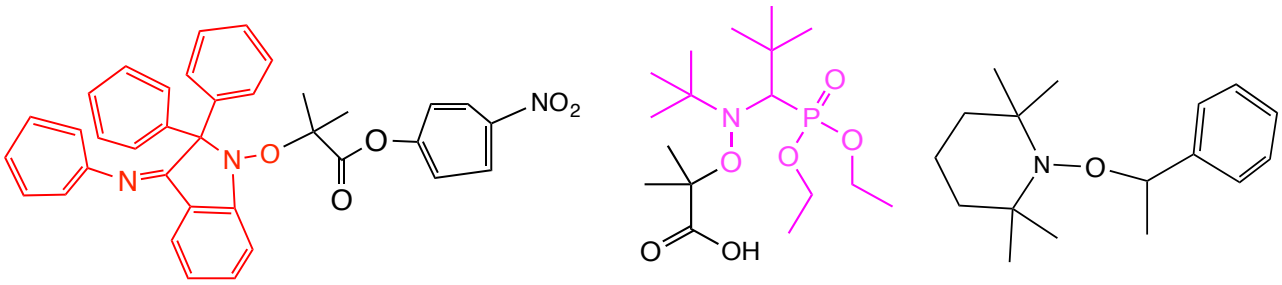
[140] Siham Telitel, 'UV-Induced Micropatterning of Complex Functional Surfaces by Photopolymerization Controlled by Alkoxyamines', *Langmuir*, 31.36 (2015), 10026–36.

[141] Armido Studer, 'The Persistent Radical Effect in Organic Synthesis', *Chemistry*, 7.6 (2001), 1159–64.

[142] Christine B. Wagner and Armido Studer, 'Synthesis of Macro(Alkoxyamines) via Hydroboration of Polyolefins with Subsequent Nitroxide Oxidation for Controlled NMP', *Macromolecular Chemistry and Physics*, 211.23 (2010), 2510–16.

[143] Lucien Marx, Gisele Volet, and Catherine Amiel, 'Well-Defined Polyoxazoline-Based Alkoxyamines as Efficient Macroinitiators in Nitroxide-Mediated Radical Polymerization of Styrene', *Journal of Polymer Science Part A: Polymer Chemistry*, 49.22 (2011), 4785–93.

[144] Bernadette Charleux and Julien Nicolas, 'Water-Soluble SG1-Based Alkoxyamines: A Breakthrough in Controlled/Living Free-Radical Polymerization in Aqueous Dispersed Media', *Polymer*, 48.20 (2007), 5813–33.



a. DPAIO alkoxyamine **b.** Blocbuilder MA **c.** TEMPO alkoxyamine

Fig. 5. The chemical structures of DPAIO alkoxyamine (a), BlocBuilder MA (b) and TEMPO alkoxyamine (c).

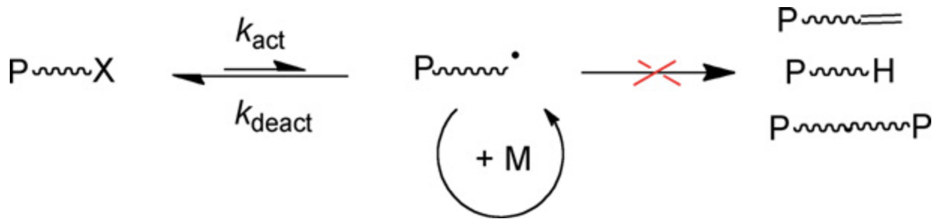
2.3. Nitroxide mediated polymerization (NMP).

Nitroxide mediated polymerization[145,146] is the main polymerization technique employed throughout the experimental synthesis of this work to obtain the final copolymeric materials. This is because the NMP is a simple technique which avoids the use of a metal catalyst or an external radical source unlike other free radical controlled polymerization techniques. The polymerization is initiated thermally in the presence of a nitroxide or alkoxyamine known as an initiator. The nitroxide or alkoxyamine also acts as a controller of the NMP process and designs the emerging macromolecules. The NMP is governed by reversible deactivation mechanism of nitroxide species or an alkoxyamine. This is based on the reversible trapping of a reactive propagating radical which is converted into a dormant species: rapid exchange between the active and dormant forms allows the polymer to grow while minimizing the

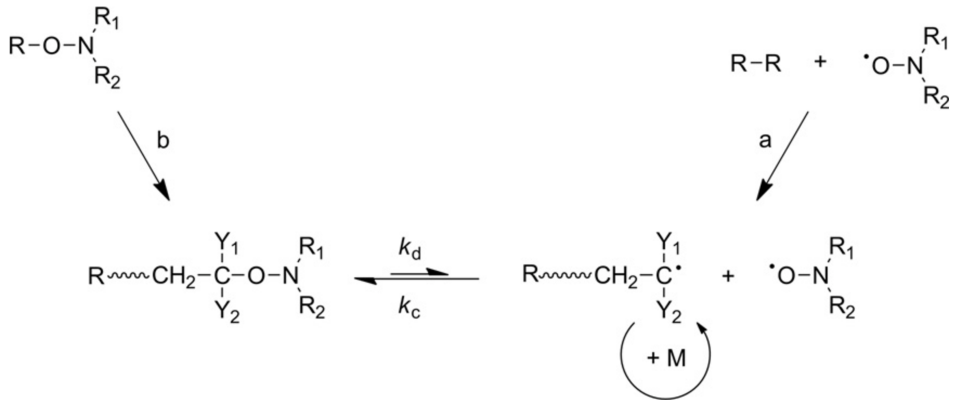
[145] Julien Nicolas, 'Nitroxide-Mediated Polymerization', *Progress in Polymer Science*, 38.1 (2013), 63–235.

[146] Graeme Moad and Ezio Rizzardo, 'Chapter 1. The History of Nitroxide-Mediated Polymerization', in *Polymer Chemistry Series*, ed. by Didier Gigmes (Cambridge: Royal Society of Chemistry, 2015), pp. 1–44.

frequency of bimolecular termination reactions. The living character[147] provides a milieu where new monomers/polymers can be further attached by covalent bonding in order to modify the properties of the existing material[148]. NMP represents an important radical polymerization technique, very effective and efficient in achieving polymers with better grafting, controlled molecular weight distribution, low polydispersity indices and good living character.



Scheme 2. General concept of controlled/living radical polymerization (CLRP). Activation–deactivation equilibrium in nitroxide-mediated polymerization.



[147] Valérie Sciannamea, Robert Jérôme, and Christophe Detrembleur, 'In-Situ Nitroxide-Mediated Radical Polymerization (NMP) Processes: Their Understanding and Optimization', *Chemical Reviews*, 108.3 (2008), 1104–26.

[148] Carlos M. R. Abreu, 'Nitroxide-Mediated Polymerization of Vinyl Chloride at Low Temperature: Kinetic and Computational Studies', *Macromolecules*, 49.2 (2016), 490–98.

Scheme 3. Bicomponent initiating system (a) and monocomponent initiating system (b).

The efficiency of the conjugation is generally higher, especially for bulky substrates, due to a lower steric hindrance and the purification of the resulting conjugates is facilitated as only the unreacted monomer has to be removed.

2.3.1. Literature review of nitroxide mediated polymerization

The art of nitroxide mediated polymerization has been vastly employed in both bulk and emulsification polymerization[149] and this technique is drastically being applied commercially. The features and achievements of NMP[150,151] since it was found (2012) summarized in a document discussed the process in detail, synthetic approaches nitroxides and alkoxyamines, its industrial applications and environmental constraints, kinetic aspects[152,153] and polymerization processes (ionic liquids[154], dispersed media, etc.). The macromolecular coupling approaches, functionalization strategies, macromolecular architectures, bio-related

[149] Julien Nicolas, Anne-Valérie Ruzette, 'Nanostructured Latex Particles Synthesized by Nitroxide-Mediated Controlled/Living Free-Radical Polymerization in Emulsion', *Polymer*, 48.24 (2007), 7029–40.

[150] Julien Nicolas, 'Nitroxide-Mediated Polymerization', *Progress in Polymer Science*, 38.1 (2013), 63–235.

[151] Rifat Jabbar, 'Nitroxide-Mediated Synthesis of Styrenic-Based Segmented and Tapered Block Copolymers Using Poly(Lactide)-Functionalized TEMPO Macromediators', *Journal of Applied Polymer Science*, 109.5 (2008), 3185–95.

[152] Marco Drache, Kerstin Mandel, and Gudrun Schmidt-Naake, 'Kinetics of Nitroxide-Controlled Radical Polymerization during the Non-Stationary State', *Polymer*, 48.7 (2007), 1875–83.

[153] Takeshi Fukuda, 'Mechanisms and Kinetics of Nitroxide-Controlled Free Radical Polymerization', 29.20 (1996), 6.

[154] Veronika Strehmel, 'Free Radical Polymerization of *n* -Butyl Methacrylate in Ionic Liquids', *Macromolecules*, 39.3 (2006), 923–30.

and hybrid materials, range of controllable monomers and polymer characterization also covered. Greci et al. homopolymerized methyl methacrylate in bulk using DPAIO alkoxyamine in a controlled NMP process at 85°C or 120°C. The first polymerization of 2-(diethylamino) ethyl methacrylate (DEAEMA) in water was done at 90°C and at ambient pressure, with a small amount of acrylonitrile[155] as a comonomer in two systems i.e., n-hydroxysuccinimidyl BlocBuilder (NHS-BB) alkoxyamine and a biocomponent imitating system composed of 2,2′-azobis [2-(2-imidazolin-2-yl) propane]- dihydrochloride (VA-044) initiator and SG1 nitroxide. The two systems demonstrated that the process was well-controlled as evidenced by low molar dispersity and evolution of the molar mass distribution and the final polymers exhibited excellent livingness[156]. Despite the challenges involved in the copolymerization between biopolymers and synthetic polymers using NMP, a tremendous amount of work has been done chemically modifying former with the latter or vice versa. An interesting publication on the synthesis of polystyrene-grafted cellulose acetate, confirmed the introduction of BlocBuilder MA on the polysaccharide backbone through 1,2-intermolecular radical addition (IRA) and subsequent grafting through controlled/living NMP under homogenous conditions[157]. Likewise, chitosan was copolymerized with methyl methacrylate in the presence of a small amount of acrylonitrile and also copolymerized with sodium 4-styrenesulfonate under heterogenous conditions using SG1-based NMP[158]. The simplicity and ease to prepare macromolecular hybrid materials such as diblock, triblock copolymers, star polymers have been reported. This review described the potential of the intermolecular radical 1,2-addition from the

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[155] Julien Nicolas, Brusseau, and Bernadette Charleux, 'A Minimal Amount of Acrylonitrile Turns the Nitroxide-Mediated Polymerization of Methyl Methacrylate into an Almost Ideal Controlled/Living System', *Journal of Polymer Science Part A: Polymer Chemistry*, 48.1 (2010), 34–47.

[156] Ali Darabi, 'Nitroxide-Mediated Polymerization of 2-(Diethylamino) Ethyl Methacrylate (DEAEMA) in Water', *Macromolecules*, 48.1 (2015), 72–80.

[157] Guillaume Moreira, 'Synthesis of Polystyrene-Grafted Cellulose Acetate Copolymers via Nitroxide-Mediated Polymerization', *Polymer Chemistry*, 6.29 (2015), 5244–53.

[158] Catherine Lefay, 'Heterogeneous Modification of Chitosan via Nitroxide-Mediated Polymerization', *Polym. Chem.*, 4.2 (2013), 322–28.

commercially available BlocBuilder MA alkoxyamine onto activated olefins[159,160,161] to synthesize either new functionalized alkoxyamines and different macromolecular architectures opening a new horizon for NMP. Synthesis of the First peptide/protein PEGylation with functional polymers designed by nitroxide-mediated polymerization demonstrated tunable reactivities towards nucleophiles depending on the choice of the alkoxyamine[162]. Biodegradable foam plastics have been prepared based on castor oil by functionalizing castor oil with maleic acid and further polymerizing the maleated castor oil[163] with styrene monomer through a free radical initiated process. The free radical process may not be in particular an NMP, however it is an indication that NMP which belongs to the family of FRP techniques can also be applied synthesis of polymers from vegetable oils.

Other faddish efficient reversible deactivation radical polymerization techniques (RDRP) are atom transfer radical polymerization (ATRP), reversible addition fragmentation chain transfer polymerization (RAFT).

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[159] Didier Gigmes, 'Intermolecular Radical 1,2-Addition of the BlocBuilder MA Alkoxyamine onto Activated Olefins: A Versatile Tool for the Synthesis of Complex Macromolecular Architecture', *Polymer Chemistry*, 2.8 (2011), 1624.

[160] Pierre-Emmanuel Dufils, 'Intermolecular Radical Addition of Alkoxyamines onto Olefins: An Easy Access to Advanced Macromolecular Architectures Precursors', *Polymer*, 48.18 (2007), 5219–25.

[161] Christine B. Wagner and Armido Studer, 'Synthesis of Macro(Alkoxyamines) via Hydroboration of Polyolefins with Subsequent Nitroxide Oxidation for Controlled NMP', *Macromolecular Chemistry and Physics*, 211.23 (2010), 2510–16.

[162] Marion Chenal, Céline Boursier, 'First Peptide/Protein PEGylation with Functional Polymers Designed by Nitroxide-Mediated Polymerization', *Polymer Chemistry*, 2.7 (2011), 1523.

[163] Hong Juan Wang and others, 'Biodegradable Foam Plastics Based on Castor Oil', *Biomacromolecules*, 9.2 (2008), 615–23.

2.3.2. Approaches of nitroxide mediated polymerization

Generally, three main approaches can be used in performing an NMP. In the "grafting form" approach[164], the polymerization takes place on the surface of the macromolecular backbone where active sites have been introduced and from where the polymer chains propagate. This approach is more ensuring in the synthesis of graft copolymers because of less steric hindrance and is preferred for the formation of well-defined block copolymers[165,166,167].

The "grafting to" approach[168] is when copolymerization occurs because propagating chains from one polymer/monomer are bonded with one or more functional groups of another polymer during the polymerization process.

Finally, the "grafting through" which is a typical approach in preparing random copolymers can be described as the formation of copolymers when monomers and active sites are put together in a system under conditions for polymerization.

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[164] Simon Kwan and Milan Marić, 'Thermoresponsive Polymers with Tunable Cloud Point Temperatures Grafted from Chitosan via Nitroxide Mediated Polymerization', *Polymer*, 86 (2016), 69–82.

[165] Qingquan Liu, 'Functional Block Copolymers from Controlled Radical and Ring Opening Polymerization', *Polymer Science Series B*, 57.5 (2015), 387–94.

[166] Sean George and others, 'Amphiphilic Block Copolymers as Stabilizers in Emulsion Polymerization: Effects of the Stabilizing Block Molecular Weight Dispersity on Stabilization Performance', *Macromolecules*, 48.24 (2015), 8913–20.

[167] Benoît H. Lessard and others, 'Poly(Styrene-Alt-Maleic Anhydride)-Block-Poly(Methacrylate-Ran-Styrene) Block Copolymers with Tunable Mechanical Properties by Nitroxide Mediated Controlled Radical Polymerization', *Macromolecular Research*, 24.8 (2016), 710–15.

[168] A.I. Khalf, D.E.El. Nashar, and N.A. Maziad, 'Effect of Grafting Cellulose Acetate and Methylmethacrylate as Compatibilizer onto NBR/SBR Blends', *Materials & Design (1980-2015)*, 31.5 (2010), 2592–98.

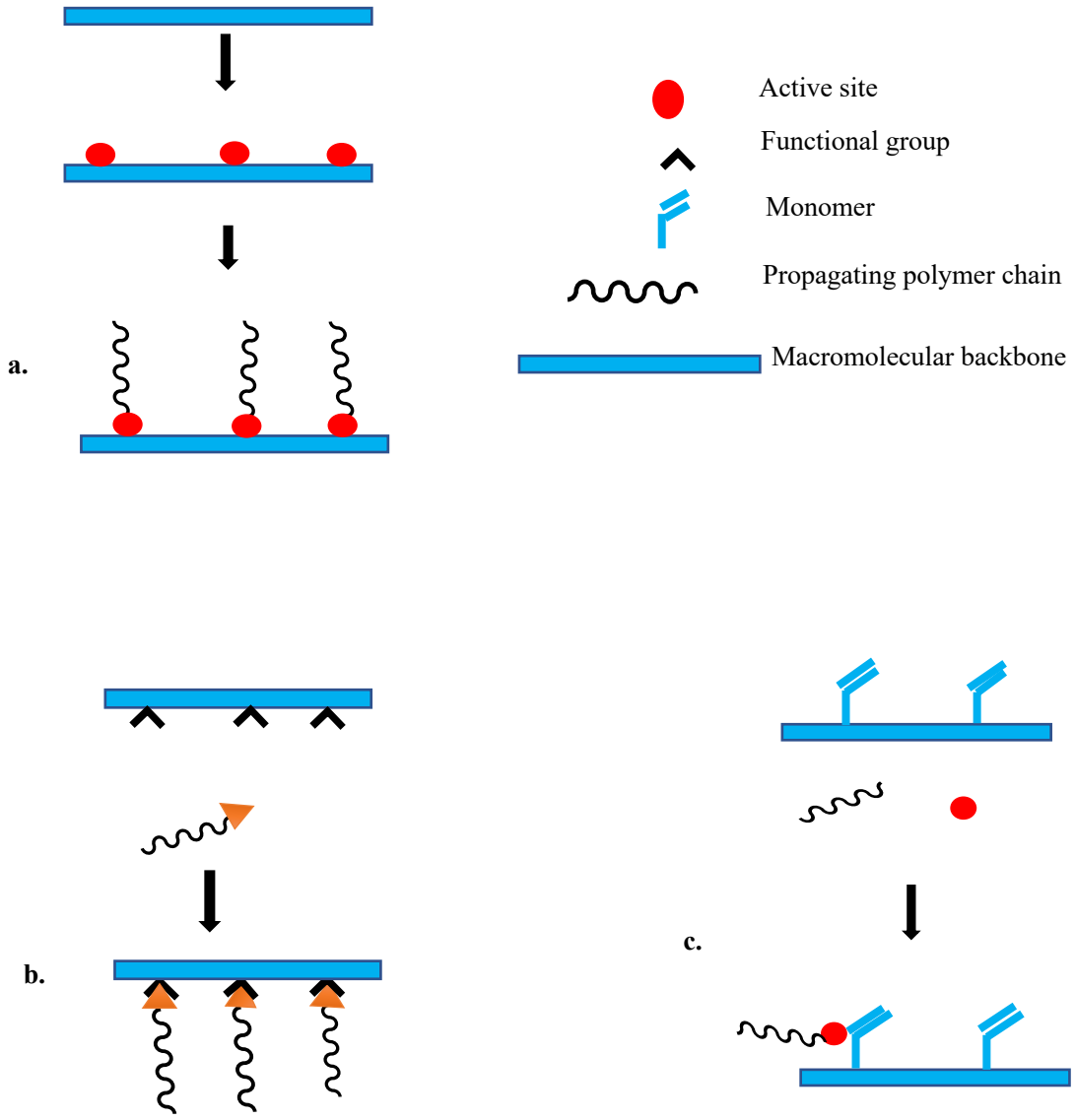
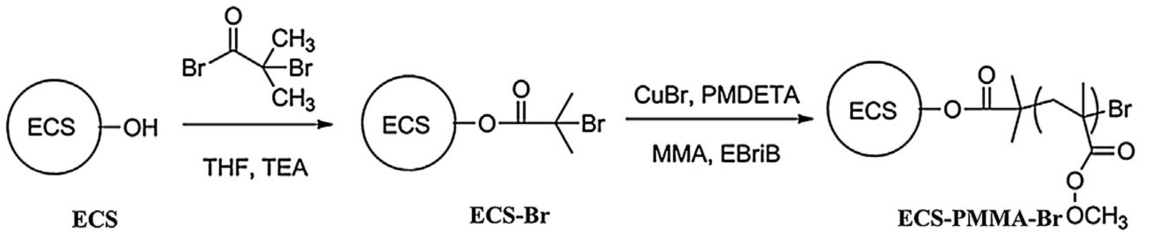


Fig. 6. Grafting from (a), grafting to (b) and grafting through (c).

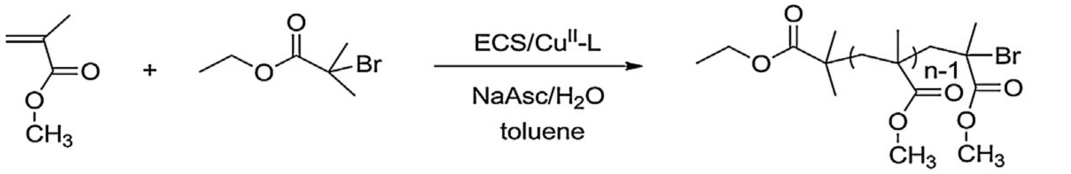
2.4. Atom transfer radical polymerization (ATRP)

ATRP[169,170,171,172] is a reversible-deactivation polymerization process which involves the formation of a carbon-carbon bond with a transition metal catalyst such as copper. The scheme (4) better below illustrates the mechanism of the process.



ECS ECS-Br ECS-PMMA-Br

(a)



methyl methacrylate ethyl 2-bromoisobutyrate EBriB Poly(methyl methacrylate) PMMA

(b)

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169 Jin Ran, 'Atom Transfer Radical Polymerization (ATRP): A Versatile and Forceful Tool for Functional Membranes', *Progress in Polymer Science*, 39.1 (2014), 124–44.

170 Veerle M. C. Coessens and Krzysztof Matyjaszewski, 'Fundamentals of Atom Transfer Radical Polymerization', *Journal of Chemical Education*, 87.9 (2010), 916–19.

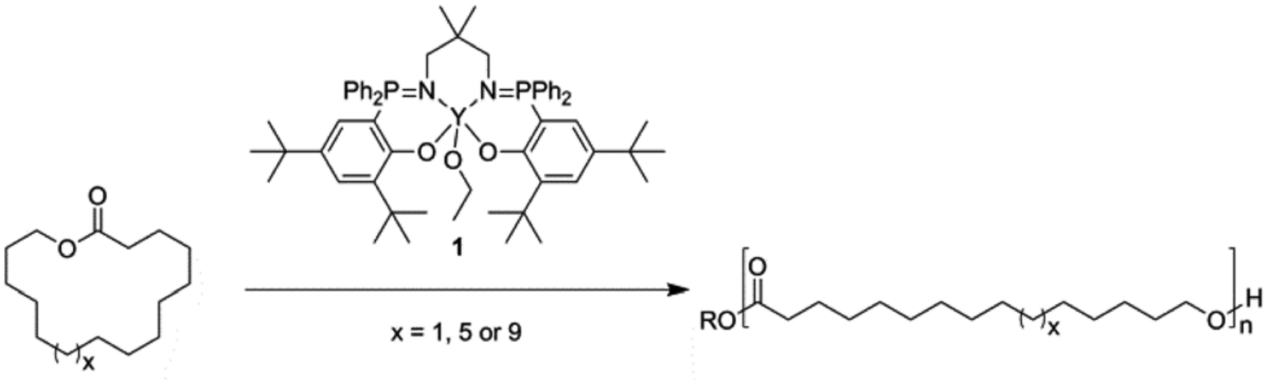
171 Bing Hu and others, 'Low Protein Fouling Polypropylene Membrane Prepared by Photoinduced Reversible Addition-Fragmentation Chain Transfer Polymerization', *Journal of Applied Polymer Science*, 123.6 (2012), 3668–74.

172 Nicolay V. Tsarevsky and Krzysztof Matyjaszewski, '"Green" Atom Transfer Radical Polymerization: From Process Design to Preparation of Well-Defined Environmentally Friendly Polymeric Materials', *Chemical Reviews*, 107.6 (2007), 2270–99.

Scheme 4. Reaction schemes showing (a) SI-ATRP of MMA on the surface of ECS. (b) AGET-ATRP of MMA using ECS loaded $CuBr_2$/PMDETA in toluene[173].

2.5. Ring opening polymerization (ROP)

ROP[174,175] is a form of chain-growth polymerization whereby the terminal end of a polymer chain acts as a reactive center allowing cyclic monomers to react by opening its ring system and form a longer polymer chain.



[173] Ankushi Bansal, 'Expanded Corn Starch as a Versatile Material in Atom Transfer Radical Polymerization (ATRP) of Styrene and Methyl Methacrylate', *Carbohydrate Polymers*, 130 (2015), 290–98.

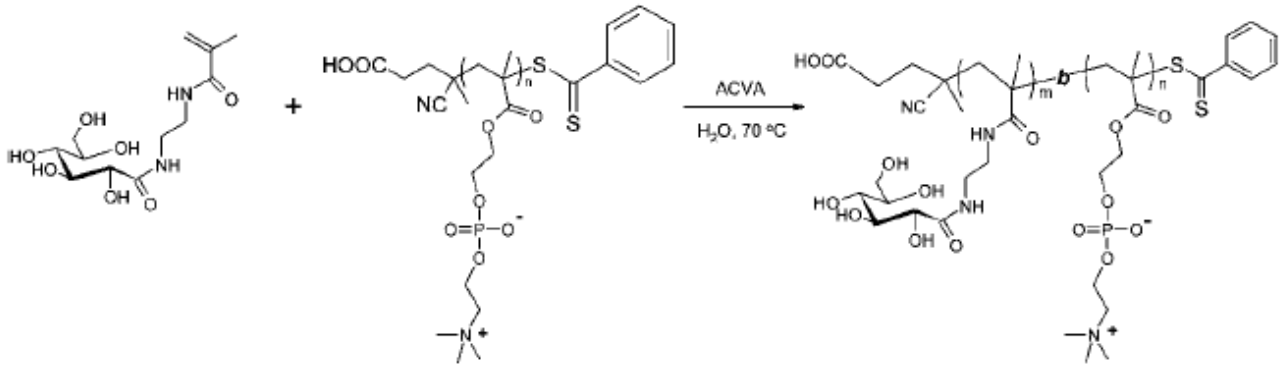
[174] Oskar Nuyken and Stephen Pask, 'Ring-Opening Polymerization—An Introductory Review', *Polymers*, 5.2 (2013), 361–403.

[175] Zongyong Zhang, Feng He, and Renxi Zhuo, 'Immobilized Lipase on Porous Silica Particles: Preparation and Application for Biodegradable Polymer Syntheses in Ionic Liquid at Higher Temperature', *Journal of Molecular Catalysis B: Enzymatic*, 94 (2013), 129–35.

Scheme 5. Yttrium phosphasalen catalysed ring-opening polymerization of macrolactones: PDL (C15), NDL (C19) and TCL (C23). Conditions: [Lactone]0/[1] = 50–200, [Lactone]0 = 0.3–3.8 M, toluene or bulk[176].

2.6. Reversible addition/fragmentation transfer polymerization

RAFT mechanism[177,178,179] precisely controls the way in which small molecules are linked together to form large polymer chains as demonstrated by the scheme (6) below.



Scheme 6. Di-block copolymerization of poly (MPC) macroCTA with GAEMA in water at 70°C in the presence of ACVA as an initiator[180]

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[176] D. Myers, 'Ring Opening Polymerization of Macrolactones: High Conversions and Activities Using an Yttrium Catalyst', *Polymer Chemistry*, 8.37 (2017), 5780–85.

[177] Sébastien Perrier, Pittaya Takolpuckdee, and Craig A. Mars, 'Reversible Addition−Fragmentation Chain Transfer Polymerization Mediated by a Solid Supported Chain Transfer Agent', *Macromolecules*, 38.16 (2005), 6770–74.

[178] Graeme Moad, Ezio Rizzardo, and San H. Thang, 'Radical Addition–Fragmentation Chemistry in Polymer Synthesis', *Polymer*, 49.5 (2008), 1079–1131.

[179] Neha Bhuchar and others, 'Detailed Study of the Reversible Addition–Fragmentation Chain Transfer Polymerization and Co-Polymerization of 2-Methacryloyloxyethyl Phosphorylcholine', *Polym. Chem.*, 2.3 (2011), 632–39.

[180] Neha Bhuchar and others, 'Detailed Study of the Reversible Addition–Fragmentation Chain Transfer Polymerization and Co-Polymerization of 2-Methacryloyloxyethyl Phosphorylcholine', *Polym. Chem.*, 2.3 (2011), 632–39.

Chapter 3. Experimental Section.

3.1. Materials

Bezoin oxime, anhydrous sulfuric acid, sodium bicarbonate, benzoic acid, acetic acid, 2 M sodium hydroxide and hydrochloric acid and distilled water were purchased at Sigma-Aldrich. α-bromoisobutyryl bromide (α-BIBB), acryloyl chloride (AC), triethylamine (TEA), dichloromethane (DCM), tetrahydrofuran (THF), acetonitrile (MeCN), dimethylformamide (DMF) were purchased from Aldrich N-tert-butyl-N-(1-diethyl phosphono-2,2-dimethylpropyl) nitroxide (SG1) and 2-methyl-2-(N-tert-butyl-N-(1-diethoxyphosphoryl- 2,2-dimethylpropyl)aminoxy) propionic acid (Blocbuilder MA) were obtained from Arkema, France. Oleic acid (OA, ≥ 99%), 2 hydroxyethyl methacrylate (HEMA, 97%), 4-dimethylaminopyridine (DMAP, 99%) and dicyclohexylcarbodiimide (DCC) 1.0 M in CH_2Cl_2, were also purchased from Sigma-Aldrich and used without any further purification. The 2,2'-azobisisobutyronitrile (AIBN, 98%, Sigma) was recrystallized from methanol. Methyl-2-methyl-3-nitro-2-nitrosopropionate (NMMA) was prepared as published. The solvents such as hexanes (mixtures of isomers), ethyl acetate (EtOAc) and methanol (MeOH) were purified by simple distillation. Diethyl ether (Et_2O) and toluene were refluxed over Na/benzophenone and freshly distilled before use. Dry dichloromethane (CH_2Cl_2) was freshly distilled over P_2O_5.

Note: All solvents were distilled or purified before use. All radical reactions were carried out in an argon atmosphere.

3.2. Synthesis of 1-hydroxyl-2-phenyl indole-2-phenyl-1H-indole-1-ol.

250 ml of sulfuric acid was measured and transferred into a round bottom flask. Then 250 g of α-benzoin oxime was weighed and added into the flask. The mixture was stirred at room temperature for 12 h. Finally, the content of the flask was poured onto 400 ml of ice

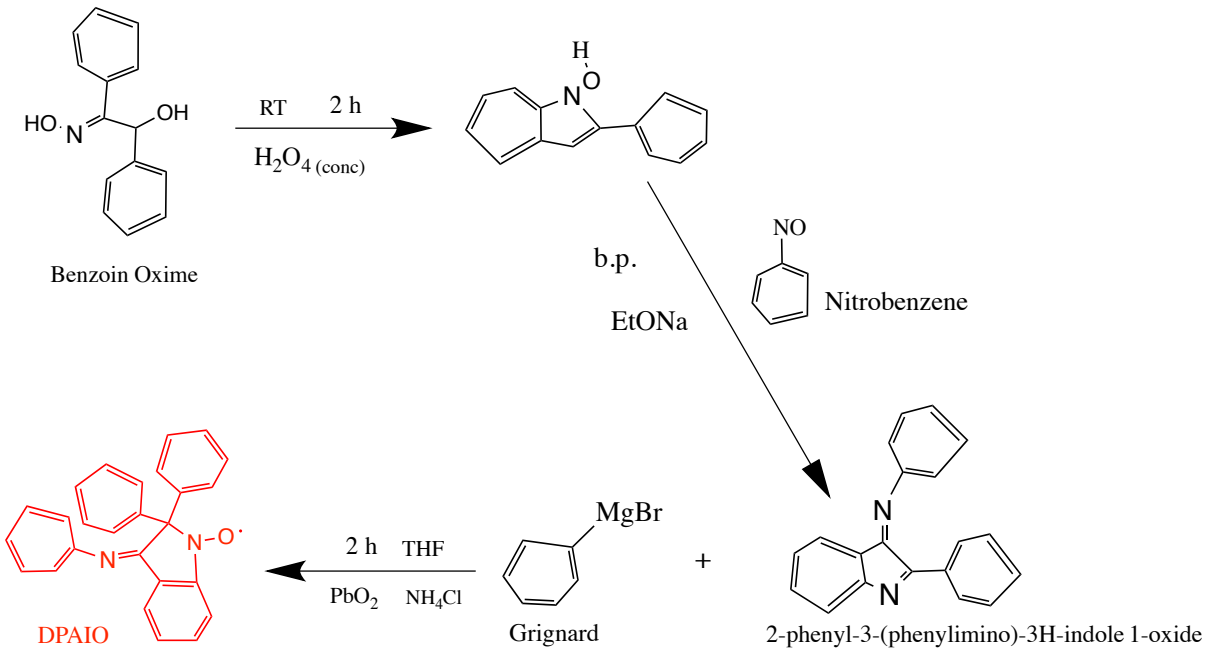
water under stirring. The resulting milky yellowish suspension was diluted with ethanol, extracted, filtered and dried on a rotavapor. Phenylindole is a derivative used for the synthesis of the nitrone.

3.3. Synthesis of 2-phenyl-3-phenylimino-3H-indole 1 oxide (nitrone)

1 g of phenylindole and 0.524 g of nitrobenzene were heated in ethanol in separate beakers simultaneously until they reached boiling point. Meanwhile sodium ethoxide solution was prepared by dissolving 0.05 g of sodium in ethanol in another beaker. Finally, the phenylindole solution was poured unto sodium ethoxide solution immediately followed by that nitrobenzene solution. The reddish-brown precipitate is formed was filtered and washed three times in ethanol, then washed with DCM and dried on the rotavapor. Absolute ethanol was used in all steps.

3.4. Synthesis of 2-phenyl-3-(phenylimino)-3H-indole 1-oxide (DPAIO nitroxide)

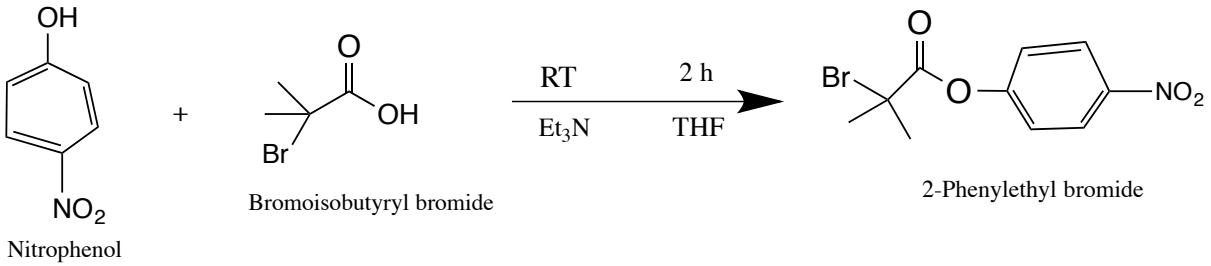
DPAIO was first synthesized by Greci et al. and the procedure is the same as it was first described. 1 g of 2-phenyl-3-(phenylimino)-3H-indole 1-oxide and dry 85 ml of THF were added in a current of argon under stirring at room temperature to a three-neck round flask equipped with an additional funnel with a pressure equalization arm. A degassed solution of the desired 9.4 ml of Grignard reactant in 30 ml of THF was poured into the funnel and added dropwise to the reaction mixture. After 1–2 h, the reaction solution was hydrolyzed with 20 ml of 1 M ammonium chloride solution and extracted with diethyl ether. The organic solution was dried over Na_2SO_4, filtered and reduced to a small volume under vacuum. 2.4 g of lead dioxide was added to this solution, the resulting mixture was stirred for 1 hour, filtered and evaporated to dryness. The residue was purified by SiO_2 chromatography using cyclohexane–ethyl acetate 8 : 2 as an eluent and crystallized from ethanol. Usually, 70–90% yields of DPAIO were obtained.



Scheme 7. Preparation of 2-phenyl-3-(phenylimino)-3H-indole 1-oxide (DPAIO nitroxide).

3.5. Synthesis of bromoderivative (2-phenylethyl bromide)

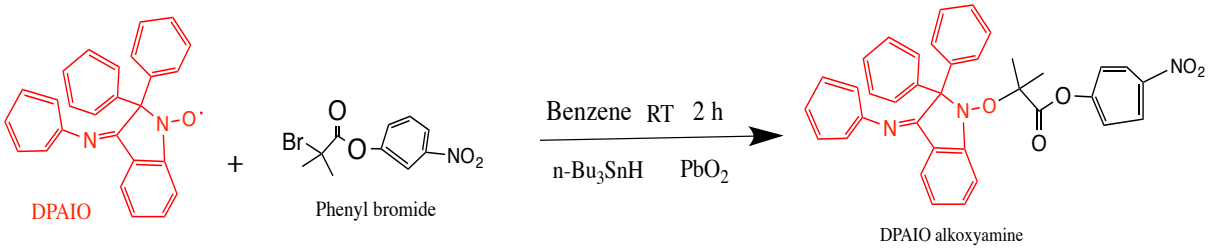
Before proceeding to the synthesis of DPAIO alkoxyamine, phenyl bromide which is one of the reagents used in the synthesis of the alkoxyamine was first synthesized. 6.260 g of nitrophenol was dissolved in THF in a round bottom flask and 6.899 ml of TEA added afterwards. 5.676 ml of α-BIBB diluted in THF was then added dropwise into the flask by means of an arm funnel. The reaction mixture was allowed to stir at room temperature for 2 h. The mixture was then filtered to remove salts and crystallized to obtain phenyl bromide.



Scheme 8. Preparation of 2-Phenylethyl bromide.

3.6. Synthesis of DPAIO alkoxyamine

Nitroxides 1a–d (0.33 mmol), bromoderivatives x–z (0.66 mmol) and PbO_2 (0.9 mmol) in 15 mL of benzene or dichloromethane were added to a three-neck round bottom flask equipped with an additional funnel with a pressure-equalization arm, in a current of argon, under stirring at room temperature. A well-degassed solution of n-Bu_3SnH (0.66 mmol) in 15 mL of benzene or dichloromethane was poured into the funnel and added dropwise to the reaction mixture. After 1–2 h, the reaction solution was filtered through celite and the filtrate was washed with water (20 mL _ 2). The separated organic layer was dried on MgSO4 and purified by column chromatography on silica gel eluting with ethyl acetate–cyclohexane 9 : 1; from the pale yellow fraction the expected alkoxyamine was isolated in 64–82% yields.



Scheme 9. Preparation of DPAIO alkoxyamine.

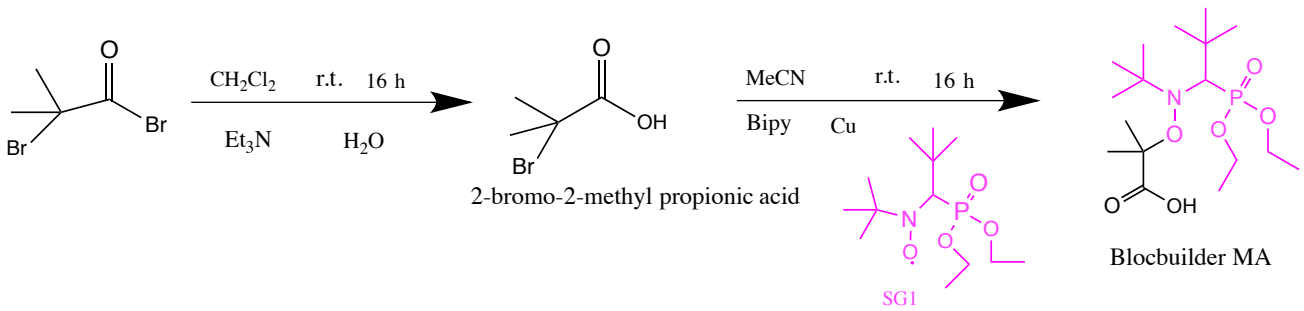
3.7. Synthesis of 2-bromo-2-methyl propionic acid

A mixture was made with 5ml of DCM, 82.26 µl of water and 667 µl of TEA. 1 g of α-BIBB diluted in a few ml of DCM was added dropwise by means of an arm funnel at a

temperature of 0°C. The reaction was stirred at this temperature for 20 min and leave overnight. A pale-yellow solution was obtained. The crude product was diluted with 5 ml DCM and the pH was brought to 9 with 1 M NaOH. The organic phase was separated from the aqueous phase and HCl was used to bring the pH to acidic. The final product was extracted using DCM and passed over anhydrous Na_2SO_4 before finally drying on the rotavapor. A colorless oil which crystallizes at room temperature was obtained.

3.8. Synthesis of blocbuilder®MA

157 mg of 2-bromo-2-methyl propionic acid, 300 mg of SG1 and 59 mg of Cu (0) were dissolved in acetonitrile in a 3 neck round bottom flask and degassed for about 20 min. 0.29 g of bipy was added and the mixture stirred under argon at room temperature for 16 h. The green precipitate obtained was diluted with 50mL EtOAc, washed with 10%HCl (3x30 mL), saturated $NaHCO_3$ (3x30mL) and sat. NaCl (30mL). The organic phase was dried over Na_2SO_4 and solvent was removed on a rotary evaporator.



Scheme 10. Preparation of Blocbuilder MA.

3.9. Synthesis of monomers

3.9.1. Synthesis of expanded corn starch (ECS)

Expanded corn starch was prepared in order to increase the pore sizes of starch molecules, therefore facilitating the incorporation of other functional groups during chemical reaction. For

the synthesis of ECS[181] was follows. 8 g corn starch was added into 150 ml of distilled water. The mixture was heated for 1 h at 90 °C under constant stirring for complete gelatinization of CS, and then 150 ml of ethanol was added drop wise to the solution of gelatinized starch and the resulting suspended particle were cooled at the room temperature. Thereafter, another 150 ml of ethanol was added drop wise for about 50 min with constant stirring. These suspended particles of CS were centrifuged followed by successive washings with ethanol and at the end, dried, to obtain porous starch particles (ECS).

3.9.2. Synthesis of starch acetate (acetylated starch)

Starch acetate (SA)[182] was prepared in order to replace the hydroxyl (OH) functional groups of starch with acetyl groups. The reason for this is because OH groups are insoluble in most organic solvents used for polymerization whereas acetyl groups are quite soluble and can significantly increase the solubility of the whole compound. The CS was dried at 50ºC for 24 h before reaction to avoid the interference of moisture. 4.4 g of dried CS and 4.4 ml of glacial acetic acid were placed into a 250 mL two-neck round-bottom flask equipped with a condenser and a magnetic stirrer. The mixture was stirred for 2 min resulting to a uniform suspension. Cold acetic anhydride was added to the mixture and stirred for 15 min before adding sulfuric acid (98%) added drop-wise to the flask contents for 10 mins. The reaction mixture was stirred for 160 min at 70ºC. By changing the chemicals ratio, it was possible to prepare esters with different degrees of substitutions (DSs). The reaction was terminated by adding the hot starch to 40 ml of distilled water. Water functions as a neutralizer to the acid. At the end of the reaction, the precipitate was filtered and washed with excess distilled water

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[181] Ankushi Bansal, Siddharth S. Ray, and Alok K. Chatterjee, 'Expanded Corn Starch a Novel Material as Macroinitiator/Solid Support in SI and AGET ATRP: GMA Polymerization', *Journal of Polymer Research*, 22.2 (2015).

[182] Seyed Heydar Mahmoudi Najafi, Maryam Baghaie, and Alireza Ashori, 'Preparation and Characterization of Acetylated Starch Nanoparticles as Drug Carrier: Ciprofloxacin as a Model', *International Journal of Biological Macromolecules*, 87 (2016), 48–54.

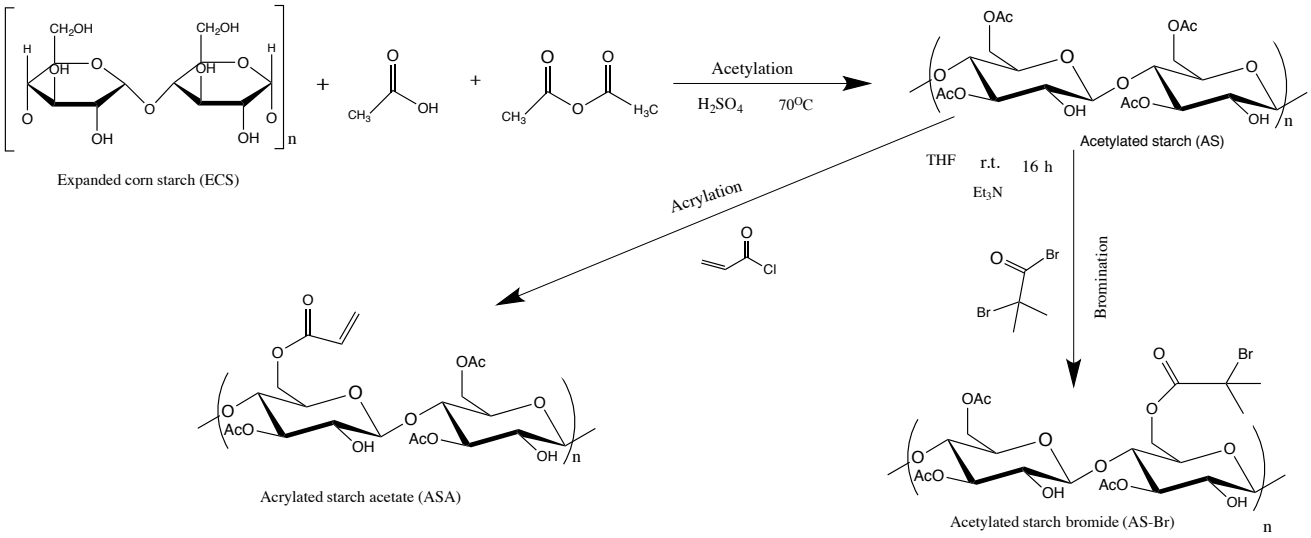
several times. The pH was checked to be neutral and then the product was at 50°C for several hours. Finally, the resultant solid was stored in an airtight container for future use.

3.9.3. Synthesis of starch acetate bromide (SA-Br)

2 g of SA was dissolved in 10 ml of distilled THF a 3 neck round bottom flask. The mixture was stirred until a homogenous solution was formed and then 5 ml of TEA was added. Argon was flushed into the flask to prevent moisture. Through an arm funnel 3 ml of α-BIBB was added dropwise at 0°C under stirring. The mixture was stirred at this temperature for 1 h and then left at r.t. to react for 16 h. The crude product was centrifuge in order to separate the unwanted salt from the liquid product. The crude product was then precipitated in excess hexane, to obtain pure white product which was dried on the rotavapor at 50°C.

3.9.4. Synthesis of acrylated starch acetate (ASA)

2 g of SA was dissolved in 10 ml of distilled THF in a 3 neck round bottom flask. The mixture was stirred until a homogenous solution was formed and then 5 ml of TEA was added. Argon was flushed into the flask to prevent moisture. Through an arm funnel 3 ml of acryloyl chloride (AC) was added dropwise at 0°C under stirring. The mixture was stirred at this temperature for 1 h and then left at r.t. to react for 16 h. The crude product was centrifuge in order to separate the unwanted salt from the liquid product. The crude product was then precipitated in excess hexane, to obtain pure white product which was dried on the rotavapor at 50°C.



Scheme 11. Preparation of starch acetate (SA), acrylated starch acetate (ASA) and starch acetate bromide (SA-Br).

3.9.5. Synthesis of cellulose acetate bromide (CA-Br)

2 g of CA was dissolved in 10 ml of distilled THF a 3 neck round bottom flask[183]. The mixture was stirred until a homogenous solution was formed and then 5 ml of TEA was added. Argon was flushed into the flask to prevent moisture. Through an arm funnel 3 ml of α-BIBB was added dropwise at 0°C under stirring. The mixture was stirred at this temperature for 1 h and then left at r.t. to react for 16 h. The crude product was centrifuge in order to separate the unwanted salt from the liquid product. The crude product was then precipitated in excess hexane, to obtain pure white product which was dried on the rotavapor at 50°C.

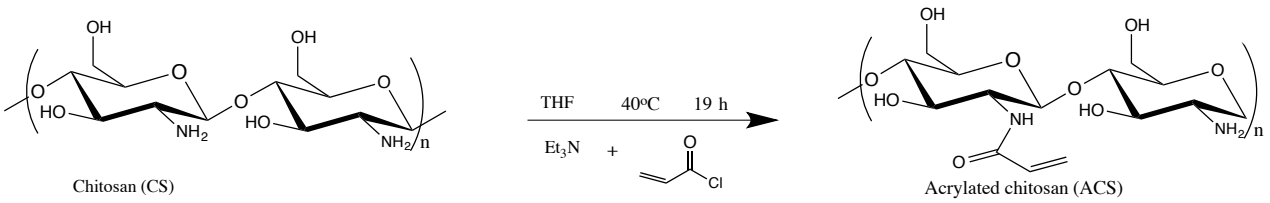
[183] Leena Nurmi and others, 'Controlled Grafting of Acetylated Starch by Atom Transfer Radical Polymerization of MMA', *European Polymer Journal*, 43.4 (2007), 1372–82.

3.9.6. Synthesis of chitosan bromide (CS-Br)

1 g of CS was dissolved in 20 ml of distilled THF a 3 neck round bottom flask. The mixture was stirred until a homogenous solution was formed and then 4.83 ml of TEA was added. Argon was flushed into the flask to prevent moisture. Through an arm funnel 2.9 ml of α-BIBB was added dropwise at 0ºC under stirring. The mixture was stirred at this temperature for 1 h and then left at r.t. to react for 19 h. The crude product was filtered, then washed with water, methanol and DCM successively. The final product was dried on the rotavapor at 50ºC.

3.9.7. Synthesis of acrylated chitosan (ACS)

0.5 g of CS was dispersed in 15 ml of distilled THF a 3 neck round bottom flask. The mixture was stirred for a while and then 3.5 ml of TEA was added. Argon was flushed into the flask to prevent moisture. Through an arm funnel 1.85 ml of acryloyl chloride (AC) was added dropwise at 5ºC under stirring. The mixture was stirred at this temperature for 1 h and then left at 40ºC to react for 19 h. The crude product was filtered, then washed with water, methanol and DCM successively. The final product was dried on the rotavapor at 50ºC.



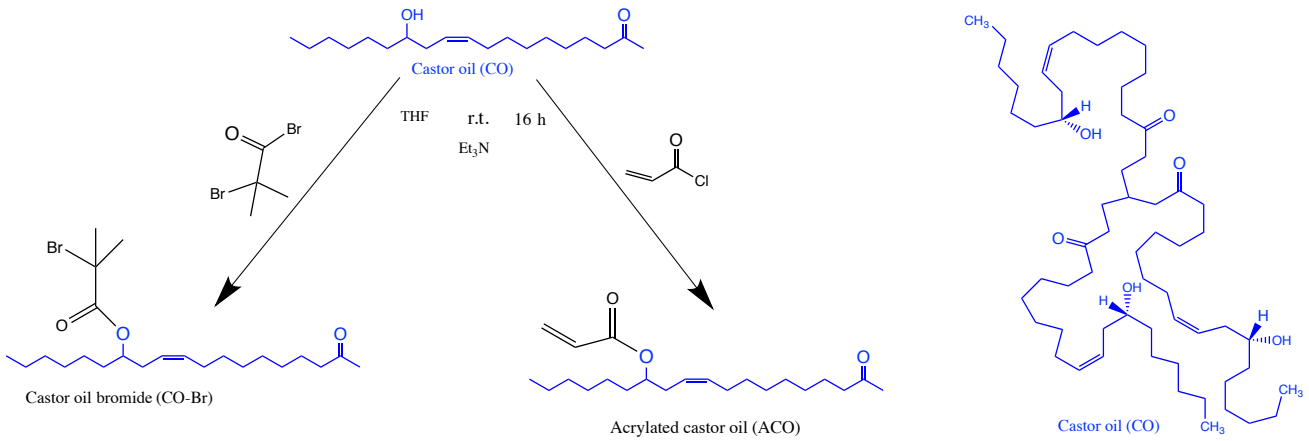
Scheme 12. Formation of acrylated chitosan (ACS).

3.9.8. Synthesis of castor oil bromide (CO-Br)

2 g of CO was dissolved in 5 ml of distilled THF a 3 neck round bottom flask. Then 9.75 ml of TEA was added to the mixture. Argon was flushed into the flask for a few minutes to prevent moisture. Through an arm funnel 5.56 ml of α-BIBB was added dropwise at 5°C under stirring. The mixture was stirred at this temperature for 1 h and then left at r.t. to react for 16 h or overnight. The crude product was dissolved in DCM and then washed with 01 M HCl. The final product was passed over anhydrous Na_2SO_4 and dried on the rotavapor at 30°C.

3.9.9. Synthesis of acrylated castor oil (ACO)

2 g of CO was dissolved in 5 ml of distilled THF a 3 neck round bottom flask. Then 9.75 ml of TEA was added to the mixture. Argon was flushed into the flask for a few minutes to prevent moisture. Through an arm funnel 5.56 ml of α-BIBB was added dropwise at 5°C under stirring. The mixture was stirred at this temperature for 1 h and then left at r.t. to react for 16 h or overnight. The crude product was dissolved in DCM and then washed with 01 M HCl. The final product was passed over anhydrous Na_2SO_4 and dried on the rotavapor at 30°C.



Scheme 13. Formation of castor oil bromide (CO-Br) and acrylated castor oil (ACO).

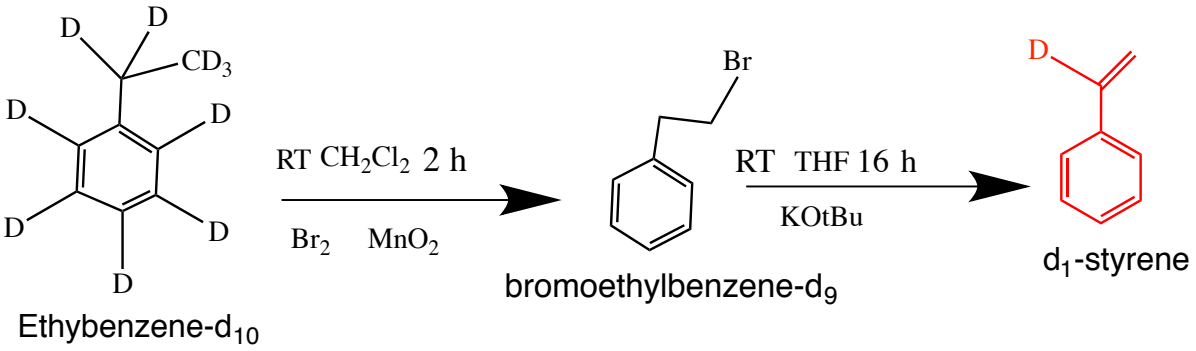
3.9.10. Synthesis of 2-(methacryloyloxy)ethyl oleate (MAEO) monomer.

The OA (2.655 mol, 750 mg) and DMAP (0.265 mmol, 32.44 mg) were dissolved in 15 mL of dry DCM, in a two- necked round bottom flask equipped with a magnetic stirrer and a dropping funnel. The solution was purged with argon at 0°C (ice bath), for 10 min. Then 2.92 ml of 1.0 M DCC solution (2.92 mmol), diluited in 5 ml of CH_2Cl_2, was added dropwise to the reaction mixture at 0°C, followed by dropwise addition of HEMA (2.655 mmol, 345.5 mg). After 20 min, the ice bath was removed and the resulting solution was stirred overnight under an argon atmosphere at room temperature. After complete consumption of the starting OA (monitored by TLC), the reaction was filtered over celite to remove the white precipitate and 10 ml of distilled water were added to the filtrate. The organic phase was washed with saturated $NaHCO_3$ (15 mL × 4) followed by brine solution (15 mL × 2) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the resulting crude oil was purified by flash chromatography on silica gel, eluting with hexanes/ethyl acetate, 95:5, to afford the MAEO monomer (1.00 g, 96 %) as a colorless oil. ^{1}H NMR (400 MHz, in $CDCl_3$): δ (ppm) = 6.15 (m, 1H, C=CH_2), 5.61 (m, 1H, C=CH_2,), 5.37 (m, 2H, -CH_2-CH=CH-CH_2-), 4.36 (m, 4H -OCH_2CH_2O-,), 2.35 (t, J = 7.63, 2H, O=CCH_2,), 2.04 (m, -CH2-CH2-CH=CH-CH2-CH2), 1.97 (s, 3H, CH_2=CCH_3), 1.64 (m, 2H, O=$CCH_2CH_2CH_2CH_2$-,), 1.36-1.28 (m, 20H), 0.90 (3H, J = 7.01 Hz, t, -CH_2CH_3). ^{1}H NMR (100 MHz, in $CDCl_3$): 173.57, 167.11, 135.99, 130.02, 129.74, 125.97, 62.47, 61.88, 34.17, 29.77, 29.70, 29.53, 29.32, 29.17, 29.10, 29.09, 27.22, 27.17, 24.92, 22.68, 18.25, 14.10. FT-IR (cm^{-1}): 3002, 2926 (C–H), 1742 (C=O), 1727 (C=O), 1639 (C=C), 1455, 1377, 1320, 1297, 1243, 1158 (C–O), 1093, 1048, 1006, 941, 882, 814, 723. GC-MS: m/z; 394 [M^+]; 376; 264; 113 (100); 95; 69; 41.

3.9.11. Synthesis of d_1-styrene

The synthesis of d_1-styrene was done in two steps. α-bromoethylbenzene was first synthesized as follows; manganese (iv) oxide (MnO_2) and bromine were added to a solution of ethylbenzene-d_{10} in DCM in a round bottom flask at 0°C. The mixture was stirred for 2 h at r.t. and then excess saturated Na_2CO_3 was added into the flask. The crude product was extracted with DCM. The organic layer was passed over $MgSO_4$, filtered and evaporated to dryness.

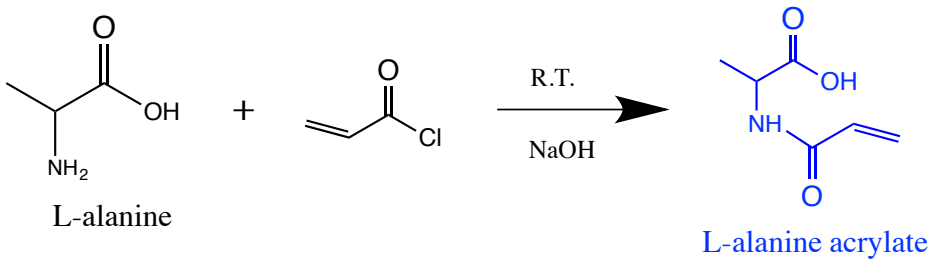
To synthesis styrene, potassium terbutyrylate (KOtBu) was dissolved in THF in a round bottom flask. Bromoethylethylbenzene was added and the mixture stirred overnight at room temperature resulting in the formation of a white precipitate. The precipitate was diluted in diethyl ether and filtered. The crude product was dried under vacuum under very low pressure since the product is highly volatile and then purified by means of SiO_2 column chromatography with pentane as the eluent. A 52% yield was obtained.



Scheme 14. Preparation of d_1-styrene.

3.9.12. Synthesis of L-alanine acrylate L-alanine acrylate (L-AlaA)

500 mg of L-alanine was dissolved in 478.8 µl of NaoH, followed by the dropwise addition of acryloyl chloride using an arm-funnel at 0°C. The reaction mixture was then stirred at room temperature for 2 h, acidified with 12 N HCl, and then extracted with ethyl acetate three times. The combined organic phase was dried over anhydrous NA_2SO4. After filtration, the filtrate was concentrated under reduced pressure. The solid was recrystallized three times from ethyl acetate.



Scheme 15. Preparation of L-alanine acrylate.

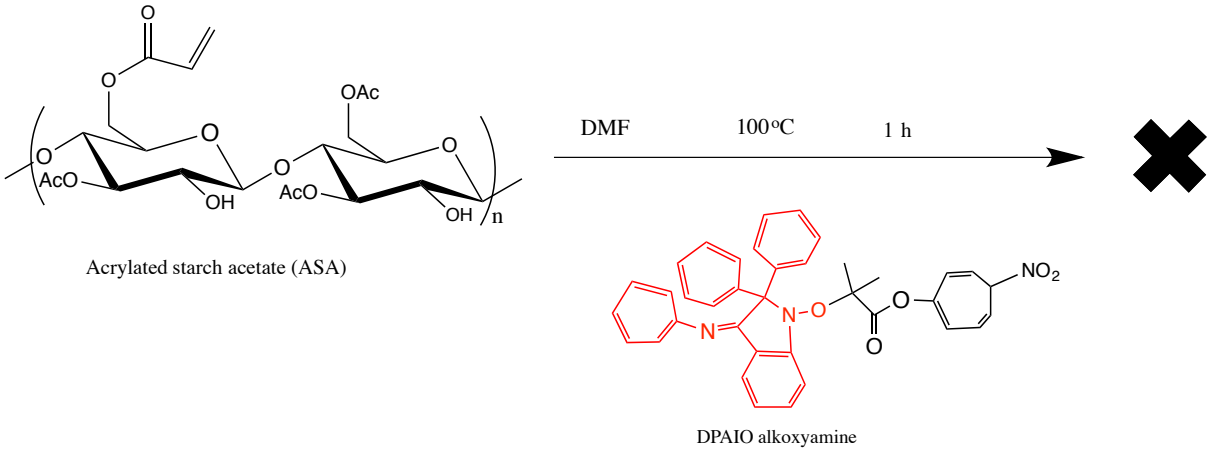
3.10. Synthesis of macroalkoxyamines (MA)

The macroalkoxyamines were synthesized by atom transfer radical addition (ATRA) and intermolecular radical addition (IRA).

3.10.1. Synthesis of starch acetate-SG1 MA

In 20 ml of THF was dissolved 150 mg of acrylated starch acetate, followed by the addition of 129 mg of blocbuilder MA. The solution was deoxygenated for about 20 min and then heated at 100°C for 1 h under argon. The reaction mixture was then cooled and the rotavapor was used to evaporate THF and the crude product was precipitated in ethyl acetate to remove any unreacted blocbuilder MA. A white solid was obtained, which was further washed with ethyl acetate three times and dried on the rotavapor at room temperature.

Note: An attempt to synthesize starch acetate-DPAIO MA was unsuccessfully (scheme 16) Acrylated starch acetate was dissolved in DMF, deoxygenated and heated at 100°C for 1 h under stirring.



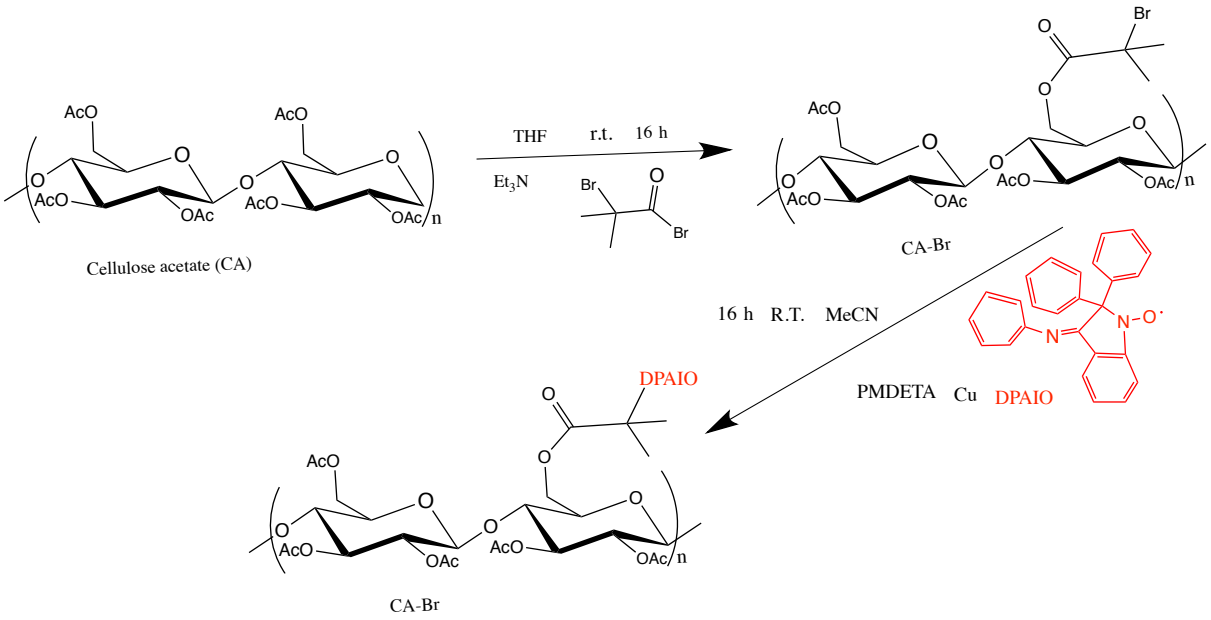
Acrylated starch acetate (ASA)

DPAIO alkoxyamine

Scheme 16. Attempted synthesis of starch acetate-DPAIO macroalkoxyamine.

3.10.2. Synthesis of CA-DPAIO MA

CA (0.5g) was dissolved 25 ml of acetonitrile, followed by the addition of 0.51g DPAIO and 40 ml of brown copper powder. The mixture was deoxygenated for about 20 min, and 157.8 µl of PMDETA was finally added into the mixture. The mixture was stirred at room temperature for about 16 h. After the reaction occurred, the mixture was neutralized with $NaHCO_3$, dissolved in ethyl acetate and washed with water several times. The final product was on the rotavapor at 50°C.



Scheme 17. Preparation of cellulose acetate bromide (CA-Br) and cellulose acetate-DPAIO MA.

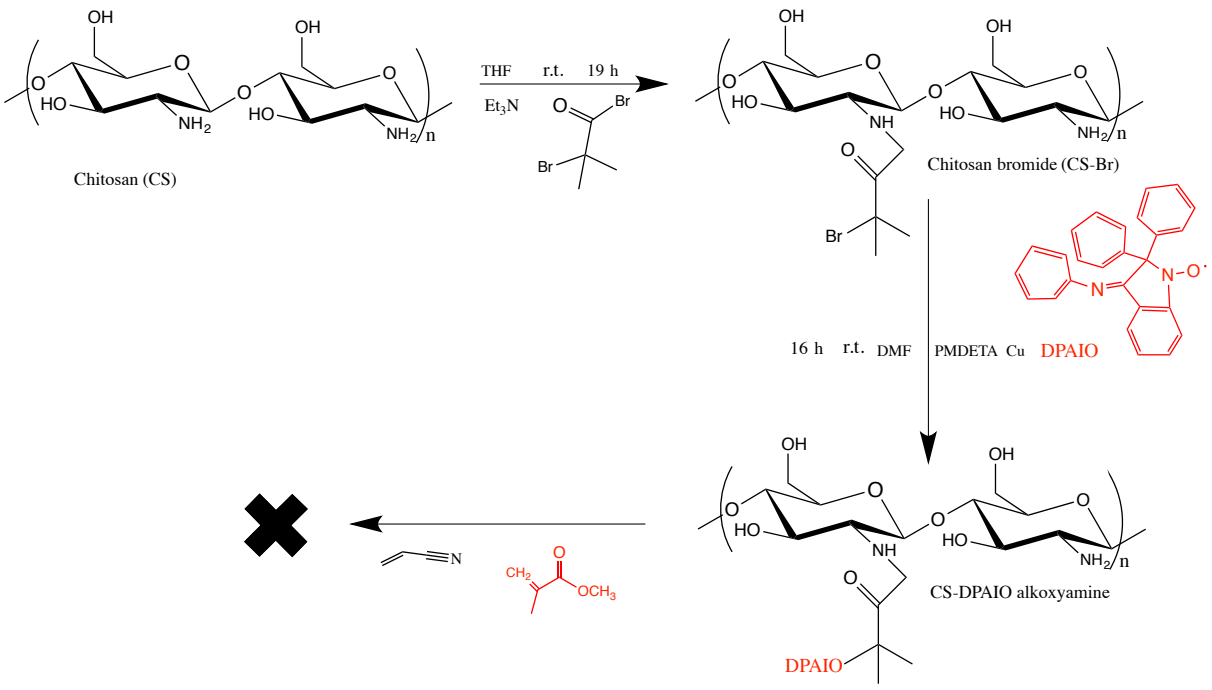
3.10.3. Synthesis of CA-SG1 MA

1 g of CA-Br was dissolved in 10 ml of MeCN, then 0.946 g of SG1 and 94.57 mg of Cu added. The mixture was deoxygenated for 20 min before introducing PMDETA. The mixture was allowed to react under argon at r.t. for about 16 h. The crude product was dissolved in ethyl

acetate, washed HCl and the volume was reduced by heating at 35°C on the rotavapor. The product was again dissolved in ethyl acetate and washed with $NaHCO_3$ in order to remove HCl. The ethyl acetate was removed by means of a rotavapor and the final product (white solid) washed with water several times before drying on the rotavapor at 50°C.

3.10.4. Synthesis of CS-DPAIO MA

200 mg of CS-Br was dissolved in 10 ml of DMF, then 126 mg of DPAIO and 31.77 mg of Cu added. The mixture was deoxygenated for 20 min before introducing PMDETA. The mixture was allowed to react under argon at r.t. for about 16 h. After the reaction, the mixture was diluted with ethyl acetate, washed with HCl followed by a subsequent wash with $NaHCO_3$ in order to bring the pH to neutral and a brown solid was obtained. The solid was filtered and washed several times with water and then dried at 50°C on the rotavapor. The synthesis was attempted using benzene as a solvent under similar conditions to no avail.



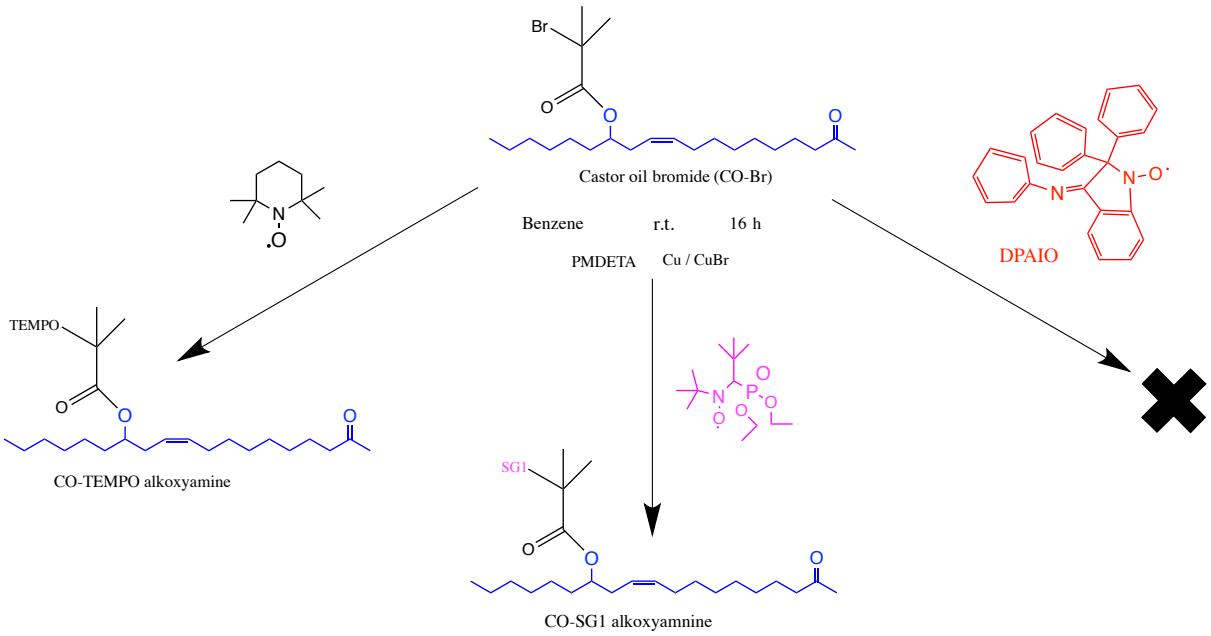
Scheme 18. Preparation of chitosan bromide (CS-Br) and chitosan-DPAIO alkoxyamine

3.10.5. Synthesis of CO-SG1 MA

Castor oil bromide (0.0728 mmol), SG1 (0.218 mmol), copper (0.218 mmol) and copper (I) bromide (0.218 mmol) were dissolved in 5.8 ml of benzene in a three-neck round bottomed flask. The reaction mixture was deoxygenated by flowing argon through the system for about 20 min and then 0.437 mmol of PMDETA was introduced into the flask. The reaction was allowed overnight (more than 16 h) at room temperature. The crude product was dissolved in 50mL EtOAc, washed with 10%HCl (3x30 mL), saturated NaHCO3 (3x30mL) and sat. NaCl (30mL). The organic phase was dried over Na_2SO4 and solvent was removed on a rotary evaporator.

3.10.6. Synthesis of CO-TEMPO MA

Castor oil bromide (0.0728 mmol), TEMPO (0.218 mmol), copper (0.218 mmol) and copper (I) bromide (0.218 mmol) were dissolved in 5.8 ml of benzene in a three-neck round bottomed flask. The reaction mixture was deoxygenated by flowing argon through the system for about 20 min and then 0.437 mmol of PMDETA was introduced into the flask. The reaction was allowed overnight (more than 16 h) at room temperature. The mixture obtained was diluted with 50mL EtOAc, washed with 10%HCl (3x30 mL), saturated NaHCO3 (3x30mL) and sat. NaCl (30mL). The organic phase was dried over Na_2SO4 and solvent was removed on a rotary evaporator.



Scheme 19. Preparation of castor oil-TEMPO and castor oil-SG1 alkoxyamines.

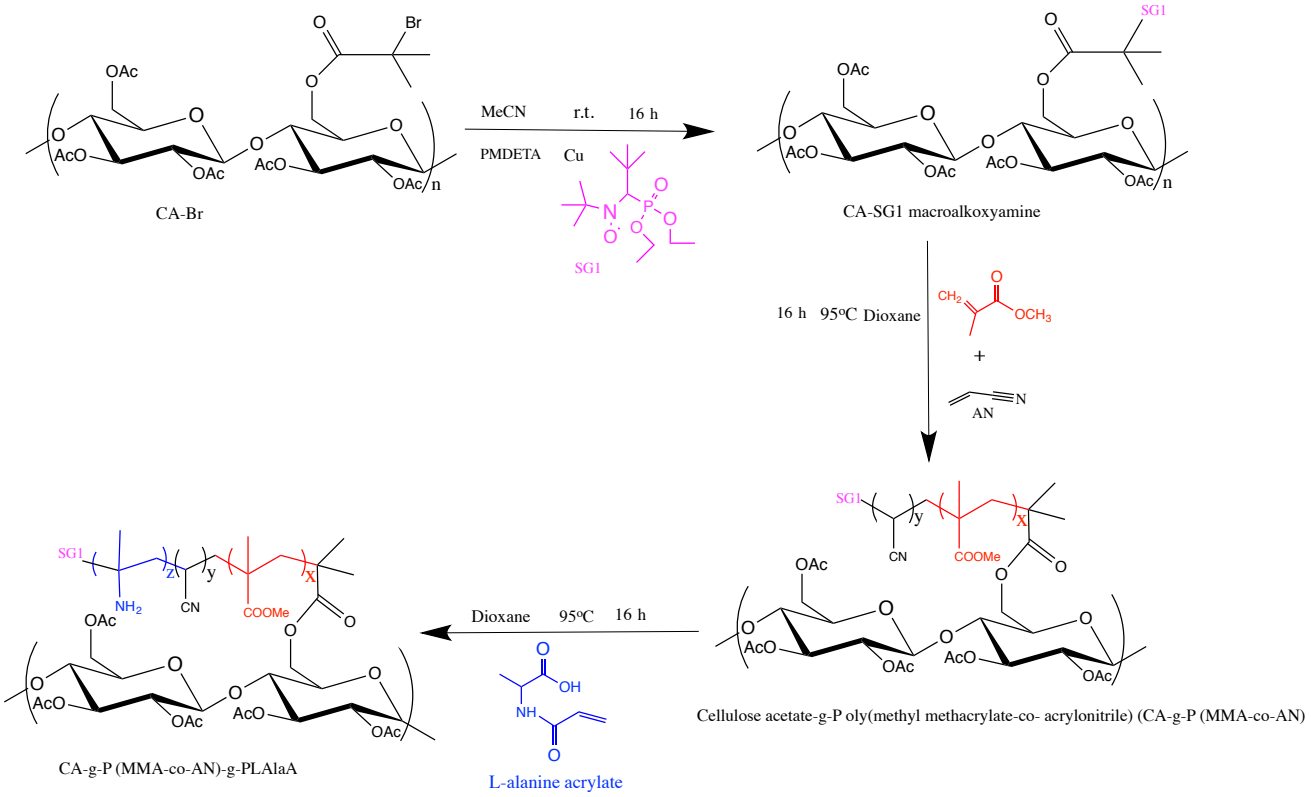
3.11. Synthesis of copolymers

3.11.1. Synthesis of cellulose acetate-co-Poly (methyl methacrylate-co-acylonitrile) (CA-co-P(MMA-co-AN)

100 mg of CA-SG1 MA, 100 mg of methyl methacrylate (MMA) and 5.3 mg of acrylonitrile (AN) were dissolved in 2 ml 1,4-Dioxane in a vial. The mixture was deoxygenated and heated at 95°C for over 16 h. The mixture was allowed to cool after 16 h and the crude product was precipitated in cold excess methanol. Another sample synthesized under the same was instead precipitated in water to obtain the final product. The final product was dried on the rotavapor at 50°C. The reaction was also successful in ethanol/water mixture under similar conditions.

3.11.2. Synthesis of cellulose acetate-co-Poly (methyl methacrylate-co-acylonitrile)-g-poly L-alanine acrylate (CA-co-P(MMA-co-AN)-g-PLAlaA)

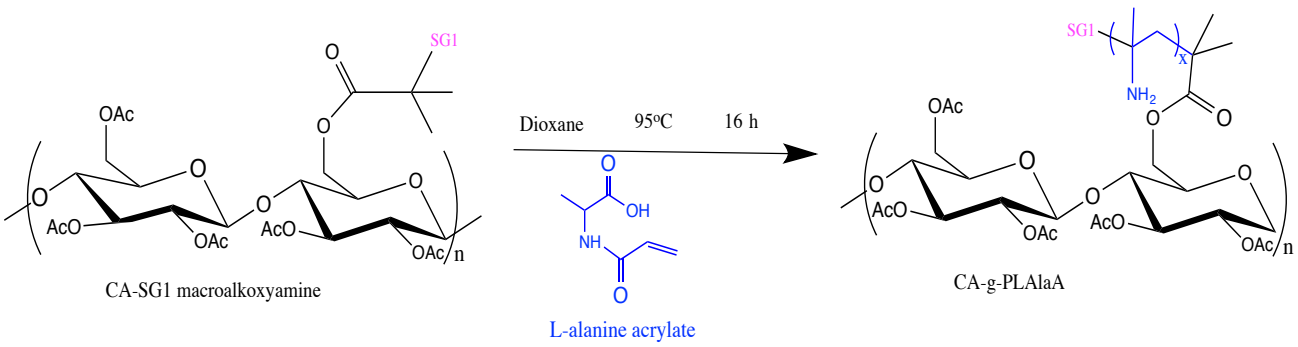
100 mg of CA-co-P(MMA-co-AN) and 50 mg of L-AlaA were dissolved in 2 ml of 1, 4-Dioxane and the mixture deoxygenated. The mixture was heated at 95°C for over 16 h. The crude product was purified by precipitating in excess cold methanol after cooling at r.t. and dried on the rotavapor at 50°C.



Scheme 20. Preparation of cellulose acetate-SG1 (CA-SG1) alkoxyamine, cellulose acetate-g-poly(methyl methacrylate-co-acrylonitrile) (CA-g-P(MMA-co-AN)) and cellulose acetate-g-poly(methyl methacrylate-co-acrylonitrile)-g-poly L-alanine acrylate (CA-g-P(MMA-co-AN)-g-PLAlaA.

3.11.3. Synthesis of cellulose acetate-co-Poly L-alanine (CA-co-PLAlaA)

100 mg of CA-SG1 MA and 50 mg of L-AlaA were dissolved in 1,4-Dioxane, then mixture was deoxygenated and heated at 95°C for over 16 h. The purification of the final product was done in excess ethanol (absolute) and the final product was dried at 50°C on the rotavapor.



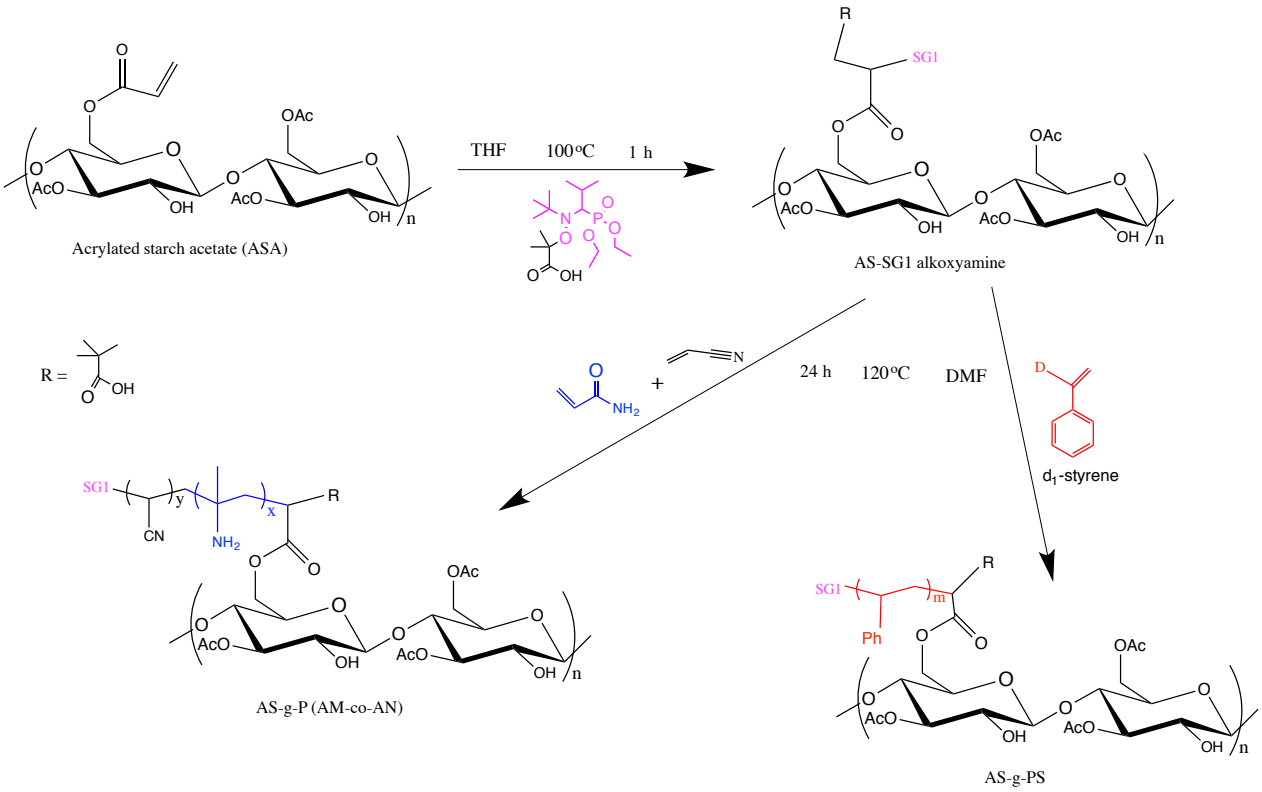
Scheme 21. Preparation of cellulose acetate-g-poly L-alanine acrylate.

3.11.4. Synthesis of starch acetate-g-Polystyrene (SA-g-PS)

50 mg of SA-Blocbuilder MA was dissolved in 5 ml of DMF and 0.165 ml of d_1-styrene added. The mixture was deoxygenated and heated at 120°C for about 24 h under vigorous stirring. The crude product cooled to r.t. and precipitated with excess cold methanol and dried at 60°C.

3.11.5. Synthesis of starch acetate-g-Poly (acrylamide-co-acrylonitrile) (SA-g-P(AM-co-AN))

100 mg of SA-Blocbuilder MA, o.414 g of acrylamide, 10wt% of acrylonitrile and 5 mole% of free SG1were dissolved in 4 ml of DMF and deoxygenated for 20 min. The mixture was then stirred under argon at 120°C for 24 h. The crude product was cooled to room temperature and precipitated in excess cold methanol and a white powder was obtained. Purifying the inal product by precipitating in excess ethanol (absolute) was also successful. The product was dried by means of rotavapor at 60°C.



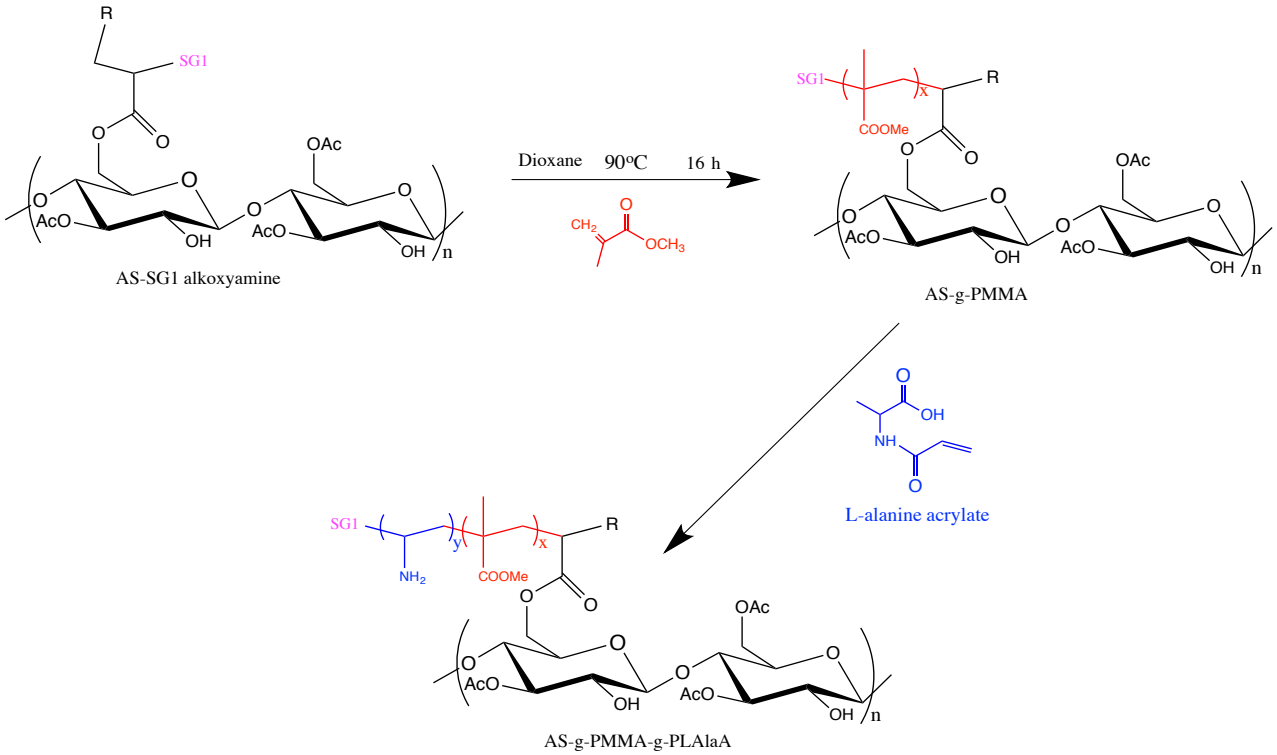
Scheme 22. Preparation of starch acetate-SG1 macroalkoxyamine, starch acetate-g-poly(acrylamide-co-acrylonitrile (SA-g-P(AM-co-AN)) and starch acetate-g-polystyrene (SA-g-PS).

3.11.6. Synthesis of starch acetate-g-Poly methyl methacrylate (SA-g-PMMA)

In a vial, 66 mg of SA-Blocbuilder MA, 200 µl of MMA, 10.6 µl of AN and 2 ml of 1,4-Dioxane were mixed to form a solution. The mixture was deoxygenated and heated at 90°C for over 16 h. The crude product was purified in excess methanol/water mixture, washed several times with methanol and dried at 50°C on the rotavapor.

3.11.7. Synthesis of starch acetate-g-Poly methyl methacrylate-g-Poly L-alanine (SA-g-PMM-g-PLAla)

100 mg of SA-g-PMMA and 50 mg of L-AlaA were dissolved in 2 ml of 1,4-Dioxane. The mixture was deoxygenated and heated at 90°C for over 16 h. The crude product was precipitated in excess cold methanol and the white solid obtained was dried on the rotavapor at 50°C.



Scheme 23. Preparation of starch acetate-g-poly (methyl methacrylate) (SA-g-PMMA) and starch acetate-g-poly (methyl methacrylate)-g-poly L-alanine acrylate (SA-g-PMMA-PLAlaA).

3.11.8. Synthesis of MAEO homopolymer

In a typical experiment, MAEO monomer (0.78 mmol, 0.5 M), AIBN (mmol, 0.038M) and freshly distilled dry toluene were added to a septum sealed glass vial. The solution was stirred at room temperature and purged with argon for 20 min. Then the reaction vial was inserted in a preheated oil bath and heated at 75°C for seven and a half hours. The reaction was quenched by cooling in ice-water bath and the resulting polymer was precipitated using a large amount of methanol. A colorless sticky homopolymer was obtained after purification by dissolving in toluene and precipitating in methanol multiple times. The final product was dried under high vacuum at room temperature. A similar procedure was followed for BlocBuilder and NMMA mediated radical polymerizations whose conditions are reported in the **table 1**.

3.12. Characterization

3.12.1. Fourrier transform infra-red (FTIR) spectroscopy

In this work, all samples were analyzed by means of a 5.3.1 Perkin-Elmer Spectrum GX FT-IR System spectrophotometer in the range between 4000 – 500 cm-1. Most of them were analyzed using the diffuse reflection method, mixing 1 mg of sample with 100 mg of an alkali halide, such as KBr, which does not contain bands in the mid-IR region of the spectrum. With this measurement method, background measurement is first performed on the KBr packed into the sample plate of the diffuse reflectance accessory. Next, the sample powder is diluted to 0.1 % to 10 % in the KBr powder and packed into the sample plate for infrared spectrum measurement. Samples dispersed in halide powder must be homogenously dispersed, with a particle size small enough not to cause scatter (theorectically < 2 microns). Spectral analysis was performed in reflectance, with a spectral resolution of 4 cm-1 and 32 scans. Each spectrum was baseline corrected and normalized. To measure IR spectra of polymer solutions, they were pipetted into an IR cell with two NaCl windows (φ 20 × 2 mm). Spectral analysis was performed in transmission mode with a spectral resolution of 4 cm-1 and 32 scans. The background spectrum was obtained using the two NaCl windows without sample solution.

3.12.2. Near magnetic resonance (NMR) spectroscopy

^{1}H NMR spectra were recorded at 400 MHz with a Varian Mercury 400 spectrometer (chemical shifts are in part per million downfield from TMS); the solvent used was $CDCl_3$. All air-sensitive reactions were carried out in heat-gun-dried glassware under an inert atmosphere of argon via standard Schlenk techniques.

3.12.3. Electron paramagnetic resonance (EPR) spectroscopy

Isotropic X-band EPR spectra were recorded on a Bruker EMX spectrometer system equipped with a microwave frequency counter and an NMR Gaussmeter for field calibration; for *g*-factor determination the whole system was standardized with a sample of perylene radical cation in concentrated sulfuric acid (g=2.00258). General EPR spectrometer settings: microwave power 5 mW, modulation amplitude 0.2 Gauss, time constant 0.64 ms, receiver gain $4.48x10^4$, sweep time 1342.177 s, conversion time 1310.720 ms. EPR spectra simulations were carried out by means of the Winsim program, freely available from NIEHS. (D. Duling, PEST Winsim Version 0.96, National Institute of Environmental Health Sciences, Triangle Park, NC, 1996).

Chapter 4. Results and discussions.

4.1. Syntheses

The polymer/monomers (substrates) from biorenewable resources were functionalized with either acryloyl chloride (AC) or bromoisobutyrly bromide (BIBB) in order to incorporate the more reactive acrylic or bromide functional groups from acryloyl chloride and bromoisobutyrly bromide respectively into the substrates through substitution of a hydrogen atom[184,185,186]. This is a "graft to" approach. Hence functionalized substrates were able to react with nitroxide species or alkoxyamines to form macroalkoxyamines under certain conditions. In the case of starch, corn starch was first of all expanded[187] in order to provide larger pores with more surface area for better incorporation of other functional groups. Acetyl groups[188] were further incorporated into the starch molecules to make it soluble into organic compounds before functionalization. Generally, the higher the degree of substitution of the acetyl groups in

[184] Hüseyin Esen and Gökhan Çayli, 'Epoxidation and Polymerization of Acrylated Castor Oil: Epoxidation and Polymerization of Acrylated Castor Oil', *European Journal of Lipid Science and Technology*, 118.6 (2016), 959–66.

[185] Ting-Ting Xin, 'Synthesis of Cellulose-graft-Poly(methyl methatcrylate) Via Homogenous ATRP', 2011, 13.

[186] Aliyu D. Mohammed, 'Synthesis of Highly-Confined CdS Nanoparticles by Copolymerization of Acryloylated Starch', *Materials Letters*, 114 (2014), 63–67.

[187] K.S. Rhee, S.H. Cho, and A.M. Pradahn, 'Expanded Extrudates from Corn Starch–Lamb Blends: Process Optimization Using Response Surface Methodology', *Meat Science*, 52.2 (1999), 127–34.

[188] M Elomaa, 'Determination of the Degree of Substitution of Acetylated Starch by Hydrolysis, 1H NMR and TGA/IR', *Carbohydrate Polymers*, 57.3 (2004), 261–67.

the starch molecule, the more soluble it becomes[189]. Polysaccharides are generally insoluble in organic solvents; therefore, soluble functional groups are often incorporated into them before functionalization. The alternative is using very strong solvents such as dimethylfomamide (DMF) in which some polysaccharides are soluble, and the reactions often take place at high temperatures.

The introduction of active sites unto the derivatives from functionalizing the polymers/monomers were done by either by atom transfer radical addition (ATRA) or by intermolecular radical addition (IRA). In ATRA, the nitroxide reacted with Cu/PMDETA ligand to form a complex catalyst during the early stages of the reaction promoting the displacement of bromide atom from the α-bromoisobutyryl bromide moiety allowing the nitroxide molecule to be chemically attached to the derivative forming a macroalkoxyamine. Meanwhile in IRA, the acrylic double bond which was incorporated into the substrates react with nitroxides species from the alkoxyamine directly to form the macroalkoxyamine.

Copolymerization was done usually using the "graft from" approach. This approach ensures the formation of the graft polymer with the polymers from the biorenewable resource (polysaccharides) being the backbone chain. At a certain temperature (≥95°C) polymerization is initiated on the surface of the backbone polymer where the active sites are present and controlled by the activation-deactivation mechanism of NMP as new polymer chains are being formed. The carbon centered radical thermally formed by the alkoxyamine decomposition was able to attack the double bond on monomeric units producing radicalic propagating chains. At the same time, such a decomposition releases nitroxide units which are able to couple with these chains yielding larger and larger macroalkoxyanines, which in turn decompose following the same mechanism with the continuous formation of radicalic polymer chains. The reason why copolymerization did not occur with DPAIO macroalkoxyamines can be attributed to the fact that, in the presence of either styrene or acrylates, which afford less hindered and stabilized propagating radical chains than methacrylates, the required temperature (>150°C) to dissociate

[189] Li Xia, 'Study on the Morphology, Crystalline Structure, and Thermal Properties of Fritillaria Ussuriensis Maxim. Starch Acetates with Different Degrees of Substitution', *Starch - Stärke*, 63.1 (2011), 24–31.

the corresponding DPAIO macroalkoxyamine is definitely too high to control the polymerization of such monomers. Moreover, it has been demonstrated that when the steric hindrance and the stabilization of the released alkyl moiety decreased, DPAIO alkoxyamines could suffer from undesired CO–N hemolytic dissociation. Obviously, a CO–N cleavage leads to an aminyl radical and an alkoxyl radical and results in a loss of control.

Table 1. Polymerization of MAEO in toluene

Entry	MAEO mmol (M)	AIBN mmol (M)	NMMA mmol (M)	BlocBuilder mmol (M)	temp. (°C)	time (h)	conv.[b] (%)	Mn[c]	Mw	PDI[c]	reten.[d] (%)
1	1.014 (0.5)	0.063 (0.031)			75	7.5	79	11100	27900	2,51	88.5
2	1014 (0.5)	0.063 (0.031)			75	14	85				91
3	1.014 (1.62)	3.33×10^{-3} (0.006)	3.33×10^{-3} (0.006)		75	7.5	54	53100	111900	2,11	90
4	1.014 (0,68)	3.67×10^{-3} (2.4×10^{-3})	3.67×10^{-3} (2.4×10^{-3})		75	7.5	48				87.1
5	1.014 (0,68)	3.67×10^{-3} (2.4×10^{-3})	3.67×10^{-3} (2.4×10^{-3})		75	5	22				88.0
6	1.014 (0,68)	3.67×10^{-3} (2.4×10^{-3})	3.67×10^{-3} (2.4×10^{-3})		75	3	18				93.7
7	0.634 (0.63)			7.6×10^{-4} (0.0076)	95	7.5	25				93.3
8[e]	0.634 (0.63)			7.6×10^{-4} (0.0076)	95	7.5	27				91.8

[a] [MAEO]/[AIBN]/[NMMA] ratio of 275/1/1,

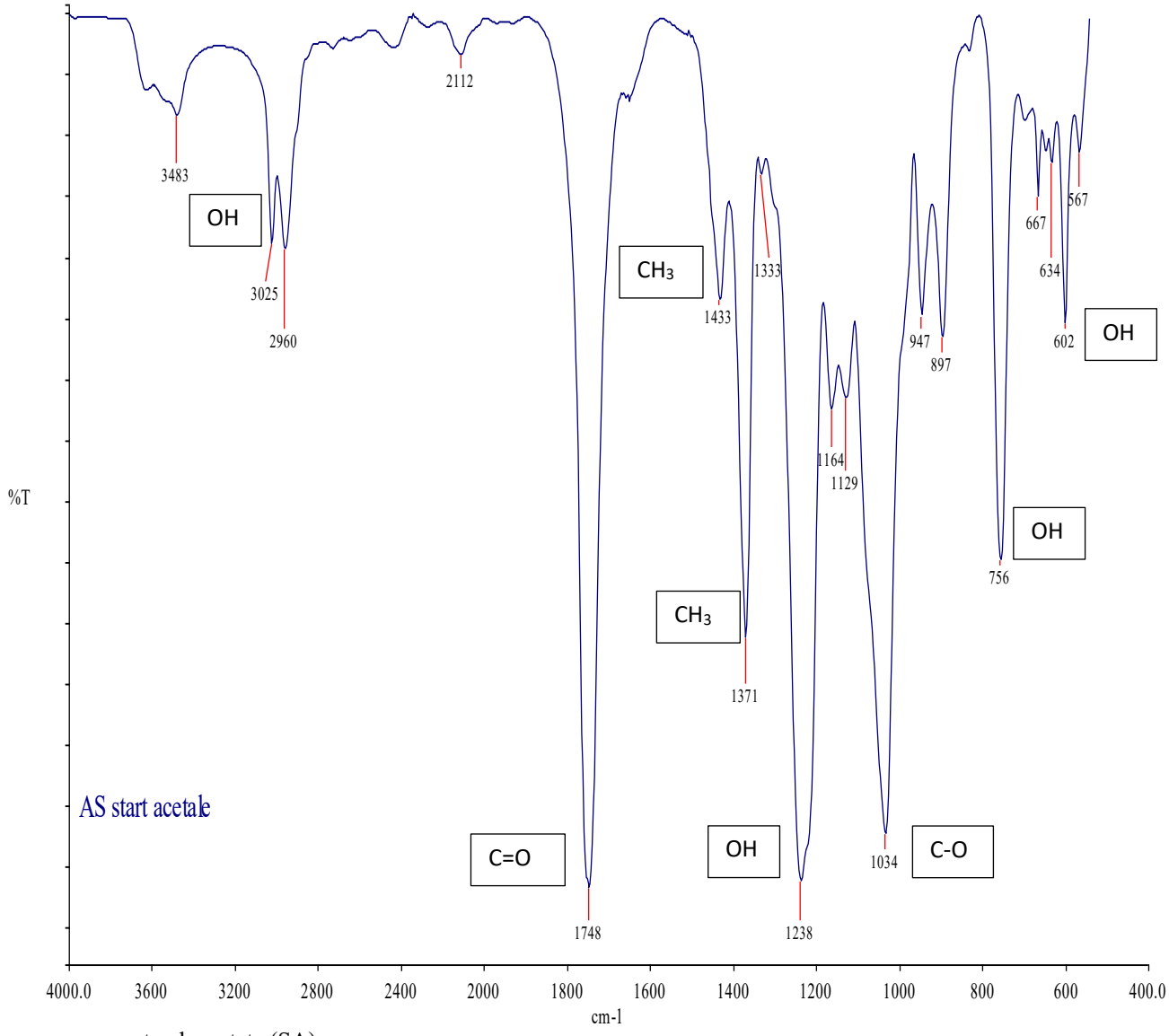
[b] Determined gravimetrically based on monomer feed; [c] Measured by GPC; [d] Retention of olefinic double bond measured by ^{1}H NMR

[e] $[SG1]_0/[BlocBuilder]_0 = 0.1$;

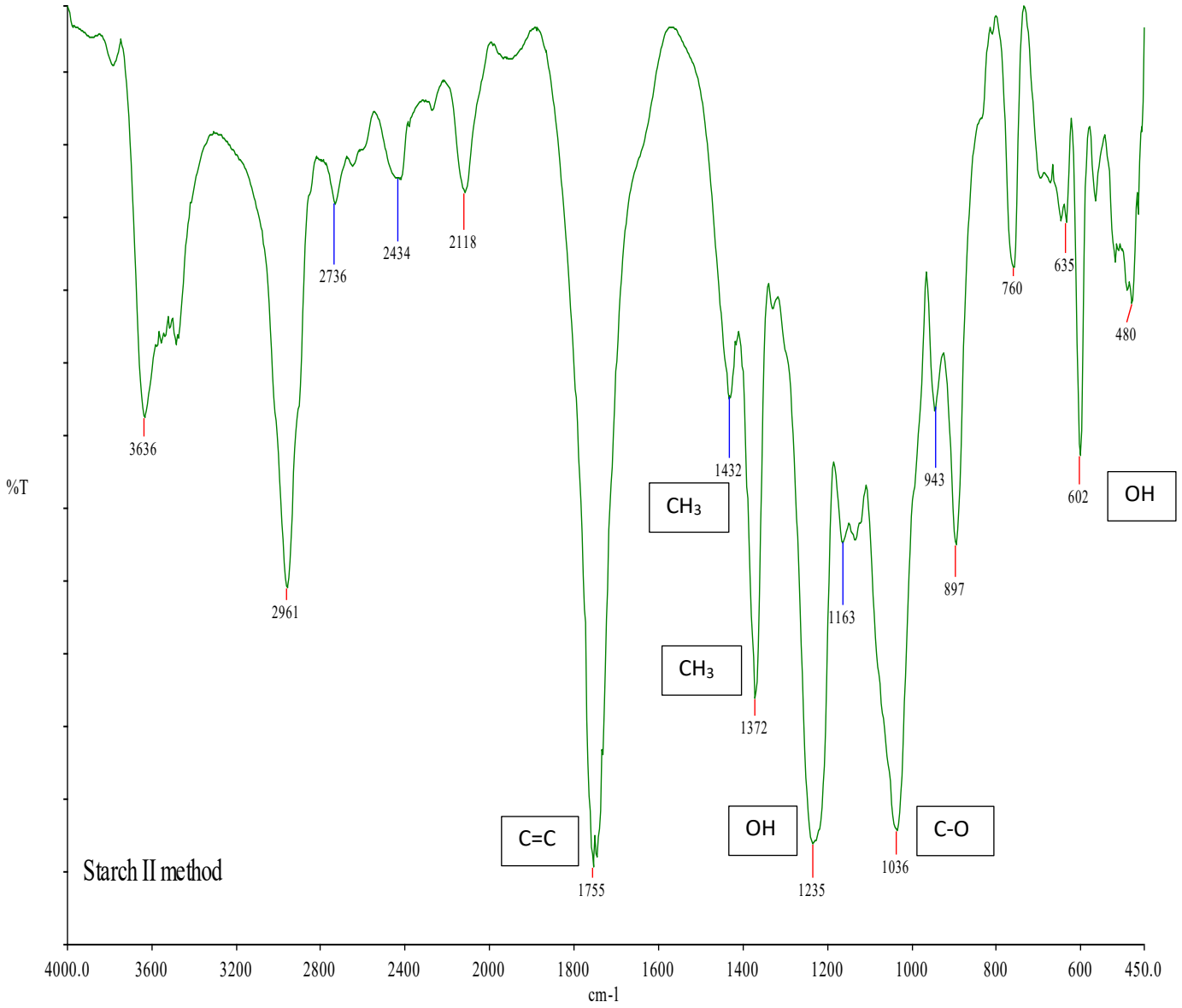
From table 1., we can deduce that both AIBN[190] and Blocbuilder can initiate a free radical homopolymerization of MAEO monomer. The fact that a higher conversion (79 %) of MAEO monomer into the homopolymer was obtained when AIBN was employed under the same conditions proves that it is more effective in polymerizing MAEO than Blocbuilder. This can be attributed to the very strong radical effect of AIBN. However, the very high PDI (2.51) of the homopolymer with AIBN shows that the polymerization process was not controlled.

[190] G. Billuart and others, 'Free Radical Emulsion Polymerization of Ethylene', *Macromolecules*, 47.19 (2014), 6591–6600.

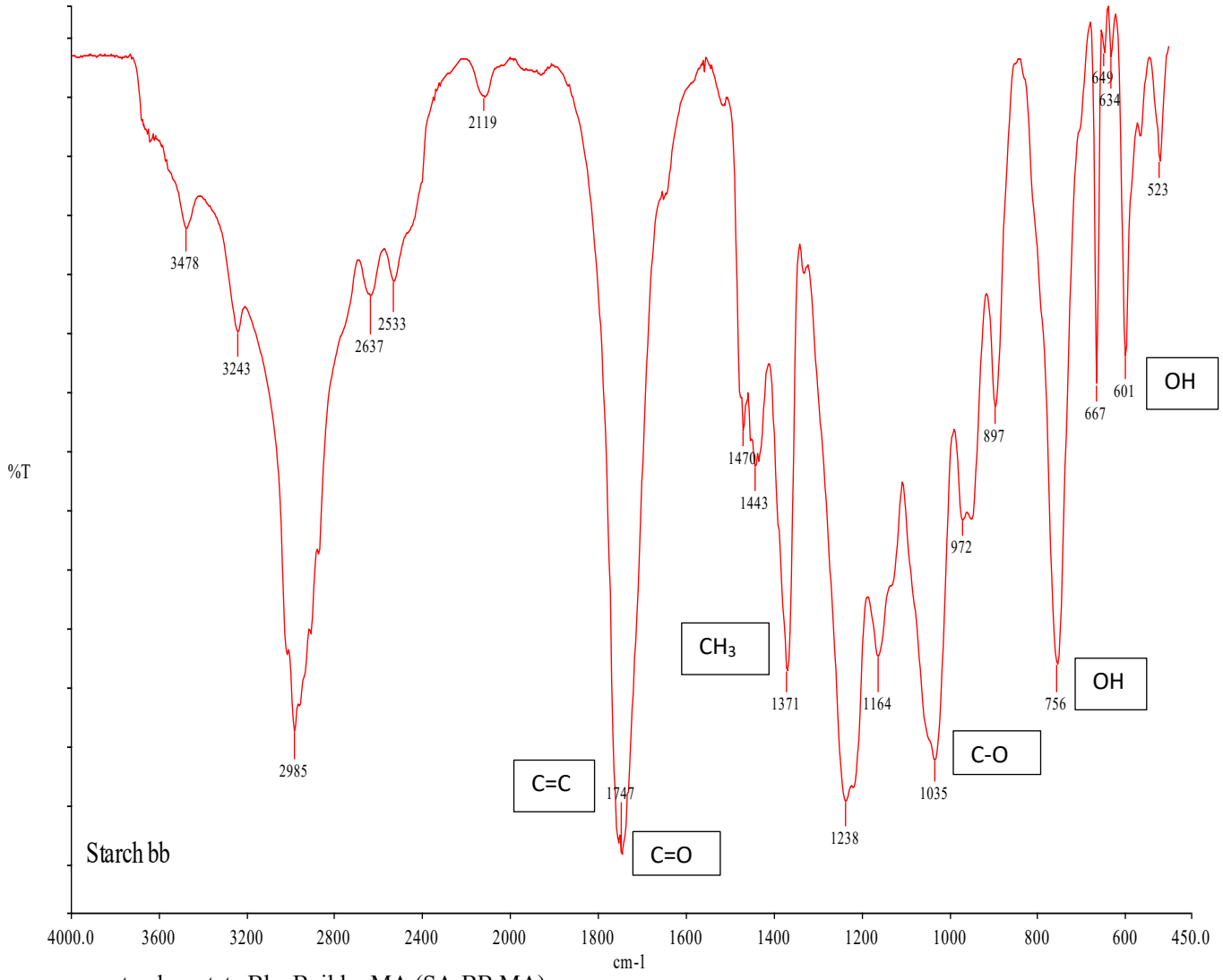
4.2. Fourrier transform infrared analyses



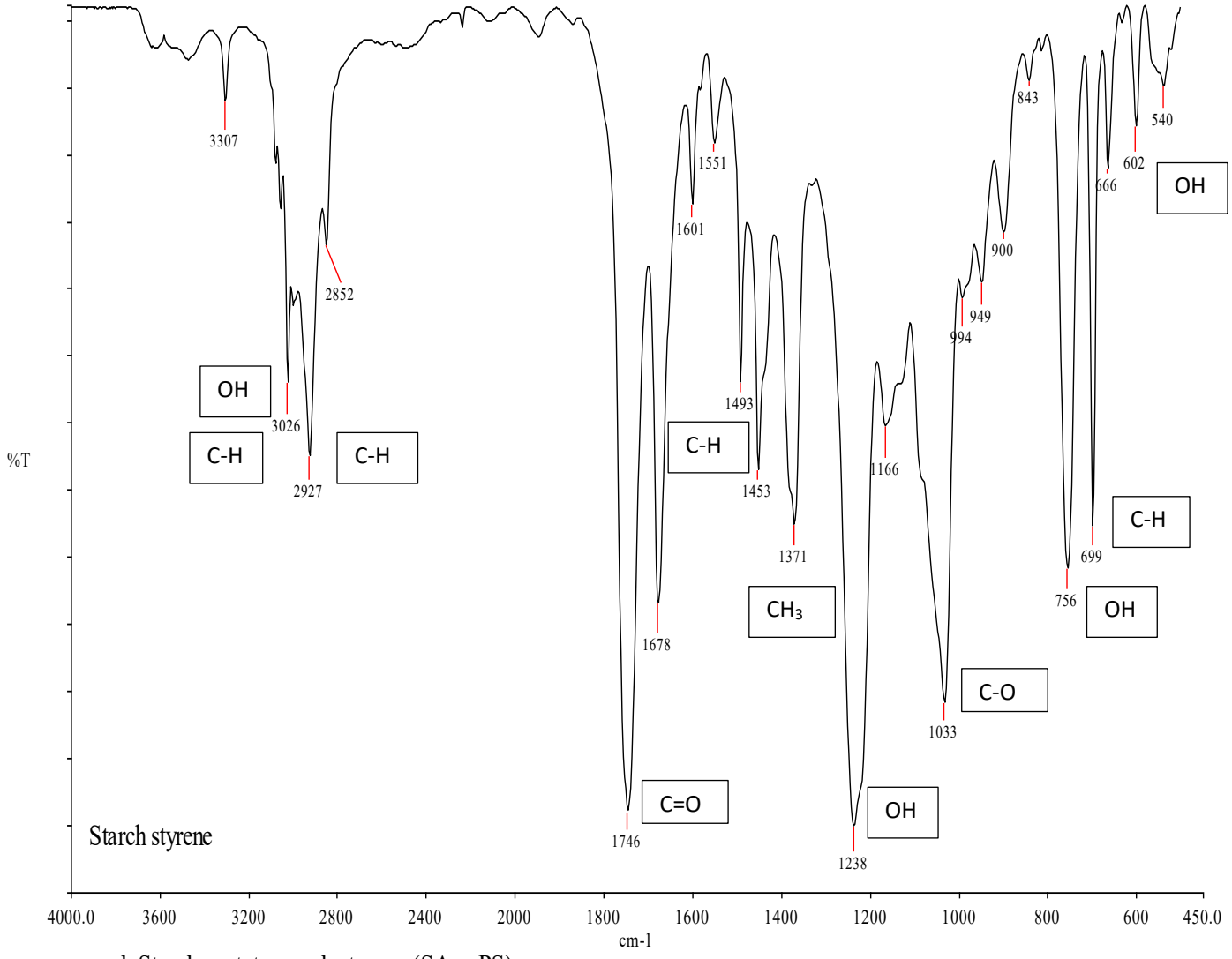
a. starch acetate (SA)



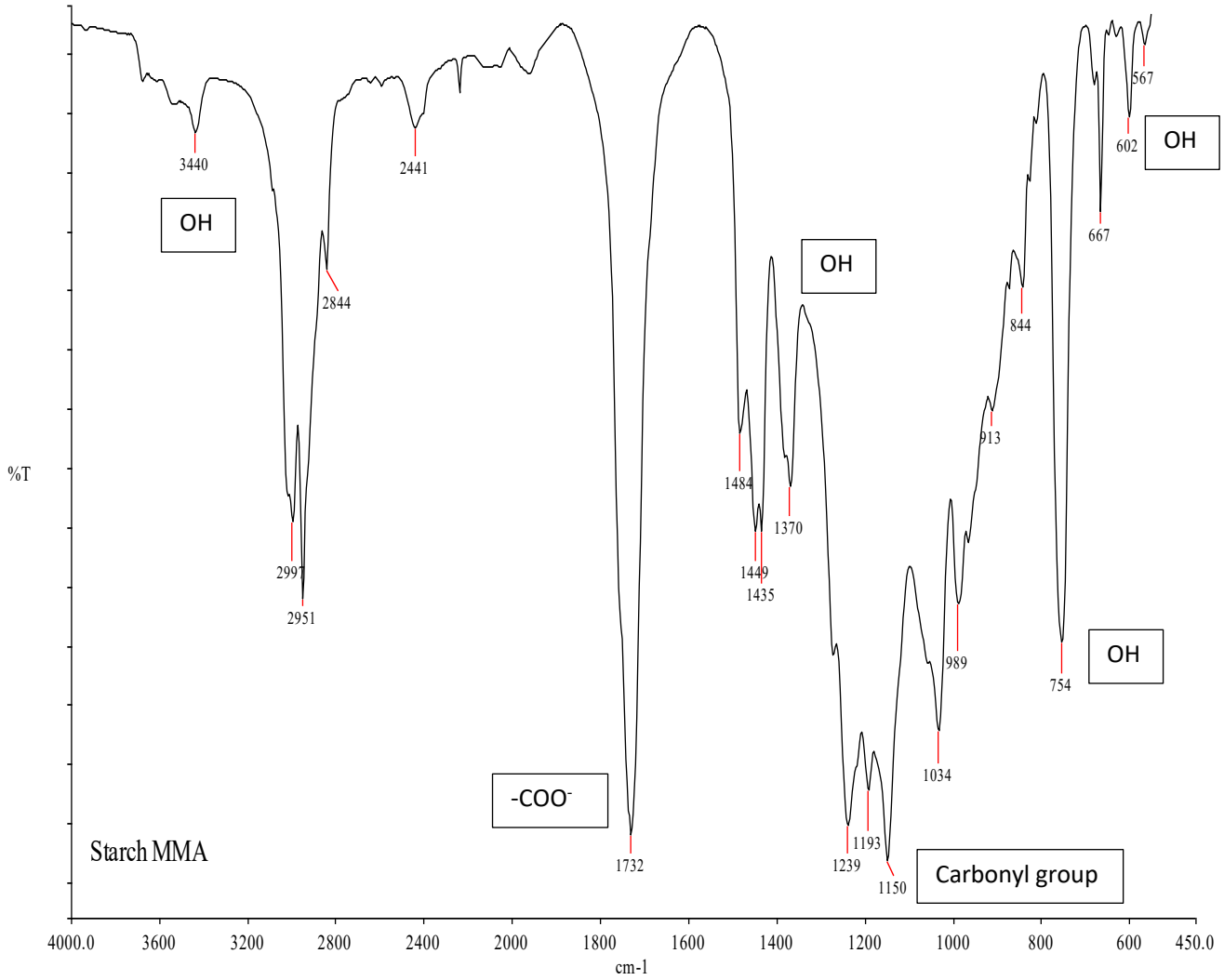
b. acrylated starch acetate (ASA)



c. starch acetate BlocBuilder MA (SA-BB MA)



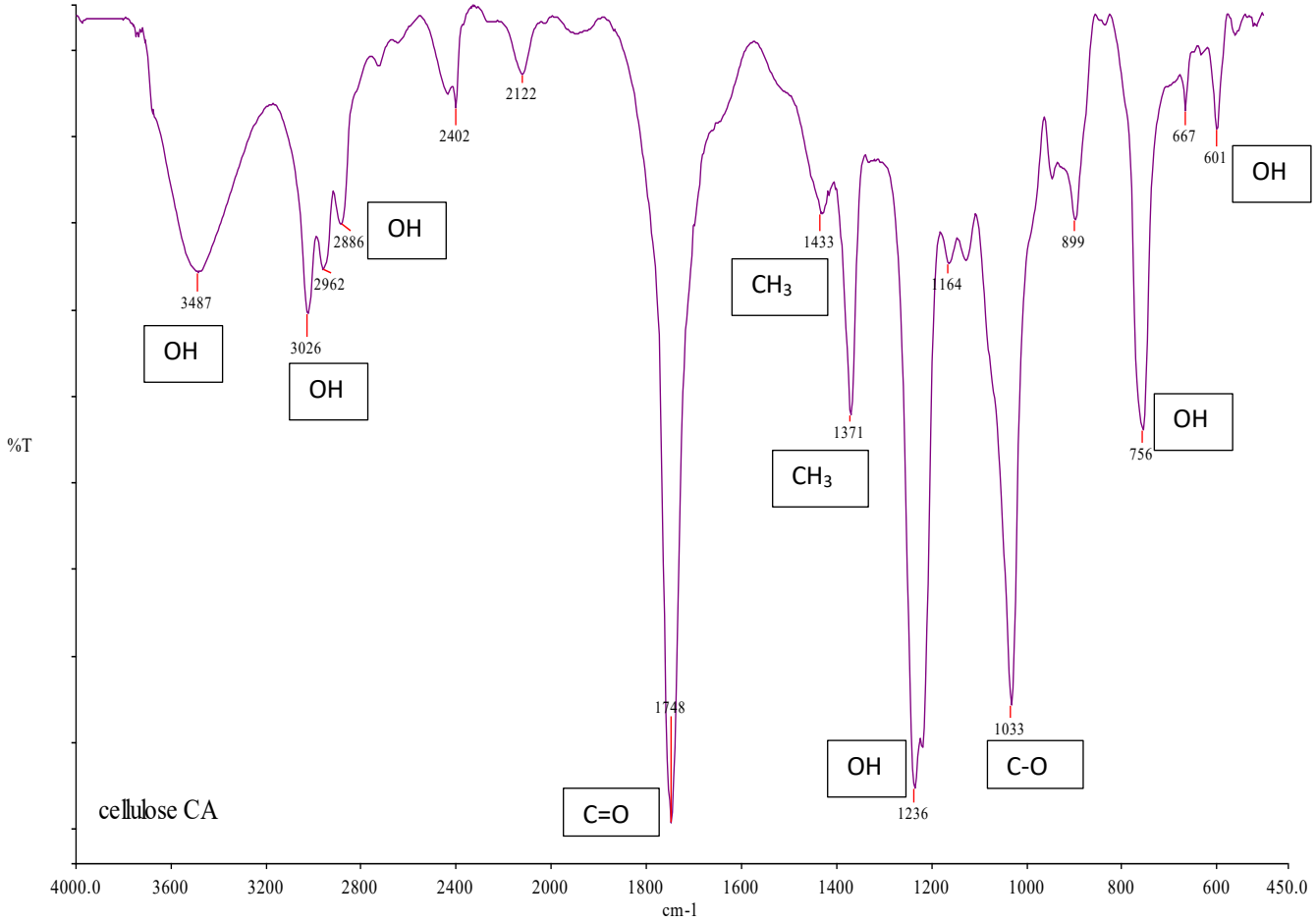
d. Starch acetate-g-polystyrene (SA-g-PS)



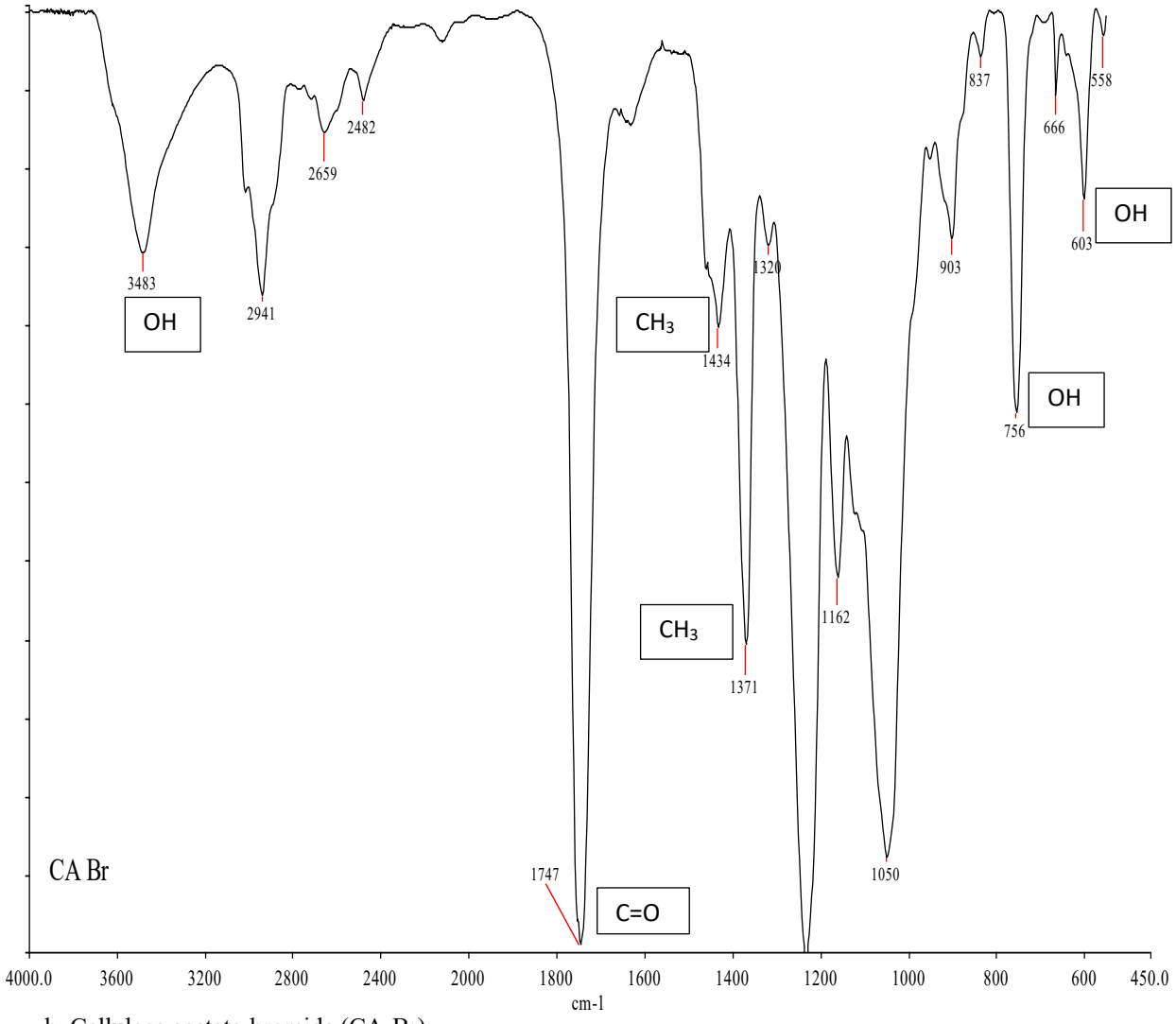
e. Starch acetate-g-poly(methyl methacrylate) (SA-g-PMMA)

Fig. 7. FTIR spectra of starch acetate (a), acrylated starch acetate (b), starch acetate-BlocBuilder MA (c), starch acetate-polystyrene (d) and starch acetate-poly(methyl methacrylate) (e).

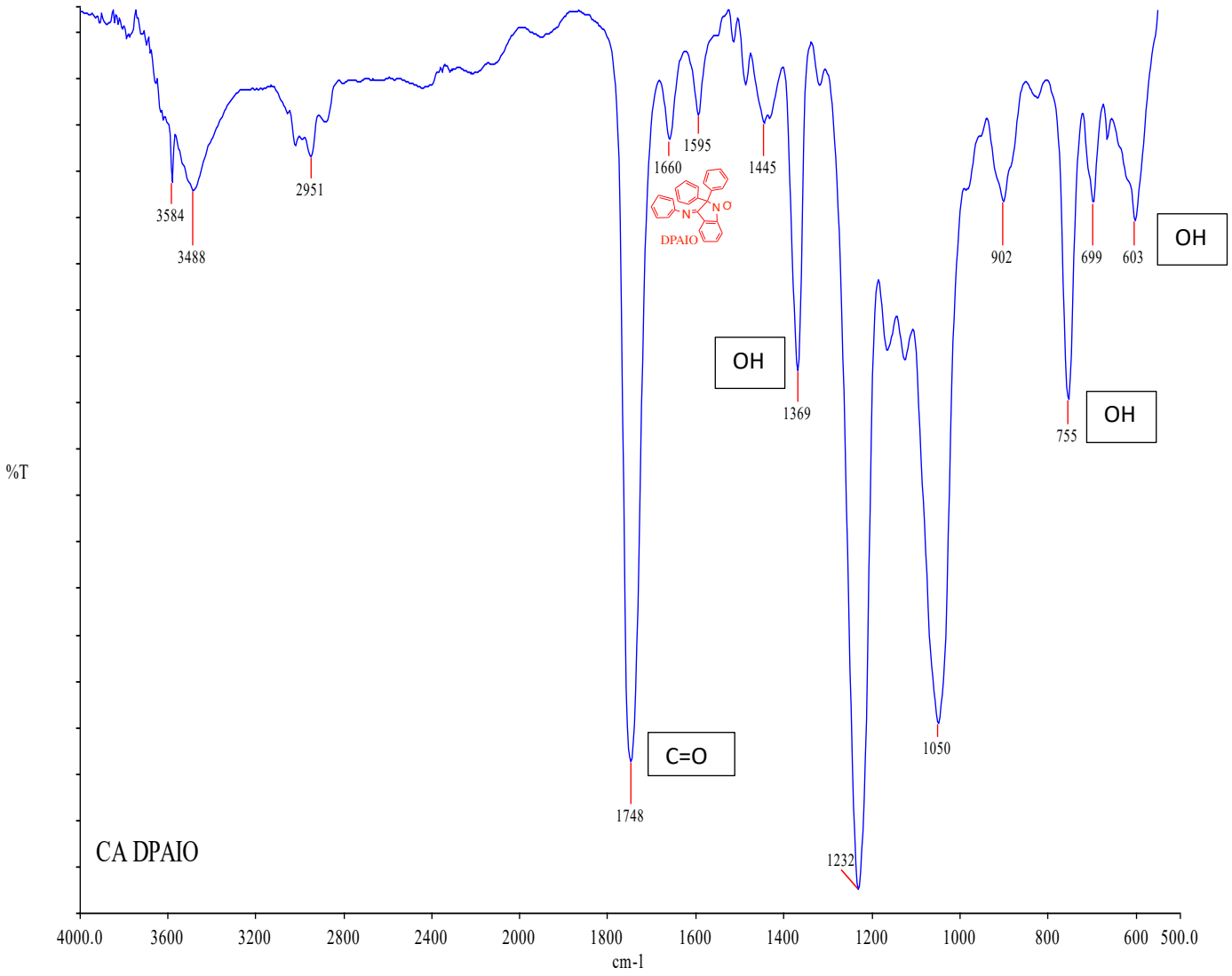
The peaks at 3483, 3025, 1238, 756 and 602 cm^{-1} belong to the OH group of starch. The absorption bands at 1745, 1433, 1371, and 1034 cm^{-1} are assigned to carbonyl C=O, CH_3 anti-symmetry bending vibration, CH_3 symmetry bending vibration and C–O stretching vibration (fig. 7a). These bonds are not found in corn starch and confirm the introduction of the acetyl groups onto starch. In fig. 7b, a new peak is observed at 1755 cm^{-1} which can be attributed to the acrylate C=C double formed after SA reacts with acryloyl chloride. Although not well isolated, there is a small new visible peak around 1054 cm^{-1} which can be ascribed to SG1/BlocBuilder on fig 7c. The C-H stretching vibrations at 3026, 2927, 1601, 1493 cm^{-1} and the aromatic deformation at 699 cm^{-1} can be attributed to polystyrene in fig 7d. For AS-g-PMMA (fig. 7e), a new peak at 1732 cm^{-1} can be observed is due to the –COO^- stretching vibration in PMMA and the characteristic peak for methyl methacrylate homopolymer at 1150 cm^{-1} from carbonyl groups confirming the graft polymer.



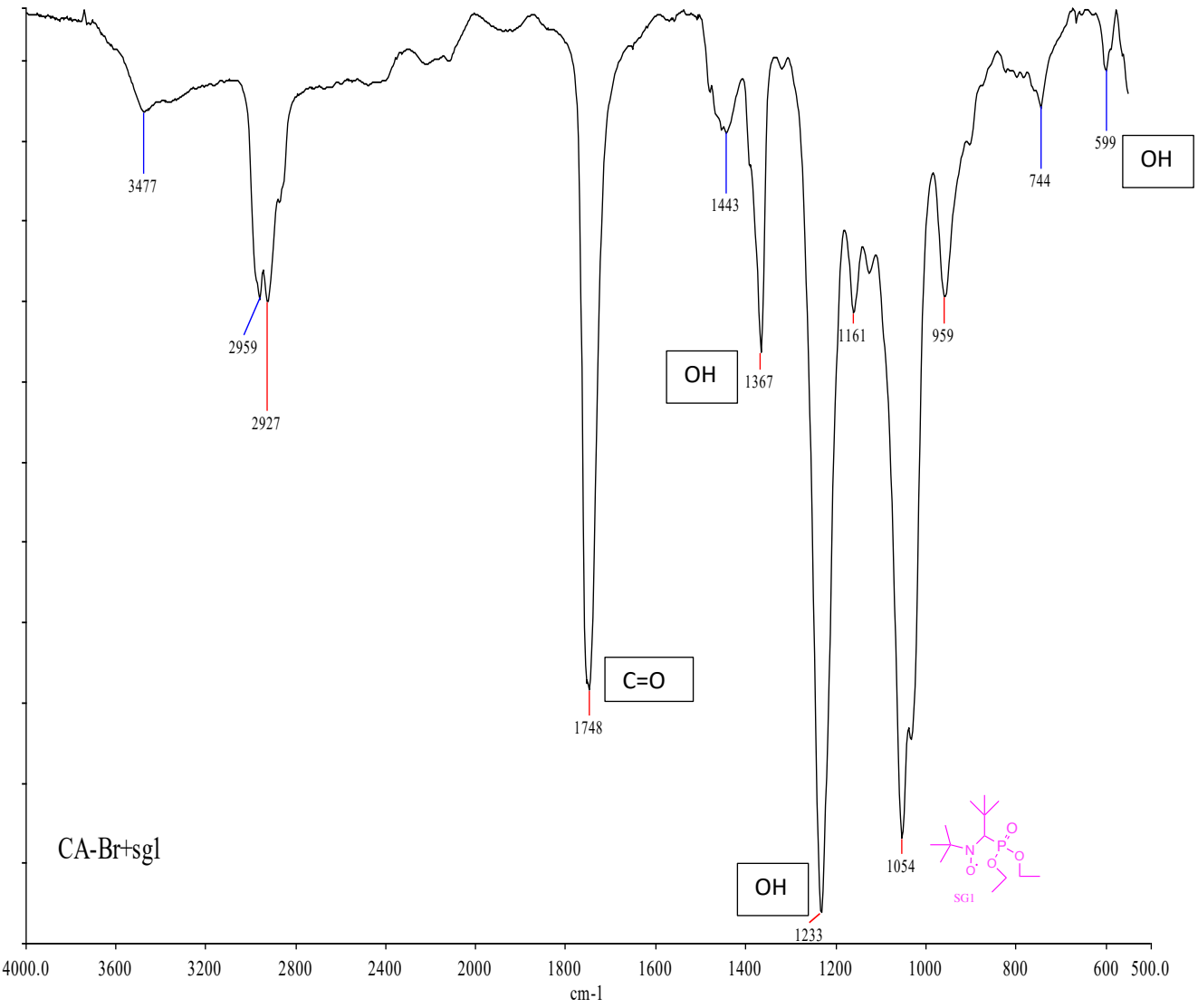
a. Cellulose acetate (CA)



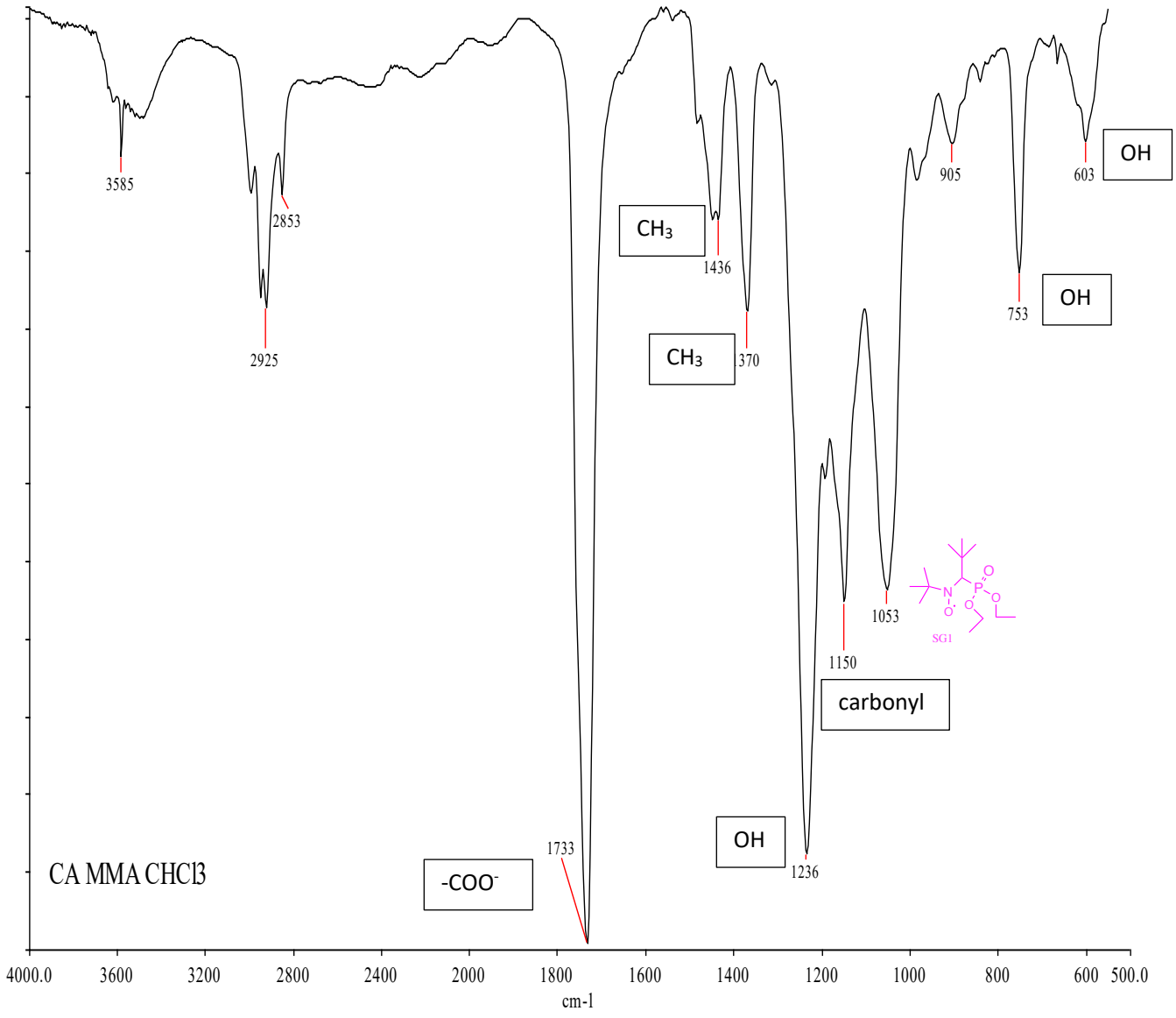
b. Cellulose acetate-bromide (CA-Br)



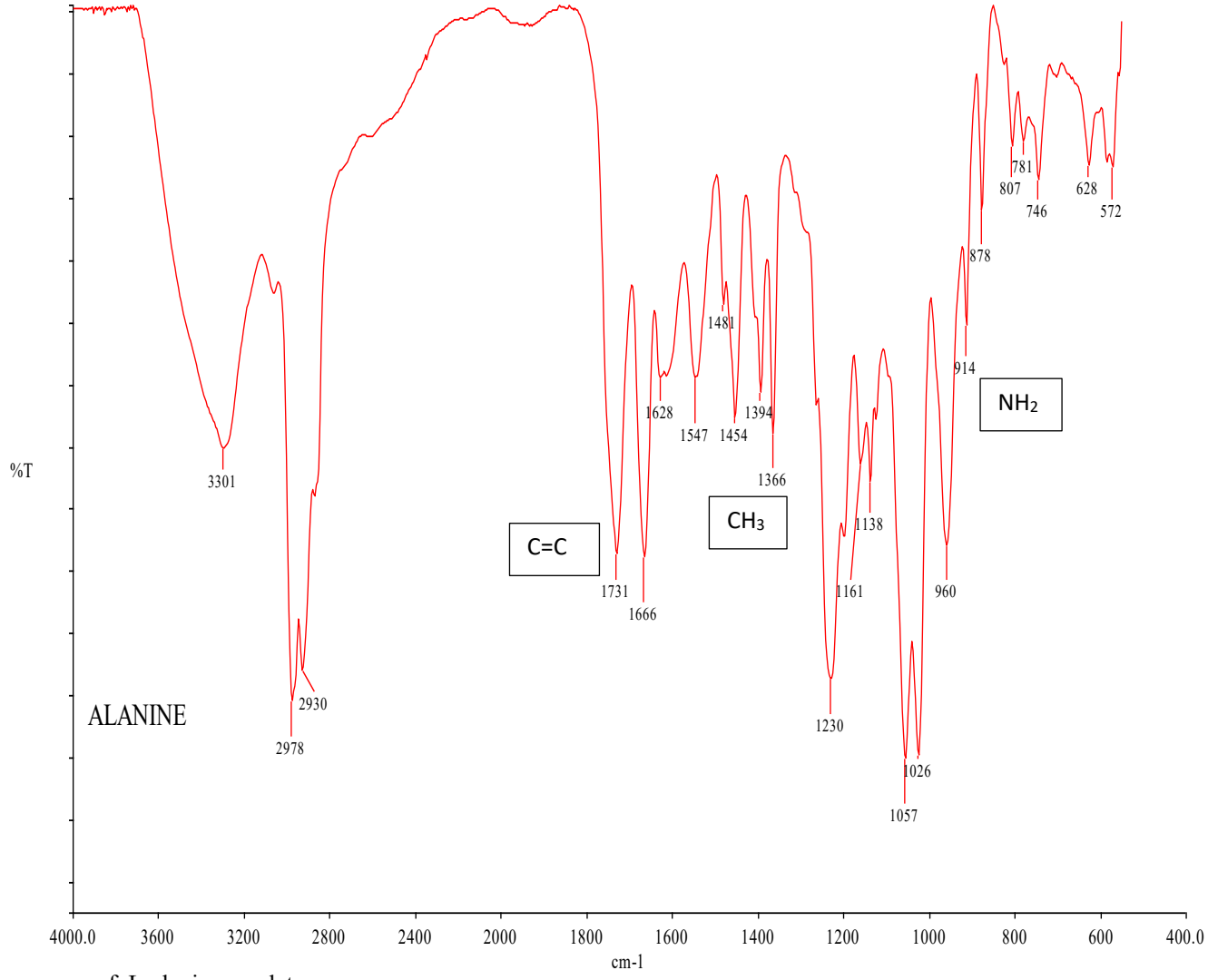
c. Cellulose acetate-DPAIO MA (CA-DPAIO MA)



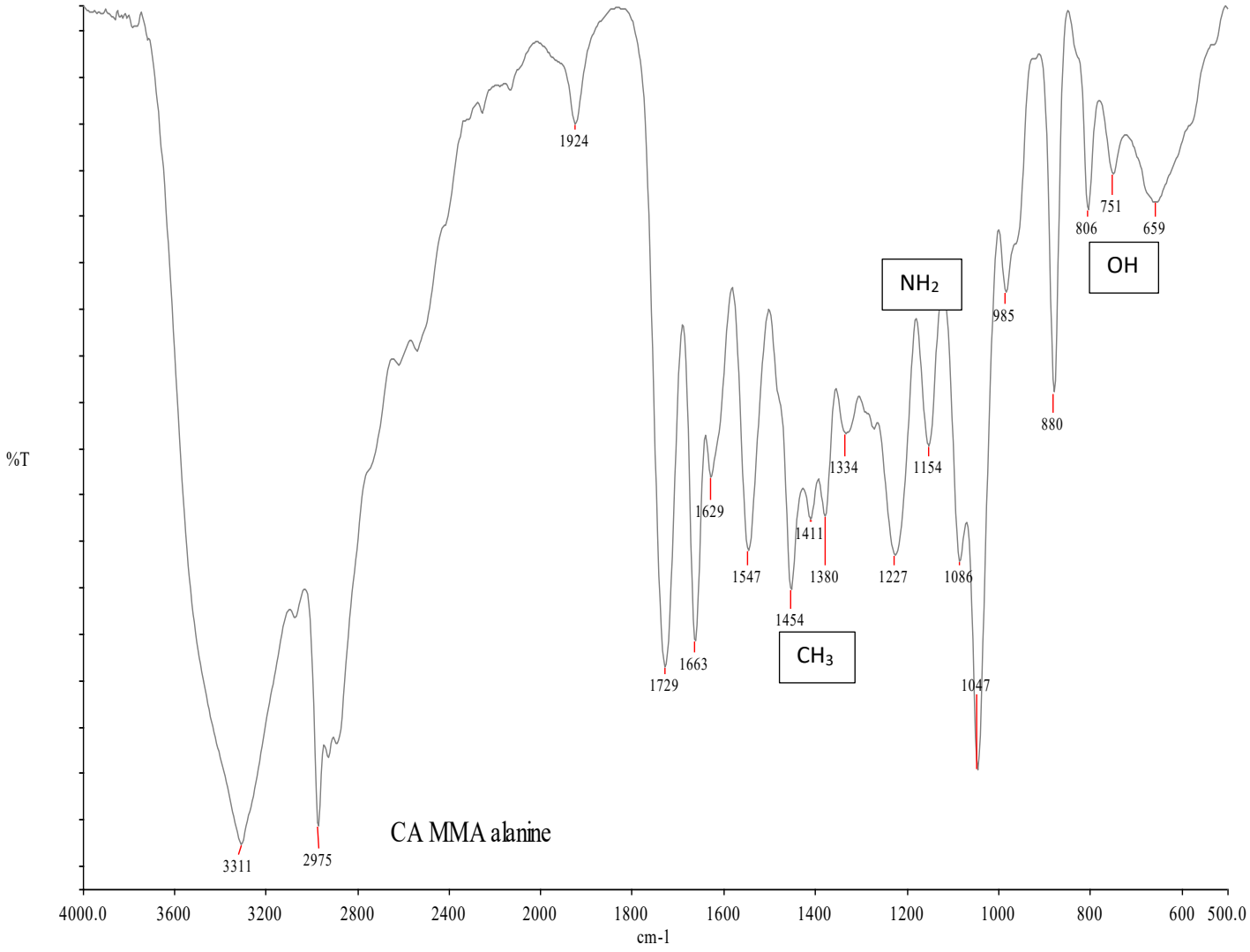
d. Cellulose acetate-SG1 MA (CA-SG1 MA)



e. Cellulose acetate-g-poly (methyl methacrylate-co-acrylonitrile) (CA-g-P(MMA-co-AN))



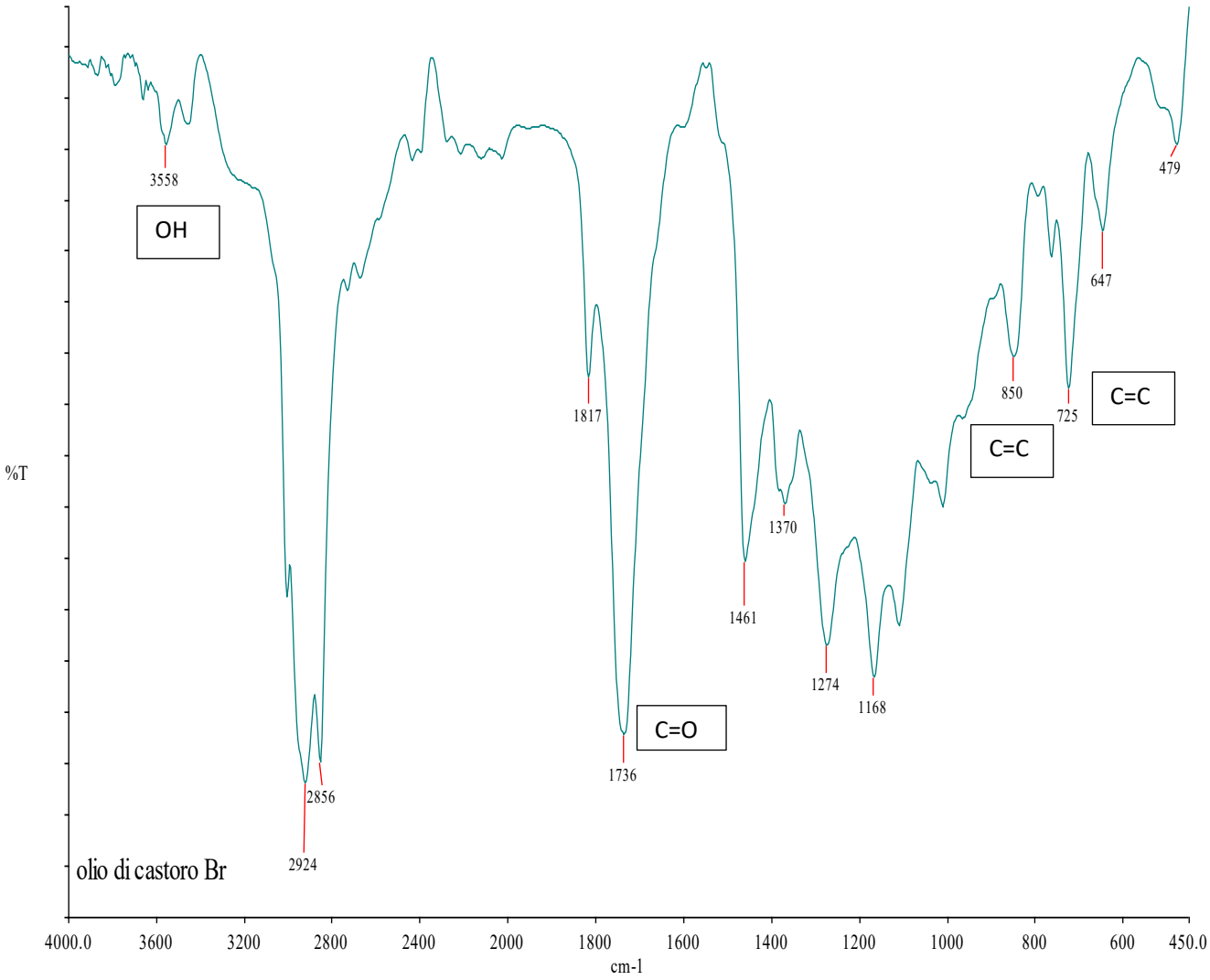
f. L-alanine acrylate



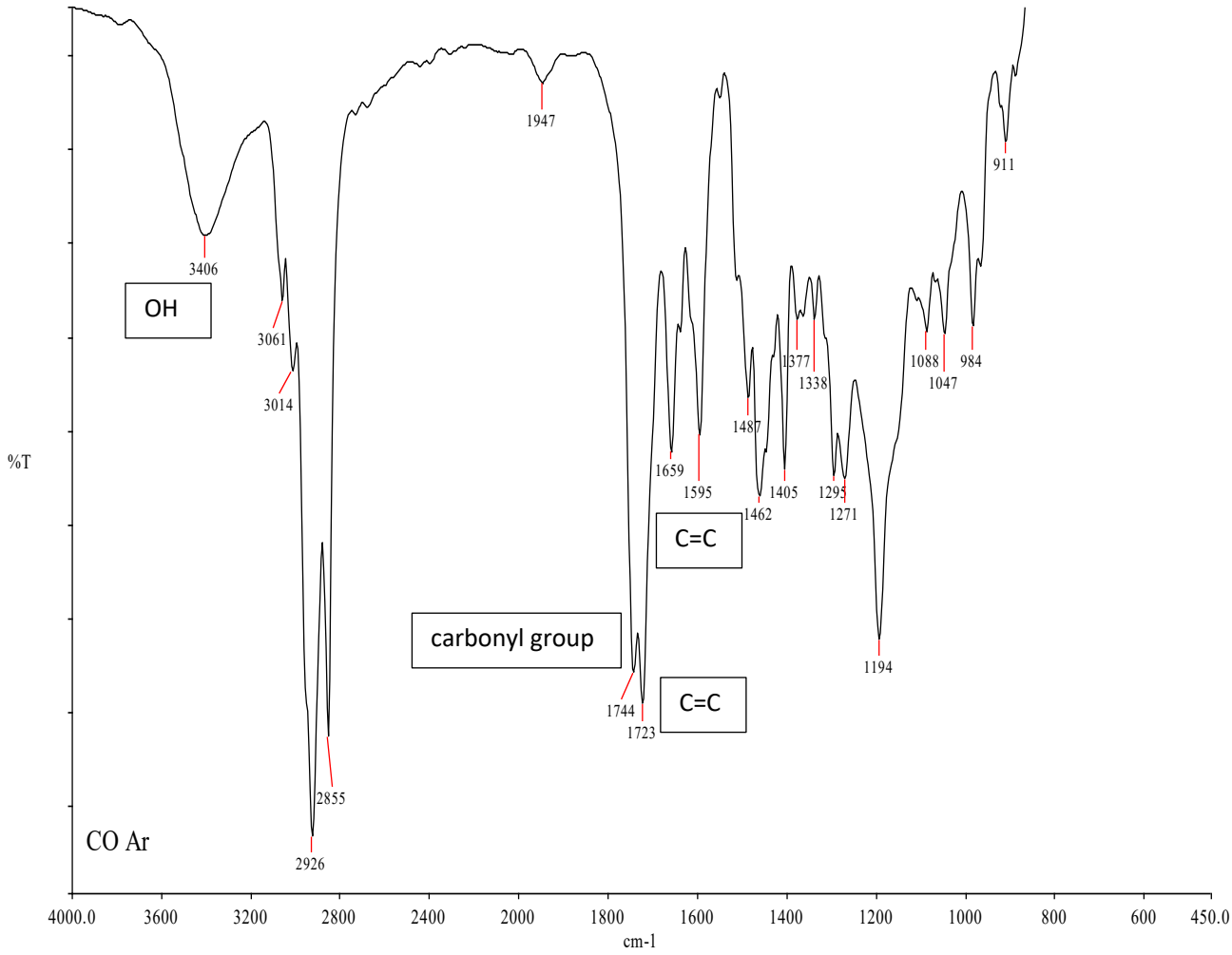
g. Cellulose acetate-g-poly(methyl methacrylate-co-acrylonitrile)-g-poly L-alanine acrylate (CA-g-P(MMA-co-AN)-g-PLAlaA).

Fig. 8. FTIR spectra of cellulose acetate (a), cellulose acetate-bromide(b), cellulose acetate (c), cellulose acetate-SG1 MA (d), Cellulose acetate-g-poly (methyl methacrylate-co-acrylonitrile) (e), L-alanine acrylate (f) and Cellulose acetate-g-poly(methyl methacrylate-co-acrylonitrile)-g-poly L-alanine acrylate (CA-g-P(MMA-co-AN)-g-PLAlaA (g).

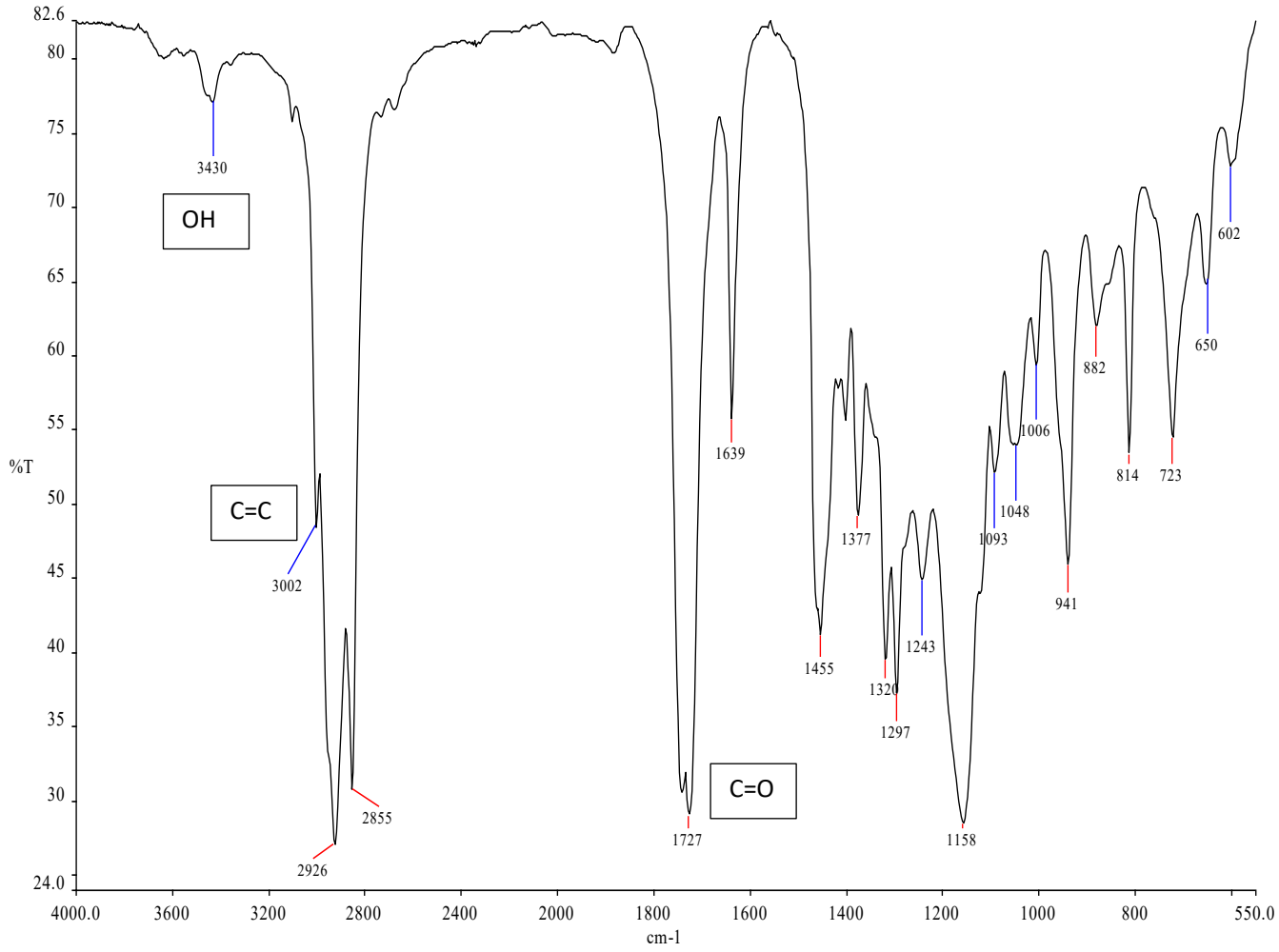
The peaks at 3487, 3026, 2886, 1369, 1236, 755 and 601 cm^{-1} are attributed to the OH groups of CA and those at 1748, 1433, 1371, and 1033 cm^{-1} show the presence of the acetyl group (fig. 8a). In fig. 8b, the band at 1748 cm^{-1} has intensified probably due to the presence of another C=O ester bond from reaction of cellulose with BIBB. The CA-DPAIO MA was confirmed by the new absorption bands at 1660, 1595 and 1485 cm^{-1} ascribed to DPAIO nitroxide (fig. 8c), meanwhile CA-SG1 the peak at 1054 cm^{-1} confirms the presence of SG1 in the macroalkoxyamine (fig. 8d). The new peak at 1733 cm^{-1} can be attributed to the -COO^- stretching vibration in PMMA and the characteristic peak for methyl methacrylate homopolymer at 1150 cm^{-1} from carbonyl groups confirming the CA-g-PMMA polymer (fig.8e), similar to the SA-g-PMM copolymer discussed above. Acrylonitrile is not observed in the spectra probably because only a very small amount was used to facilitate the polymerization process. Fig. 8f shows the peaks at 1731, 1454, 1366 and 914 cm^{-1} which can be attributed to the acrylate double bond, the CH_3, -COO and NH_2 groups respectively. The spectrum of fig. 8g shows the introduction of new peaks at 1454, 1411 and 1154 cm^{-1} belonging to the symmetrical and asymmetrical CH_3, and NH_2 groups of alanine respectively.



a. castor oil-bromide (CO-Br)



b. acrylated castor oil (ACO)

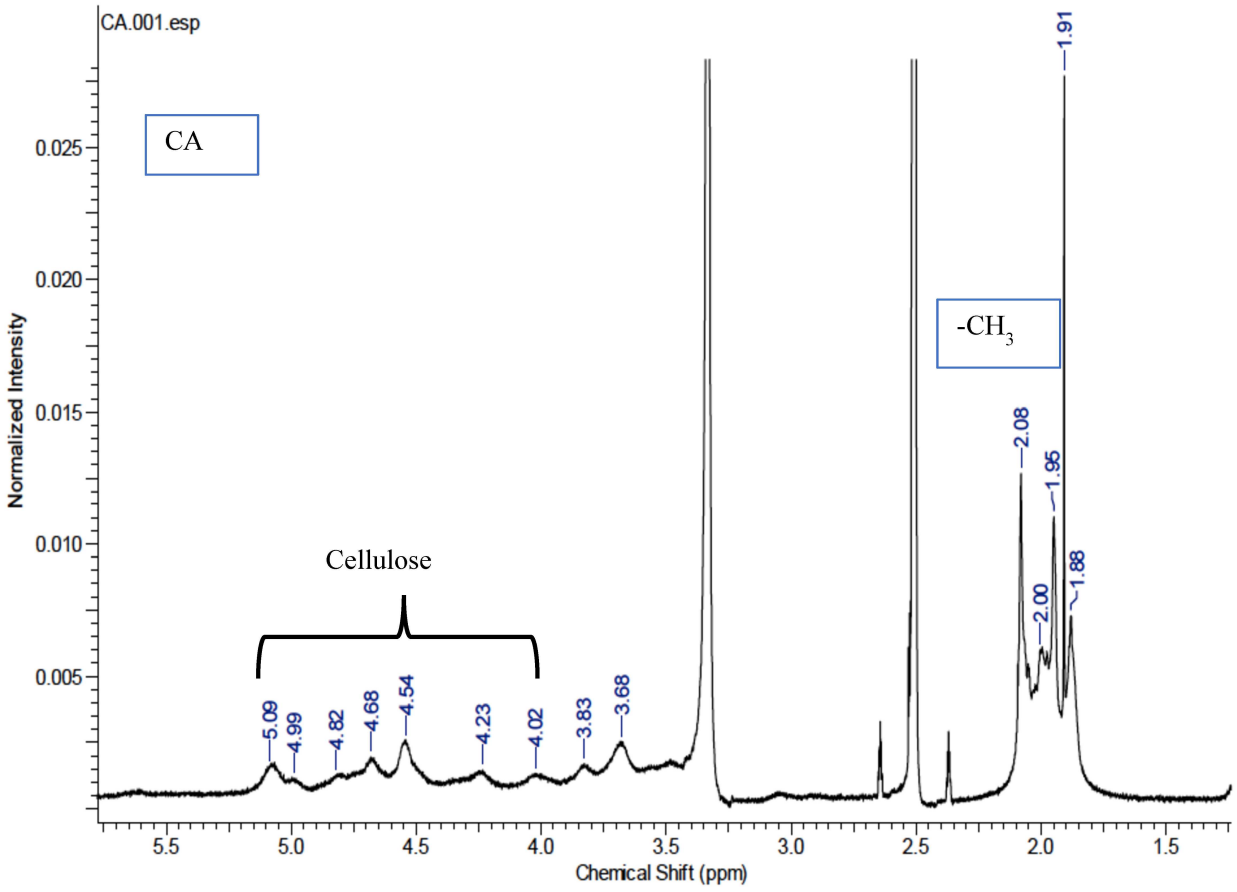


c. 2-(methacryloyloxy)ethyl oleate (MAEO) monomer

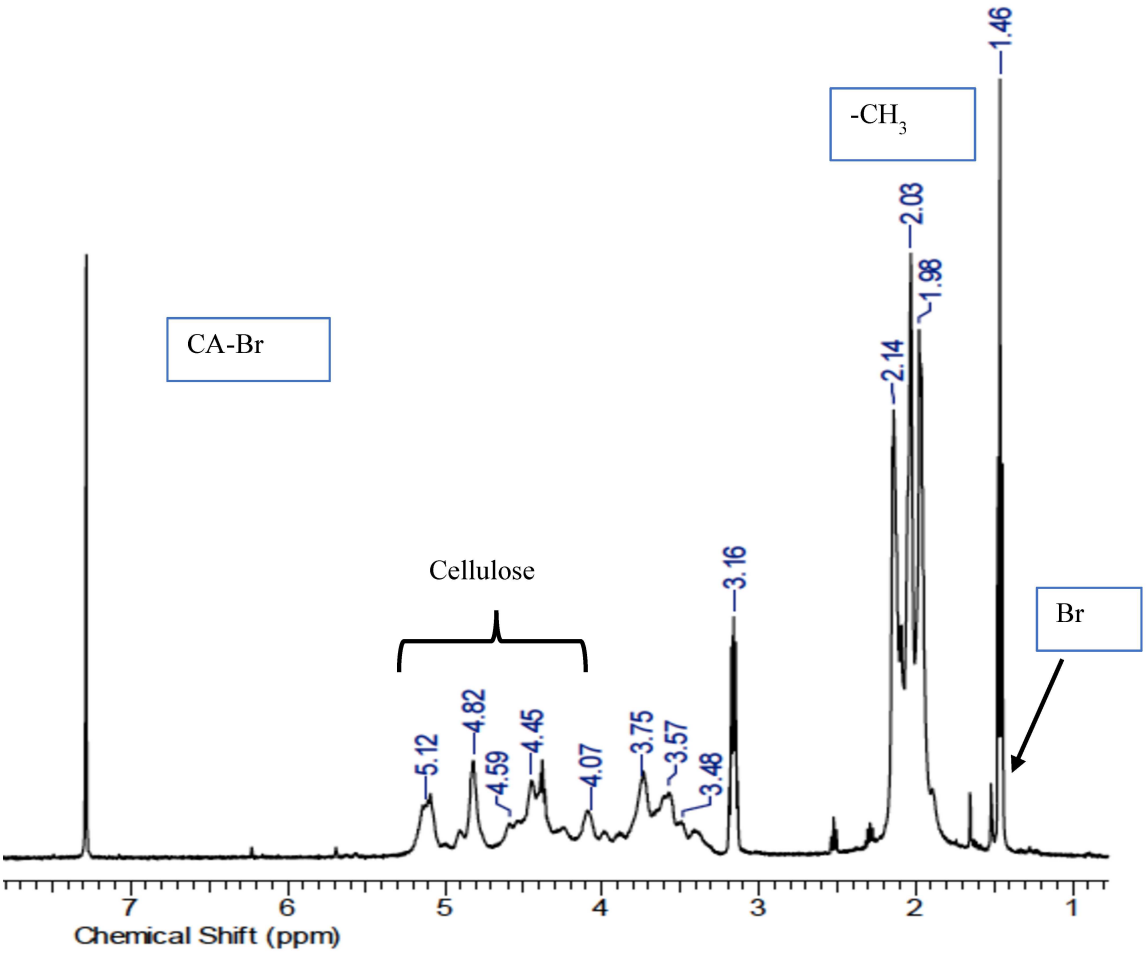
Fig. 9. FTIR spectra of castor oil bromide (a), acrylated castor oil (b) and 2-(methacryloyloxy)ethyl oleate monomer(c).

The peak at 1736 cm^{-1} represents the ester bond formed from the bromination of castor oil. Those peaks at 725 and 850 are attributed to the C=C bond, while the peak at 3558 cm^{-1} belongs to the OH group. Acrylated castor oil spectrum (fig. 9b) reveals the OH group at 3406 cm^{-1}, the C=C bond at1659, and the carbonyl ester group at 1744 cm^{-1}. The peak at 1723 cm^{-1} represents the acrylate ester. In fig. 9c, the peaks at 3430, 3002 and 1727 cm^{-1} can be attributed to OH group from castor oil, C=C and C=O ester bonds respectively.

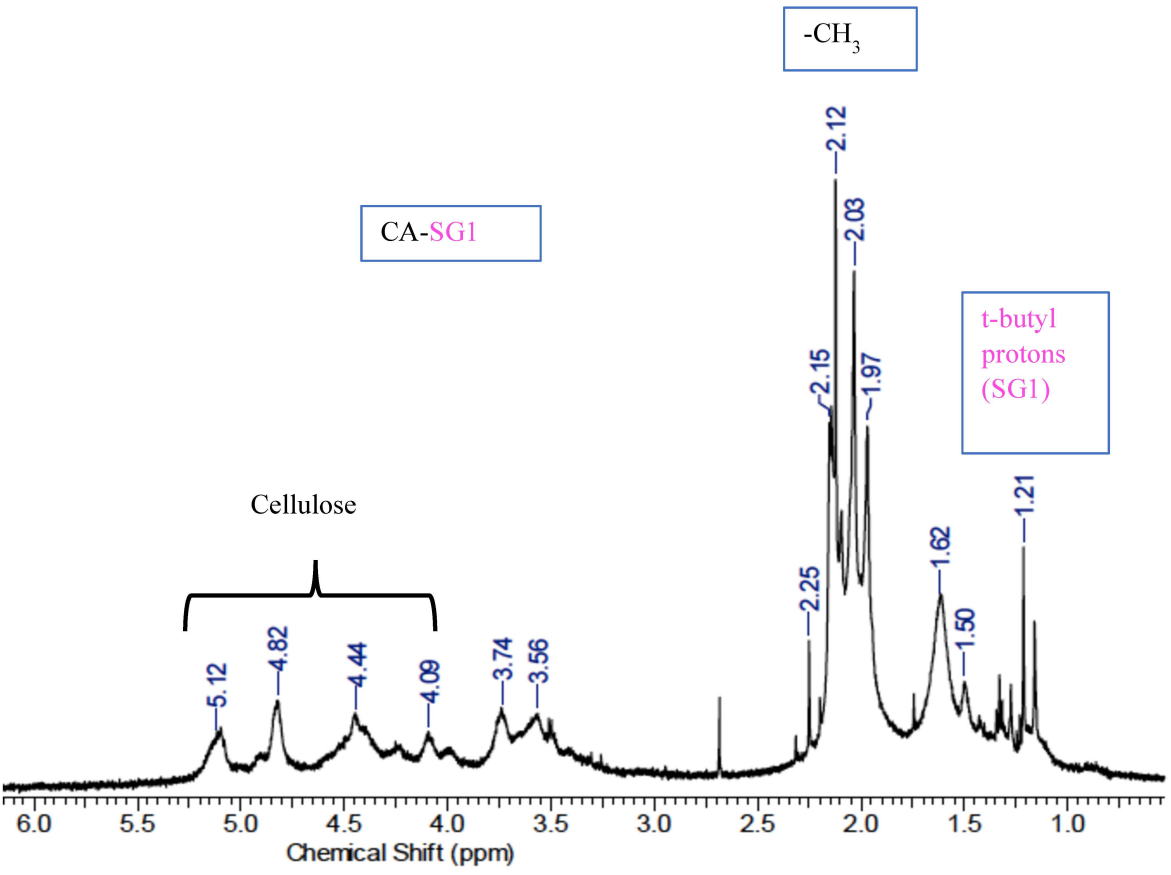
4.3. Nuclear magnetic resonance (NMR) analyses



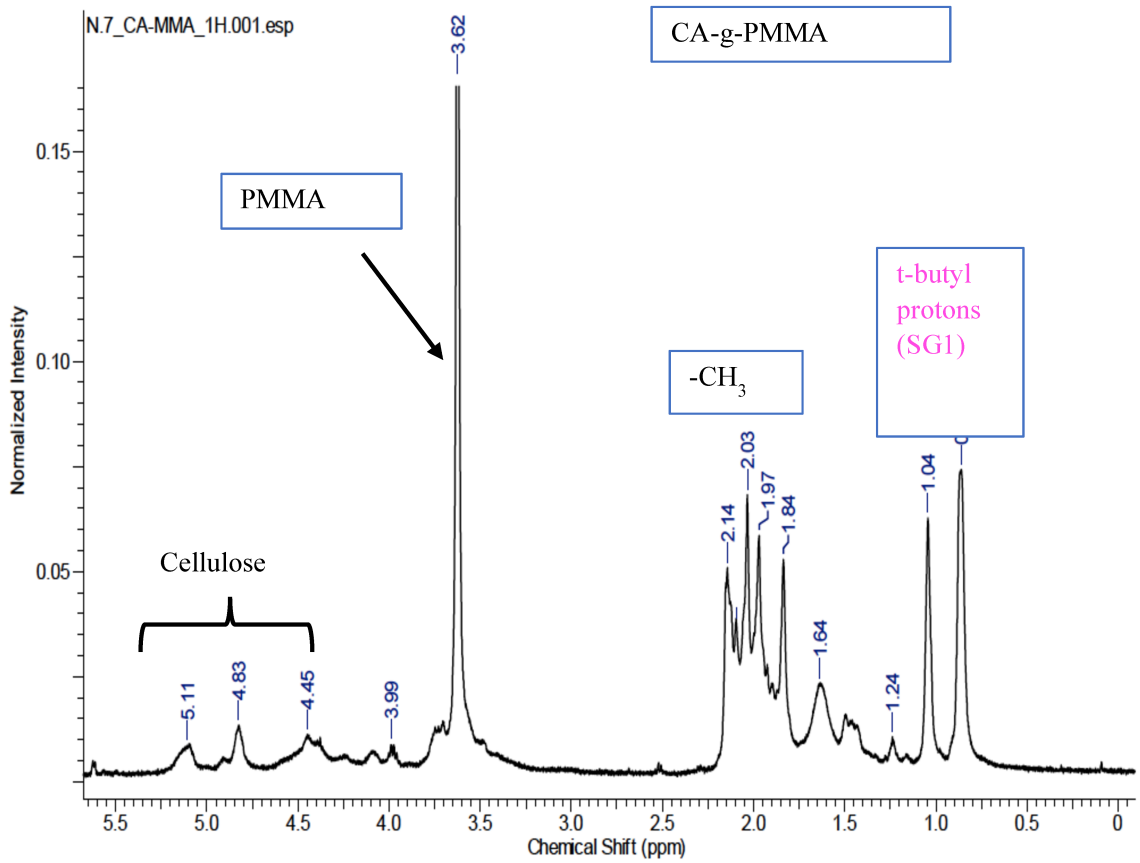
a.


-CH3
CA-Br
Cellulose
Br
1.46
2.03
1.98
2.14
3.16
5.12
4.82
4.59
4.45
4.07
3.75
3.57
3.48
7
6
5
4
3
2
1
Chemical Shift (ppm)

b.


-CH3
CA-SG1
t-butyl protons (SG1)
Cellulose
5.12
4.82
4.44
4.09
3.74
3.56
2.25
2.15
2.12
2.03
1.97
1.62
1.50
1.21
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical Shift (ppm)

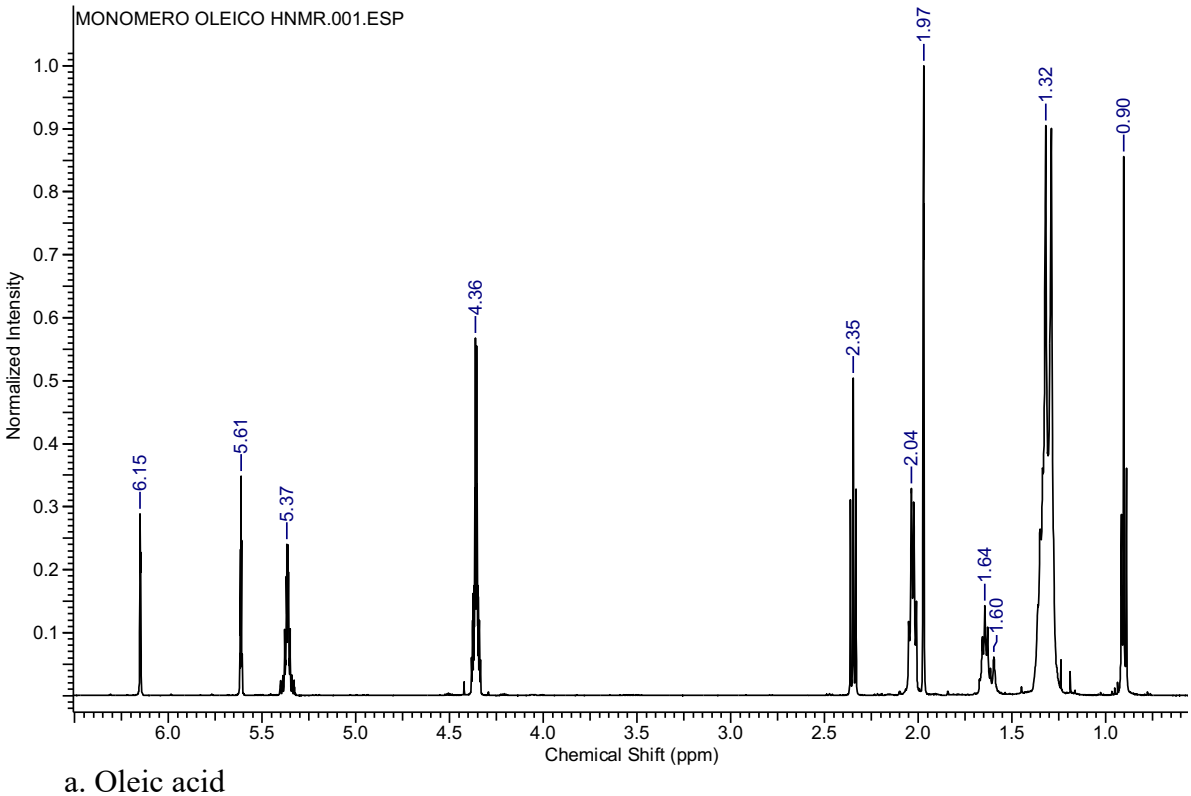
c.



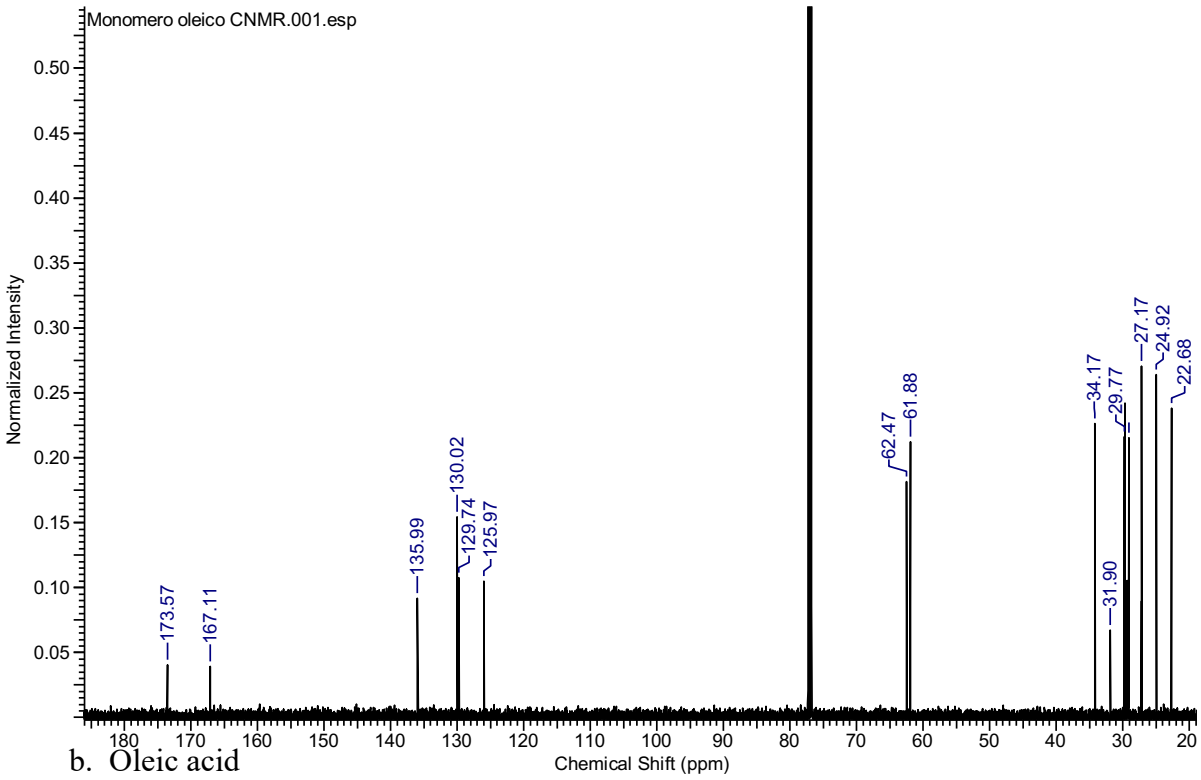
d.

Fig. 10. ^{1}H NMR spectra of CA (a), CA-Br (b), CA-SG1 (c) and CA-g-(PMMA-co-AN) (d).

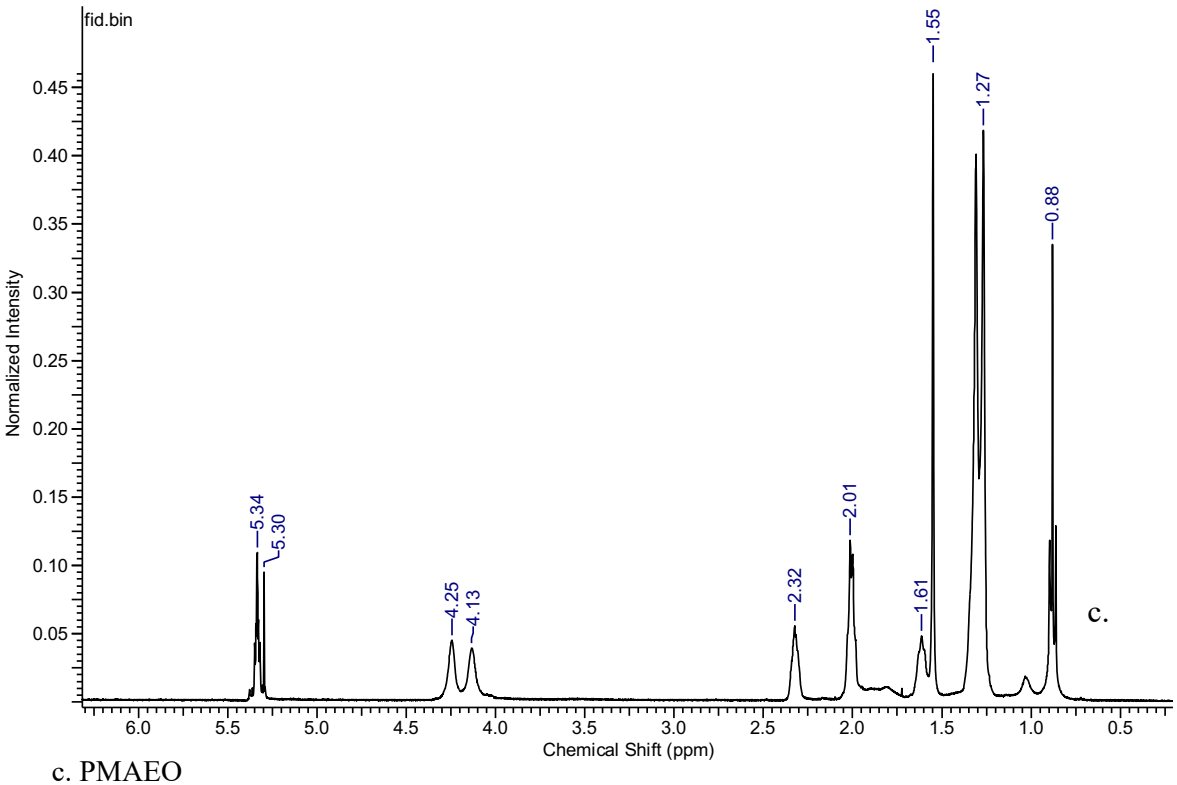
The peaks from 4.0 to 5.0 ppm in fig. 6a represent the hydroxyl groups of cellulose while acetyl groups are represented by the area around 2 ppm. A new peak appears at 1.46 ppm which represents the bromide indicating the formation of CA-Br (fig. 6b). t-butyl protons from SG1 made their appearance at around 1.0 ppm in fig. 6c showing the formation of an alkoxyamine. In the CA-g-PMMA polymer, the presence of PMMA is indicated at 3.62 ppm confirming the formation of the copolymer. The presence of t-butyl protons from SG1 in the copolymer indicates that it possesses a living character and further polymerization with other monomers is possible.


MONOMERO OLEICO HNMR.001.ESP
Normalized Intensity
Chemical Shift (ppm)
6.15
5.61
5.37
4.36
2.35
2.04
1.97
1.64
1.60
1.32
0.90

a. Oleic acid


Monomero oleico CNMR.001.esp
Normalized Intensity
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
173.57
167.11
135.99
130.02
129.74
125.97
62.47
61.88
34.17
31.90
29.77
27.17
24.92
22.68
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
Chemical Shift (ppm)

b. Oleic acid



c. PMAEO

Fig. 11. ^{1}H NMR spectra of oleic acid (a), ^{1}C NMR spectra of oleic acid (b) and ^{1}H NMR spectra of poly(2-methacryloyloxy)ethyl oleate) (PMAEO (c).

The peak at 5.37 ppm (fig. 11a) can be assigned to oleic acid and that at 5.34 ppm in fig. 11c corresponds to the side chain double bonds in PMAEO.

4.4. Differential scanning calorimetry (DSC) analyses

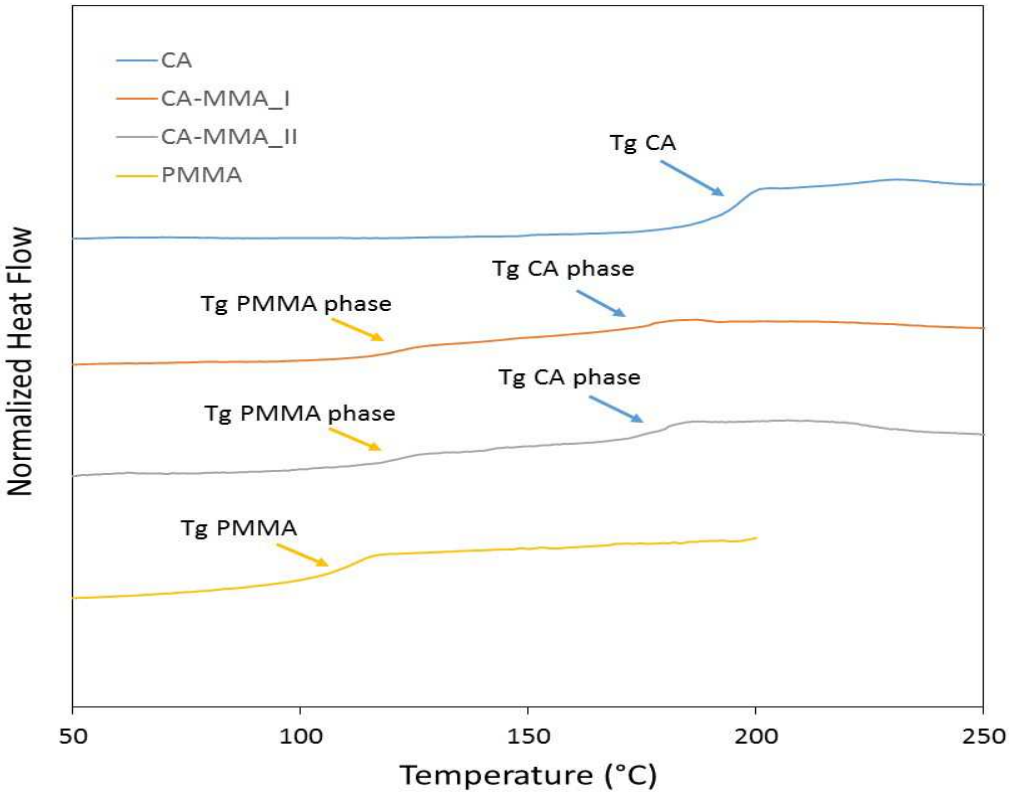


Fig 12. Differential scanning calorimetry analyses of CA, PMMA and CA-g-PMMA.

DSC tests were performed from 50 ° C to 250 ° C with a scanning speed of 20 ° C / min. The results are in all cases compatible with the structure of "grafted copolymers". For both CA-MMA samples, two Tgs for two separate phases were recorded; a phase rich in PMMA with Tg around 120 °C and a rich CA phase with Tg around 175 °C. Concerning these values, the Tg of the phase richer in PMMA is higher compared to that of the PMMA homopolymer. Furthermore, the Tg of the sample richer in CA is lower with respect to that of the pure CA homopolymer, also indicating a partial miscibility between the two polymers. The fact that both

samples were obtained by precipitation in different solvents (methanol and water) makes these results even more interesting.

4.5. Mass spectrometry analysis of MAEO monomer

Mass spectra were recorded on a gas chromatography with a mass-selective detector, utilizing electron ionization (EI) at an ionizing energy of 70 eV. The monomer was solubilized in dichloromethane and the solution injected into the GC-MS.

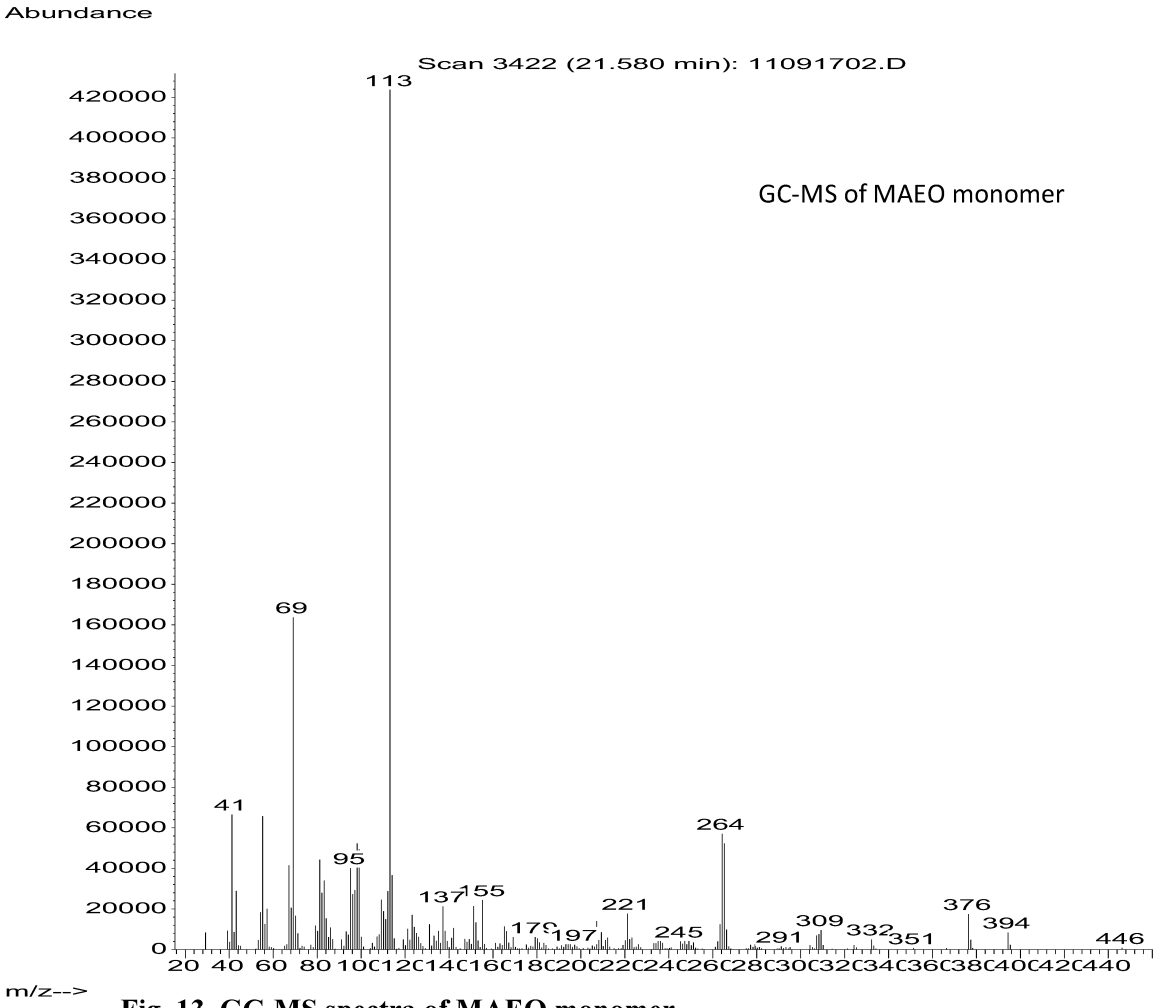
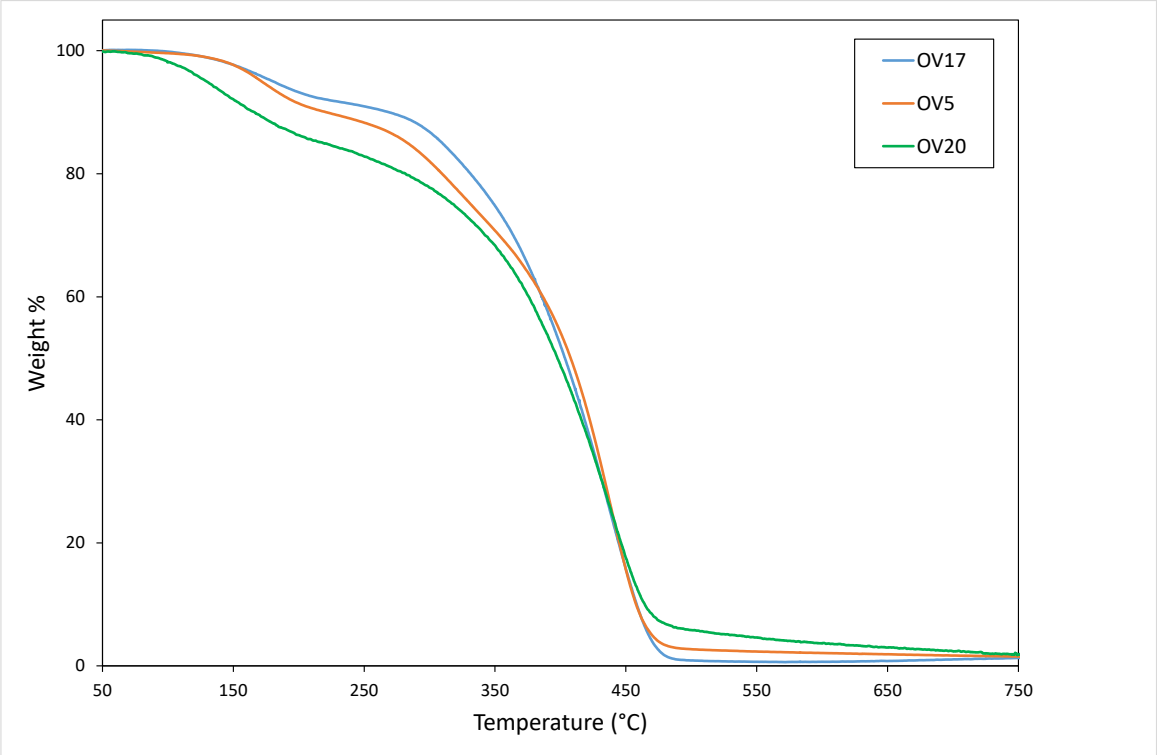
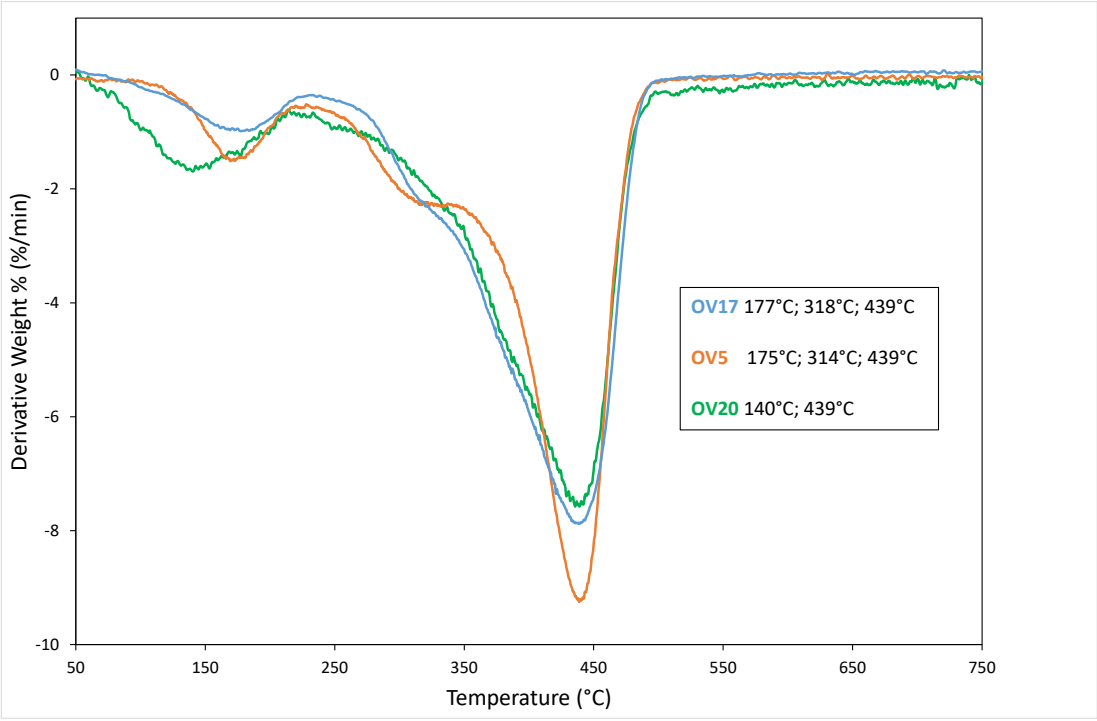


Fig. 13. GC-MS spectra of MAEO monomer.

4.6. Thermogravimetric analyses of MAEO homopolymers.



a.



b.

Fig. 14. Thermogravimetric analyses of MAEO homopolymers.

The thermogravimetric analyses were done with MAEO homopolymers synthesized with AIBN (OV17), BlocBuilder (OV5) and AIBN/NMA (OV20) respectively (fig. 14a). The tests were conducted in nitrogen (flow 40mL / min) and at 10 ° C / min. It can be deduced that the weight of all three homopolymers decrease with increase in temperature. This can be attributed to the thermal degradation of oleic acid in the homopolymer. We can observe that, heating the homopolymers around 500°C led to the complete breakdown of the OV17, while OV5 and OV20 still had about 5wt% and 10wt% of homopolymer respectively. This means that the homopolymer synthesized with AIBN has a faster rate of thermal decomposition than the other two samples. From fig. 14b, we can deduce that the major degradation of OV17, OV5 and OV20 occur around (177°C, 318°C, 439°C), (175°C, 314°C, 439°C) and (140°C, 439°C) respectively. No significant degradation was observed after heating above 500°C for all three samples.

4.7. Electron Paramagnetic Resonance Analyses

The EPR was used to confirm the formation nitroxides and macro(alkoxyamines) and determine the livingness of the polymers. The samples were dissolved in either THF or tert-butylbenzene depending on their solubility and the type of alkoxyamine, and then heated from 30°C to 120°C. In all samples, there were no increase in signals or peaks at 30°C, implying that they were inactive and around 70°C, the signals of the spectra increased with increase in temperature. The optimum signals for the samples were obtained between 90°C and 120°C. The formation of DPAIO(alkoxyamine), BlocBuilder MA, SA-BlocBuilder MA, CS-DPAIO MA, CA-SG1 MA, CO-TEMPO MA, CO-SG1 MA and the living character of CA-g-P(MMA-co-AN) polymer was also confirmed.

Chapter 5. Conclusions.

Several copolymers were synthesized starting with biorenewable raw materials, which were then grafted to synthetic ones through the widely known nitroxide mediated polymerization process. However, most of the results on the copolymers in this study are preliminary and have to be confirmed further by different analytical techniques.

Cellulose acetate-g-poly(methacrylate) copolymer on its part, was confirmed by both NMR and DSC analyses, and it is the first time this copolymer is synthesized through nitroxide mediated polymerization. This work was presented at the Joint event on 5th International Conference on Bioplastics and 6th World Congress on Biopolymers and has been published as **Cellulose acetate-graft-poly(methyl methacrylate): A graft from approach of nitroxide mediated radical polymerization (NMP**), J Chem Eng Process Technol. Likewise, the results obtained from the syntheses of poly(2-(methacryloyloxy)ethyl oleate) (PMAEO) which were confirmed by NMR and TG analyses are the first as well and this work is also being documented for publication. Future works will include the investigation of the properties and characteristics of the obtained polymers in order to find their most suitable applications and also synthesize more polymers from biorenewable resources through the NMP technique.

In this study, monomers from starch, cellulose, chitosan, MAEO, just to name a few were polymerized by using the nitroxide mediated polymerization technique and this is just one of the multifold studies which accredits the effectiveness and efficiency of the nitroxide mediated polymerization process. It also in conformity with others published works which have demonstrated that through nitroxide mediated polymerization, materials of different shapes, sizes and structures can be produced as shown in the case of cellulose acetate-g-poly(methyl methacrylate)-g-polyL-alanine acrylate. The fact that nitroxide mediated polymerization offers architectural tuning opportunities in polymeric materials, provides these materials with versatility and expands their scope of application. Additionally, nitroxide mediated polymerization is a green process free from metals and harsh agents (scarvengers), since the process is initiated and controlled by radical species only. The use of commercial SG1/BlocBuilder MA was very essential in the syntheses of macroalkoxyamines and in the

NMP process because of efficiency and effectiveness due to its high dissociation constant. The best results of this work were obtained when SG1/BlocBuilder was employed.

Some challenges were encountered during the syntheses and experimental work. Polysaccharide which are generally insoluble in organic solvents used for polymerization had to be acetylated before functionalization. DPAIO nitroxide which was our first choice nitroxide is not commercial and has very high dissociation constant and requires a very high temperature to initiate a polymerization process, thus was not suitable. The alternative was commercial SG1/BlocBuilder MA which was offered to us by our friends and collaborators at Aix-Marseille Université. Nonetheless, UNIVPM on its part provided also provided the resources for me to attend conferences/congresses, equipment, a conducive environment and a great team to facilitate my work.

Despite the challenges which led to the incompletion of **the green polymer cycle (fig. 1)** experiment, green, smart, versatile, functional biodegradable polymeric materials can be produced from biorenewable resources through nitroxide mediated polymerization. This statement can be backed by both the literature search and experimental work in this study. As much there is a tremendous progress in the research, production and application of biodegradable polymers from biorenewable resources (fig.2 and fig. 3), more research is necessary complete thoroughly comprehend and apply the green polymer cycle commercially especially biodegradable polymers are expected to completely replace synthetic one someday. Applying the green polymer cycle industrially with more than 50% efficiency will provide a huge environmental and economic benefit globally. I hope this study enormously contributes to the field of material science and engineering and its continuation provides an astronomical influence industrially as well.

In this light, I strongly recommend this work as basis for further research in biodegradable polymers from biorenewable resources.

Chapter 6. Project: Development of degradable hydrogels PLA-b-PNIPAAm-co-PEGMA for ischemic stroke recovery.

This is a project done at the joint research unit UMR 7273, "Institut de Chimie Radicalaire" (ICR), centre national de la recherche scientifique (CNRS), Aix-Marseille Université (AMU), Marseille as part of collaboration agreement with Università Politecnica delle Marche (UNIVPM) Ancona and part of the requirements to obtain a European PhD (Doctor Europaeus). The project was principally funded by AMU.

6.1. Introduction

Stroke[191,192], either caused by the partial occlusion of vascular structure within the brain (*i.e.* ischemic stroke) or by a weakened blood vessel leak (*i.e.* hemorrhagic), is one of the main causes of death and disability worldwide[193]. Stroke induced-deficits, ranging from motor to cognitive, cannot be fully treated by the current clinical therapeutic strategies[194,195] , which mainly consist of direct clot lysis followed by a long-term rehabilitation. In order to enhance recovery, experimental therapies are focused on treating locally the brain infarction area by

[191] Amy J. Gleichman and S. Thomas Carmichael, 'Astrocytic Therapies for Neuronal Repair in Stroke', *Neuroscience Letters*, 565 (2014), 47–52.

[192] Stephen Grupke, 'Understanding History, and Not Repeating It. Neuroprotection for Acute Ischemic Stroke: From Review to Preview', *Clinical Neurology and Neurosurgery*, 129 (2015), 1–9.

[193] Dariush Mozaffarian, 'Executive Summary: Heart Disease and Stroke Statistics—2016 Update', 8.

[194] Toby B. Cumming, Randolph S. Marshall, and Ronald M. Lazar, 'Stroke, Cognitive Deficits, and Rehabilitation: Still an Incomplete Picture', *International Journal of Stroke*, 8.1 (2013), 38–45.

[195] Caroline Pin-Barre and Jérôme Laurin, 'Physical Exercise as a Diagnostic, Rehabilitation, and Preventive Tool: Influence on Neuroplasticity and Motor Recovery after Stroke', *Neural Plasticity*, 2015 (2015), 1–12.

biomolecular injections[196] and cell transplantations. However, such approaches have failed in their translation to the clinic, mainly due to the limited amount of injected molecules reaching the lesion site and the poor survival of transplanted cells[197].

An alternative strategy is the implantation into the cerebral infarcted area of biocompatible materials so as to promote tissue reconstruction. Additionally, biomaterials can be used as a delivery device to release pharmacological treatment and/or encapsulated cells and even increase their respective therapeutic efficiency[198,199,200]. Engineered materials that are displaying mechanical properties close to the ones of the nervous system, such as

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[196] Amit Alexander, 'Polyethylene Glycol (PEG)–Poly(N-Isopropylacrylamide) (PNIPAAm) Based Thermosensitive Injectable Hydrogels for Biomedical Applications', *European Journal of Pharmaceutics and Biopharmaceutics*, 88.3 (2014), 575–85.

[197] Roger Y Tam, 'Regenerative Therapies for Central Nervous System Diseases: A Biomaterials Approach', *Neuropsychopharmacology*, 39.1 (2014), 169–88.

[198] Anup Tuladhar, Cindi M. Morshead, and Molly S. Shoichet, 'Circumventing the Blood–Brain Barrier: Local Delivery of Cyclosporin A Stimulates Stem Cells in Stroke-Injured Rat Brain', *Journal of Controlled Release*, 215 (2015), 1–11.

[199] Jin Zhong and others, 'Hydrogel Matrix to Support Stem Cell Survival After Brain Transplantation in Stroke', *Neurorehabilitation and Neural Repair*, 24.7 (2010), 636–44.

[200] Douglas J Cook, 'Hydrogel-Delivered Brain-Derived Neurotrophic Factor Promotes Tissue Repair and Recovery after Stroke', *Journal of Cerebral Blood Flow & Metabolism*, 37.3 (2017), 1030–45.

hydrogels[201,202,203,204,205], are generally desired to support cell adhesion and differentiation[206]. Those hydrogels must be biodegradable[207,208] in order to prevent chronic complications (*e.g.* tissue scarring)[209] and injectable. Indeed, thermosensitive materials that can be injected at room temperature and gel at body temperature are highly required to minimize the surgery invasiveness. Although numerous hydrogels have already been engineered to treat stroke[210], none of them is combining all the above-mentioned properties to our knowledge.

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[201] Manuel Gregoritza and others, 'Controlled Antibody Release from Degradable Thermoresponsive Hydrogels Cross-Linked by Diels–Alder Chemistry', *Biomacromolecules*, 18.8 (2017), 2410–18.

[202] Andre R. Massensini, 'Concentration-Dependent Rheological Properties of ECM Hydrogel for Intracerebral Delivery to a Stroke Cavity', *Acta Biomaterialia*, 27 (2015), 116–30.

[203] Sytze J. Buwalda, Tina Vermonden, and Wim E. Hennink, 'Hydrogels for Therapeutic Delivery: Current Developments and Future Directions', *Biomacromolecules*, 18.2 (2017), 316–30.

[204] Todd R. Hoare and Daniel S. Kohane, 'Hydrogels in Drug Delivery: Progress and Challenges', *Polymer*, 49.8 (2008), 1993–2007.

[205] Eve Ruel-Gariépy and Jean-Christophe Leroux, 'In Situ-Forming Hydrogels—Review of Temperature-Sensitive Systems', *European Journal of Pharmaceutics and Biopharmaceutics*, 58.2 (2004), 409–26.

[206] Pavla Jendelová, 'Current Developments in Cell- and Biomaterial-Based Approaches for Stroke Repair', *Expert Opinion on Biological Therapy*, 16.1 (2016), 43–56.

[207] Min Hee Park, 'Biodegradable Thermogels', *Accounts of Chemical Research*, 45.3 (2012), 424–33.

[208] Huayu Tian, 'Biodegradable Synthetic Polymers: Preparation, Functionalization and Biomedical Application', *Progress in Polymer Science*, 37.2 (2012), 237–80.

[209] Lina Ratiba Nih, Stanley Thomas Carmichael, and Tatiana Segura, 'Hydrogels for Brain Repair after Stroke: An Emerging Treatment Option', *Current Opinion in Biotechnology*, 40 (2016), 155–63.

[210] Emily R. Aurand, Kyle J. Lampe, and Kimberly B. Bjugstad, 'Defining and Designing Polymers and Hydrogels for Neural Tissue Engineering', *Neuroscience Research*, 72.3 (2012), 199–213.

Developing a hydrogel presenting all the required properties can be obtained by combining polymers with complementary characteristics. For example, Abandansari *et al.* synthesized a block copolymer made of poly(ethylene glycol) (PEG), poly(caprolactone) (PCL) and poly(N-isopropylacrylamide) (PNIPAAm) so as to benefit from the hydrophilicity, degradability and the thermosensitivity[211] of the respective constituting polymers[212]. Their pentablock copolymer, namely PNIPAAm-*b*-PCL-b-PEG-b-PCL-*b*-PNIPAAm, was synthesized by the combination of controlled ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP). Such material undergoes sol-gel transition between room temperature and human body temperature and shows no apparent cell cytotoxicity. Moreover, it is able to effectively release bioactive molecules. Nevertheless, no therapeutic application was performed and only a hypothesis of the gelation process was proposed. More precisely, the authors suggested that by increasing the temperature above the lower critical solution temperature (LCST)[213] (*i.e.* temperature at which the PNIPAAm segment becomes hydrophobic), the block copolymer forms aggregate micelles resulting in a free-standing physical gel. Pertici et al. synthesized a similar copolymer structure using a combination of ROP and nitroxide mediated polymerization (NMP), replacing PCL with PLA to obtain faster degradation. Results demonstrated that the copolymer hydrogel was degradable, non-toxic (neuronal cells), able to incorporate riluzole (neuroprotective drug) [214] and injectable in the brain (in vivo in rats). Nonetheless, with the pentablock architecture, it is expected that after

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[211] Jianjun Guan, 'Protein-Reactive, Thermoresponsive Copolymers with High Flexibility and Biodegradability', *Biomacromolecules*, 9.4 (2008), 1283–92.

[212] Hamid Sadeghi Abandansari, 'Preparation of Injectable and Thermoresponsive Hydrogel Based on Penta-Block Copolymer with Improved Sol Stability and Mechanical Properties', *Polymer*, 54.4 (2013), 1329–40.

[213] Emily Ho, Anthony Lowman, and Michele Marcolongo, 'Synthesis and Characterization of an Injectable Hydrogel with Tunable Mechanical Properties for Soft Tissue Repair', *Biomacromolecules*, 7.11 (2006), 3223–28.

[214] Shashi K. Verma, 'Enhancement in the Neuroprotective Power of Riluzole Against Cerebral Ischemia Using a Brain Targeted Drug Delivery Vehicle', *ACS Applied Materials & Interfaces*, 8.30 (2016), 19716–23.

the degradation of PCL or PLA hydrophobic PNIPAAm remains in the body system while hydrophilic PEG[215] may be eliminated. PNIPAAm may cause further complications in the body system.

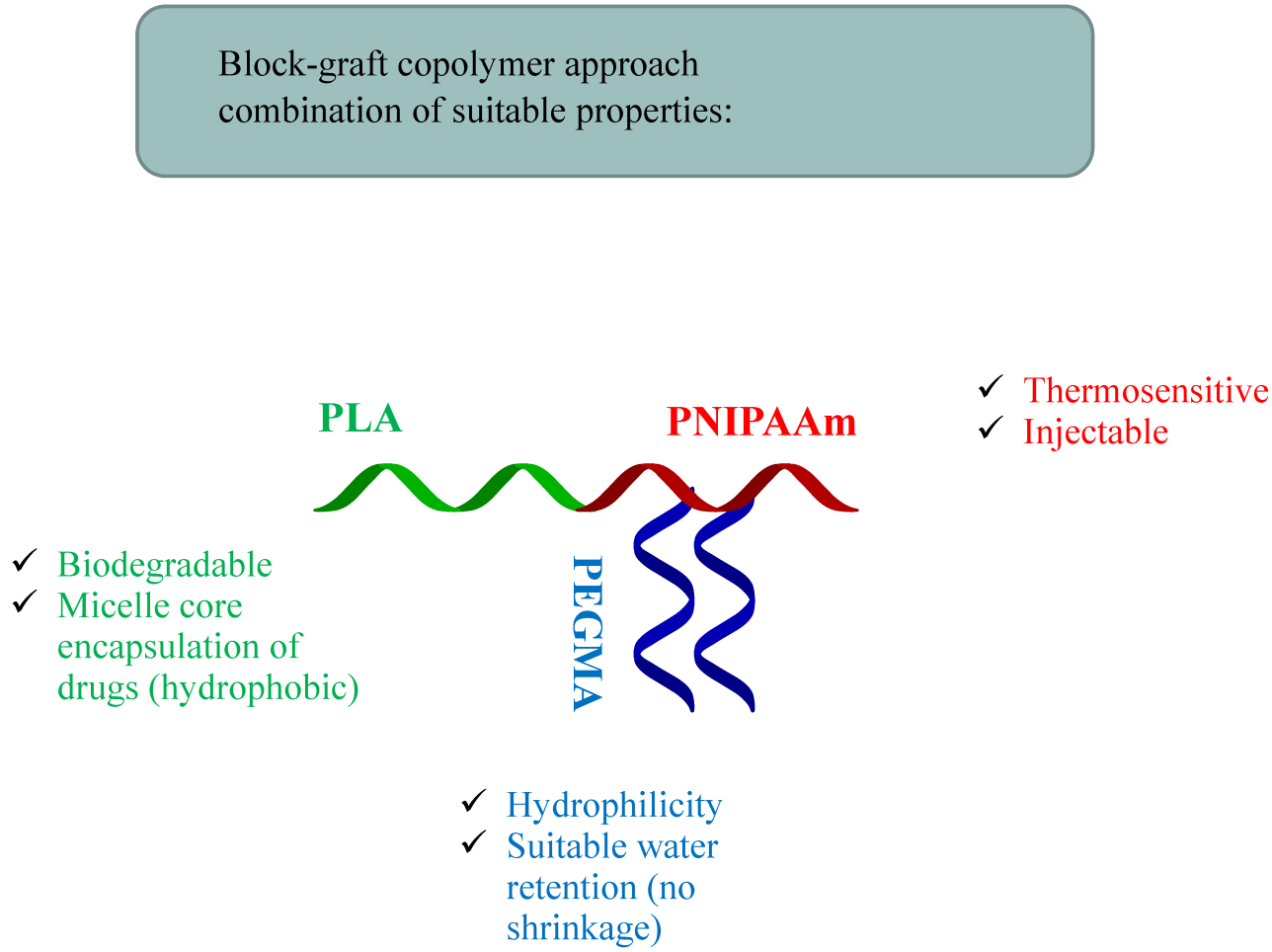

Fig. 15. The block-graft copolymer approach.

[215] Ali Darabi, 'PEGylation of Chitosan Via Nitroxide-Mediated Polymerization in Aqueous Media: PEGylation of Chitosan Via Nitroxide-Mediated Polymerization in Aqueous Media', *Macromolecular Reaction Engineering*, 10.1 (2016), 82–89.

Different macromolecular architectures of PNIPAAm-based[216,217,218,219] thermosensitive hydrogels have been synthesized using different techniques in the past, but very few by nitroxide mediated radical polymerization (NMP). The use of SG1 can be an asset over other controlled radical polymerization techniques due to reported innocuous character of this nitroxide and its derived alkoxyamines[220], as well its ability to be involved in radical additions. The synthesis of the macromolecule can be easily obtained with a controlled process. We successfully applied the NMP technique using SG1 in the synthesis of a novel

[216] Meidong Lang, 'Injectable Hydrogel as Stem Cell Scaffolds from the Thermosensitive Terpolymer of NIPAAm/AAc/HEMAPCL', *International Journal of Nanomedicine*, 2012, 4893.

[217] Il Keun Kwon and Takehisa Matsuda, 'Photo-Iniferter-Based Thermoresponsive Block Copolymers Composed of Poly(Ethylene Glycol) and Poly(N-Isopropylacrylamide) and Chondrocyte Immobilization', *Biomaterials*, 27.7 (2006), 986–95.

[218] Hai-Hui Lin and Yu-Ling Cheng, 'In-Situ Thermoreversible Gelation of Block and Star Copolymers of Poly(Ethylene Glycol) and Poly(*N* -Isopropylacrylamide) of Varying Architectures', *Macromolecules*, 34.11 (2001), 3710–15.

[219] H. Malonne, 'Preparation of Poly(N-Isopropylacrylamide) Copolymers and Preliminary Assessment of Their Acute and Subacute Toxicity in Mice', *European Journal of Pharmaceutics and Biopharmaceutics*, 61.3 (2005), 188–94.

[220] Marion Chenal, Simona Mura, 'Facile Synthesis of Innocuous Comb-Shaped Polymethacrylates with PEG Side Chains by Nitroxide-Mediated Radical Polymerization in Hydroalcoholic Solutions', *Macromolecules*, 43.22 (2010), 9291–9303.

amphiphilic[221,222,223] copolymer architecture, polylactide-block-P(NIPAAm-co-poly(ethylene glycol) methacrylate) (PLA-b-P(NIPAAm-co-PEGMA)) (Fig 15). This copolymer possesses all the parameters required for an ideal biomaterial recovery of ischemic stroke;

[221] Sean George, 'Amphiphilic Block Copolymers as Stabilizers in Emulsion Polymerization: Effects of the Stabilizing Block Molecular Weight Dispersity on Stabilization Performance', *Macromolecules*, 48.24 (2015), 8913–20.

[222] Lisa M. Ryno, 'Amphiphilic Graft Copolymers from End-Functionalized Starches: Synthesis, Characterization, Thin Film Preparation, and Small Molecule Loading', *Biomacromolecules*, 15.8 (2014), 2944–51.

[223] O. V. Borisova, 'Synthesis of Amphiphilic Block-Gradient Copolymers of Styrene and Acrylic Acid by Nitroxide Mediated Polymerization', *Polymer Science Series C*, 57.1 (2015), 86–93.

injectable[224,225,226], thermosensitive[227,228,229], biodegradable[230,231], biocompatible[232]. Moreover, the block-graft structure of the copolymer facilitates the elimination of PNIPPAm from the body system after the degradation of PLA. The reason is because hydrophilic PEGMA chains are covalently bonded to PNIPAAm as branches, therefore when the PLA backbone which is also covalently bonded to PNIPAAm degrades, the whole PNIPAAm-g-PEGMA structure becomes hydrophilic (solution) due to the strong hydrophilic nature of PEGMA and the absence of hydrophobic PLA.

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[224] Zhenqing Li, 'Injectable, Highly Flexible, and Thermosensitive Hydrogels Capable of Delivering Superoxide Dismutase', *Biomacromolecules*, 10.12 (2009), 3306–16.

[225] Jeong-A. Yang, 'In Situ-Forming Injectable Hydrogels for Regenerative Medicine', *Progress in Polymer Science*, 39.12 (2014), 1973–86.

[226] Malgosia M Pakulska, Brian G Ballios, and Molly S Shoichet, 'Injectable Hydrogels for Central Nervous System Therapy', *Biomedical Materials*, 7.2 (2012), 024101.

[227] Zuwei Ma, 'Thermally Responsive Injectable Hydrogel Incorporating Methacrylate-Polylactide for Hydrolytic Lability', *Biomacromolecules*, 11.7 (2010), 1873–81.

[228] Maja Radivojša Matanović, Julijana Kristl, and Pegi Ahlin Grabnar, 'Thermoresponsive Polymers: Insights into Decisive Hydrogel Characteristics, Mechanisms of Gelation, and Promising Biomedical Applications', *International Journal of Pharmaceutics*, 472.1–2 (2014), 262–75.

[229] Debashish Roy, William L. A. Brooks, and Brent S. Sumerlin, 'New Directions in Thermoresponsive Polymers', *Chemical Society Reviews*, 42.17 (2013), 7214.

[230] Jian Yang, 'A Thermoresponsive Biodegradable Polymer with Intrinsic Antioxidant Properties', *Biomacromolecules*, 15.11 (2014), 3942–52.

[231] J Piantino, 'An Injectable, Biodegradable Hydrogel for Trophic Factor Delivery Enhances Axonal Rewiring and Improves Performance after Spinal Cord Injury', *Experimental Neurology*, 201.2 (2006), 359–67.

[232] Zhanwu Cui, 'Degradation, Cytotoxicity, and Biocompatibility of NIPAAm-Based Thermosensitive, Injectable, and Bioresorbable Polymer Hydrogels', *Journal of Biomedical Materials Research Part A*, 98A.2 (2011), 159–66.

Hydrogels can also be used in the treatment (cleaning) of paintings and concrete. To treat concrete, hydrogels can be applied to improve curing, in anti-dusting hardening and as sealants to control moisture thereby replacing multiple products and reducing cost. Besides, they are environmentally friendly and take less time and labor to be applied. Moreover, they are compatible with coatings, additives and epoxy designs for bare concrete and can penetrate deep into slabs preventing the risk of de-lamination. Furthermore, appropriate treatment of hydrogels on existing concrete will bridge micro-cracking up 0.5 mm serving as a pro-active maintenance in the early phase of concrete aging. Such treatments carry a long-term warranty of up to 15 years and will endure for the life of the concrete.

Fig. 16. Semi-IPN hydrogel (H65) on a travertine stone. On the left, the hydrogel applied in vertical position; on the right, the hydrogel removal is shown. It is worth noting that the stone surface is wet only in correspondence with the contact area.

Cleaning of artworks are usually done traditionally with the use of detergent solutions or pure solvents[233]. Scarce environmental safety and poor selectivity are some the problems faced with when treating with organic solvents. Moreover, detergents in solvents can diffuse into a deeper and broader area of the artifact. Furthermore, lack of proper control would cause penetration of pure solvents into the artifact's porous structure eventually leading to swelling or leaching of binders and varnishes, with unknown long-term effects[234]. Recently, researchers have found out that polymeric hydrogels with good hydrophilic properties and mechanical strength can replace traditional solvents in the cleaning of paintings. Although hydropilic some polymer hydrogels[235] such as poly(2-hydroxyethyl methacrylate) P(HEMA) lack sufficient mechanical strength, they can be cross-linked with other suitable polymer networks to produce tuned or modified polymers with much improved properties.

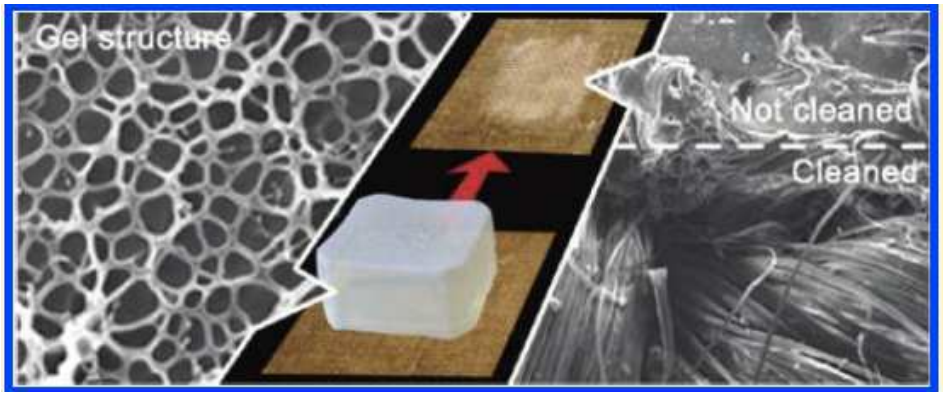


Fig. 17. Image of a hydrogel cleaning test.

[233] Joana Domingues, 'Innovative Method for the Cleaning of Water- Sensitive artifacts: Synthesis and Application of Highly Retentive Chemical Hydrogels', 8.

[234] Giacomo Pizzorusso, 'Physicochemical Characterization of Acrylamide/Bisacrylamide Hydrogels and Their Application for the Conservation of Easel Paintings', *Langmuir*, 28.8 (2012), 3952–61.

[235] Joana A. L. Domingues, 'Innovative Hydrogels Based on Semi-Interpenetrating p(HEMA)/PVP Networks for the Cleaning of Water-Sensitive Cultural Heritage Artifacts', *Langmuir*, 29.8 (2013), 2746–55.

PEGMA is hydrophilic and water retentive, and when grafted with hydrophobic PLA, the resulting polymer may be suitable for the treatment of art works. The study of the hydrogels for biomedical purposes has provided me with an insight to pursue its use for architectural and environmental applications. This field of studies can be further researched and exploited.

6.2. Synthesis

PLA-b-P(NIPAAm-co-PEGMA) was prepared in a three-step process starting with ROP (Scheme 25). In the presence of stannous octoate catalyst, 2-hydroxyethyl acrylate (HEA) initiated the ring opening of lactide (LA) to form a PLA-HEA polymer. Next, Blocbuilder MA was added onto PLA-HEA through IRA leading to a functionalized macroalkoxyamine initiator, PLA-SG1, with a nearly 100% functionalization yield. Applying a temperature of 100^{o}C resulted in the decomposition of Blocbuilder MA, thereby breaking the C-O bond and releasing the alkyl moiety which was added to the double bond, followed by recombination of SG1. In the final step, NIPAAm and PEGMA monomers were polymerized from PLA-SG1 through NMP at 120^{o}C, which led to the formation of PLA-b-P(NIPAAm-co-PEGMA).

6.2.1. Materials

Poly (ethylene glycol) (PEG, Mn=2000 g/mol) monohydroxy was purchased from Sigma-Aldrich, D-Lactide (LA) was purchased from Corbion Purac, packaged under vacuum, and then stored under argon. Stannous octoate ($Sn(Oct)_{2,}$ 95%), toluene, 2-hydroxyethyl acrylate (HEA), methacryloy chloride (85%), 1,4-dioxane, N-Isopropylacrylamide and dichloromethane (DCM) were also purchased from Sigma-Aldrich. Triethylamine (TEA) was purchased from Acros Organics, and Blocbuilder MA was provided by Arkema (France).

6.2.2. Synthesis of Polylactide-2-hydroxyethyl acrylate (PLA-HEA)

10 g (69.38 mmol) of Lactide (LA) was weighed into a round bottom flask and placed under vacuum. 0.33 g (2.89 mmol) of 2-hydroxyethyl acrylate (HEA) and 289.10 μmol of stannous octoate (SnO_2) were also weighed in a vial and placed under vacuum. Argon was then flowed through the reactants every hour four times to deoxygenate the content. Toluene was added into the flask and the content of the vial transferred was also transferred into it. The flask was then immersed into an oil bath at 100^oC and the reaction was allowed to run under stirring for 2 h. Samples were taken at 5, 10, 15, 20, 30, 40, 60, 90 and 120 mins for analyses. After 2 h, the reaction was stopped by cooling the flask with ice for several minutes. Toluene was removed by means of a rotavapor and the crude product diluted with THF. The reaction mixture was then precipitated in cold pentane to obtain pure PLA-HEA which was filtered by means of a funnel and dried under vacuum.

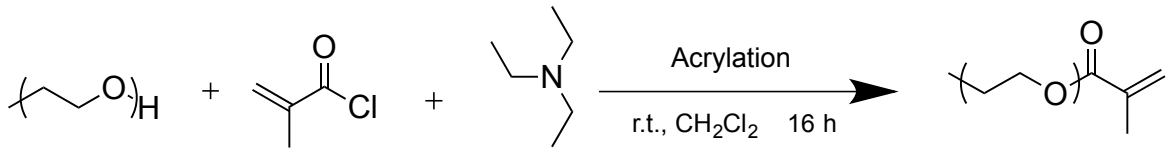
6.2.3. Synthesis of PLA-SG1 macroinitiator/alkoxyamine

978.98 μmol of PLA-HEA and 9.79 mmol of Blocbuilder MA were weighed in vials. 2ml of 1, 4-dioxane was added for every gram of PLA-HEA and the reaction mixture was degassed for about 20 mins. The vials were placed in an oil bath at 100^oC and the reaction mixture was stirred for 1 h. After which the reaction was stopped by cooling the vials with ice and the reaction mixture was precipitated in excess cold ethanol. Pure PLA-SG1 macrointiator was obtained and dried under vacuum.

6.2.4. Synthesis of poly (ethylene glycol methacrylate) PEGMA monomer

10 g (4.96 mmol) of poly (ethylene glycol) (PEG), Mn=2000, was weighed in a round bottom flask equipped with an arm funnel and dichloromethane (DCM) was added into the flask to dissolve PEG and form a homogenous solution. 5.2 g (49.64 mmol) of triethylamine (TEA) was added into the reaction mixture. Argon was bubbled through the mixture to remove water vapor. Then 49.64 mmol of methacyloyl chloride was added dropwise through the arm funnel at 0^oC under stirring. The reaction was allowed to run overnight at room temperature. DCM was removed by means of rotavapor and the crude product was diluted with THF and centrifuge to separate the salt from the salt formed from the reaction mixture. The crude solution

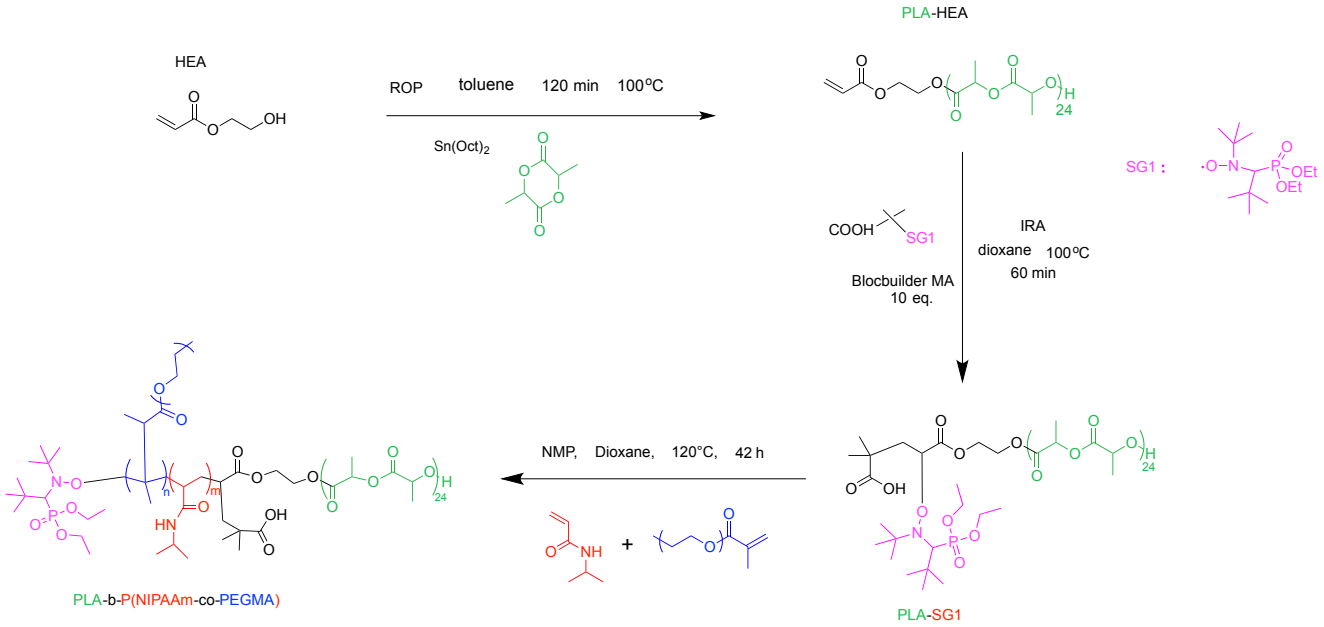
was precipitate in 90% diethyl ether and 10% ethanol mixture. The pure product was filtered by means of a funnel dried under vacuum.



Scheme 24. Acrylation process for the synthesis of poly (ethylene glycol methacrylate).

6.2.5. Synthesis of PLA-b-P(NIPAAm-co-PEGMA) copolymer

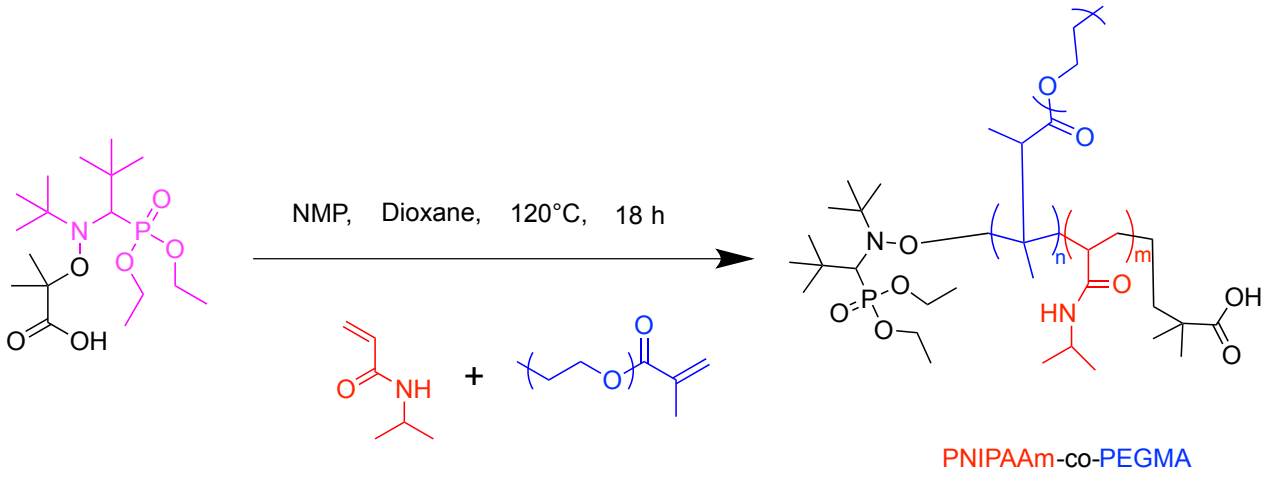
125.48 μmol of PLA-SG1macroinitiator, 5.85 mmol of PEGMA and 17.23 mmol of NIPAAm were put in a vial and 5 mL of 1, 4-Dioxane added into the vial. The reactants were dissolved and degassed for about 20 mins. The mixture was then immersed in an oil bath at 120^{o}C and stirred for 42 h. Samples were collected at 5 mins, 30 mins, 18 h, and 41 h for analyses. After 42 h, the crude product was precipitated in excess cold diethyl ether and the pure product was filtered and dried under vacuum.



Scheme 25. Formation of PLA-HEA through ring opening polymerization (ROP), formation of PLA-SG1 through 1,2 intermolecular radical addition (IRA) and formation of PLA-b-PNIPAAm-co-PEGMA through nitroxide mediated polymerization (NMP).

6.2.6. Synthesis of P(NIPAAm-co-PEGMA)

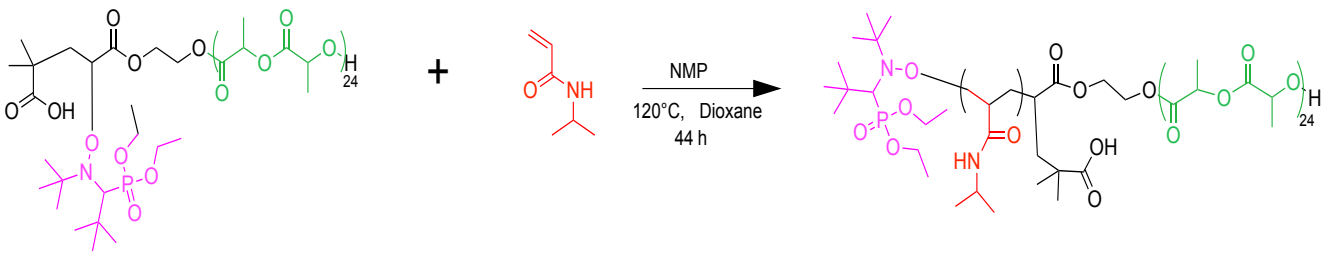
47.2 mg of Blocbuilder MA, 750.2 mg of PEGMA and 2.96 g of NIPAAm were dissolved in 3 ml of dioxane in a vial. The mixture was deoxygynated by flowing in argon for about 15 min and then placed in an oil bath at 120°C and stirred for 18 h. The crude product was purified by adding dropwise onto cold diethyl ether under vigorous stirring. The final product was filtered and dried under vacuum at stored below 0°C.



Scheme 26. Nitroxide mediated polymerization synthesis of PNIPAAm-co-PEGMA.

6.2.7. Synthesis of polylactide-b-poly (N-isopropylamide) (PLA-b-PNIPAAm)

503.4 mg of PLA-SG1macroinitiator, 1.96 g of PNIPAAm and were put in a vial and 3 mL of 1, 4-Dioxane added. The reactants were dissolved and degassed for about 20 mins. The mixture was then immersed in an oil bath at 120^{o}C and stirred for 44 h. Samples were collected at 5, 15, 30, 50, 80 mins, 16 and 24 h as the reaction proceeded for analyses. After 44 h, the crude product was precipitated in excess cold diethyl ether and the pure product was filtered and dried under vacuum.



Scheme 27. Nitroxide mediated polymerization synthesis of PLA-b-PNIPAAm.

6.3. Characterization

6.3.1. Nuclear magnetic resonance (NMR)

^{1}H and ^{31}P nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance 400 MHz spectrometer to confirm polymerizations, end-chain functionalizations and degradation. The deuterated solvents used were deuterated chloroform ($CDCl_3$) and deuterated dimethyl sulfoxide (DMSO-d_6).

6.3.2. Size exclusive chromatography (SEC)

Size exclusion chromatography (SEC) experiments, highlighting change in polymer length after polymerization reaction or degradation, were performed on a Varian PL-GPC 120 apparatus, which was composed of a PL-AS-MT autosampler, an Agilent 1100 series pump, a degasser, an injection valve, a column oven and a refractive index (RI) detector. The following columns were used: one pre-column and two PL Resipore columns (300 mm Å~ 7.8 mm). The injection loop, the columns and the RI detector were in the same oven thermostated at 70 °C. The eluent was a solution of 0.1 M LiBr in N,N-dimethylformamide (DMF) filtered through a 0.45 µm nylon membrane and the flow rate was fixed at 0.7 $mL.min^{-1}$. The samples were prepared in a mixture of eluent and toluene (0.25 vol%) as the flowmarker, filtered through a 0.2 µm nylon filter (Interchim) and placed in an autosampler preheated at 50 °C. The sample concentration was 0.25 wt%. Calibration curves were established with poly (methyl methacrylate) (PMMA) standards purchased from Agilent.

6.3.3. Dynamic light scattering (DLS)

DLS analysis has been performed on 0.1 wt% copolymer solutions in PBS (pH 7.4). The hydrodynamic diameter of the micelles and their size distribution (PDI) were measured using a Zetasizer Nano ZS apparatus (Malvern, UK). After equilibration at 15 °C, the temperature

was incremented in steps of 0.5 to 45 °C. For each step, an equilibration period was fixed at 3 min before performing 2 measurements that were then averaged.

6.3.4. Inverted test tube method

The inverted test tube method was used in determining the sol-gel transition phase of the copolymers in aqueous solution. Furthermore, it was used to prove the effect of PLA on the gelation temperature of the copolymer. 15 wt% of each sample A ((PLA-b-P(NIPAAm-co-PEGMA)) and sample B (PNIPAAm-co-PEGMA) in normal phosphate buffer saline (PBS) solution were prepared and placed in separate vials. The content in each vial was made homogenous by proper mixing and stored at 4^{o}C to prevent the degradation of PLA and waited for 5 days for it to dissolve well. Both samples were heated from 10^{o}C to 50^{o}C and photographs taken.

6.3.5. Degradation test

Hydrogel degradation kinetics was investigated in physiological conditions at 37 °C in PBS pH 7.4. Typically, the block copolymer (150 or 200 mg) was dissolved in 1 mL PBS in a 5 mL test tube and set at 37 °C until complete gelation (*i.e.* 15 or 20 wt% copolymer concentration).

6.4. Result and Discussions

6.4.1. Syntheses

The synthesis of the copolymers was based on the strategies of ring opening ROP[236,237], IRA[238] and NMP as shown on scheme 2. PLA-b-P(NIPAAm-co-PEGMA) was prepared in a three-step process starting with ROP (Scheme 25). In the presence of stannous octoate catalyst, 2-hydroxyethyl acrylate (HEA) initiated the ring opening of lactide (LA) to form a PLA-HEA polymer. Next, BlocBuilder alkoxyamine was added onto PLA-HEA through IRA leading to a functionalized macroalkoxyamine initiator, PLA-SG1, with a nearly 100% functionalization yield. Applying a temperature of 100ºC resulted in the decomposition of BlocBuilder MA, thereby breaking the C-O bond and releasing the alkyl moiety which was added to the double bond, followed by recombination of SG1. In the final step, NIPAAm and PEGMA monomers were polymerized from PLA-SG1 through NMP at 120ºC, which led to the formation of PLA-b-P (NIPAAm-co-PEGMA) (scheme 2). PEGMA monomer was synthesized separately through an acrylation process from PEG, Mn=2000 g/mol using methacryloyl chloride. The methacryloyl chloride was distilled to get rid of the dimers[239]. P(NIPAAm-co-PEGMA) as a reference was synthesized through NMP from BlocBuilder as initiator, as described in scheme 26.

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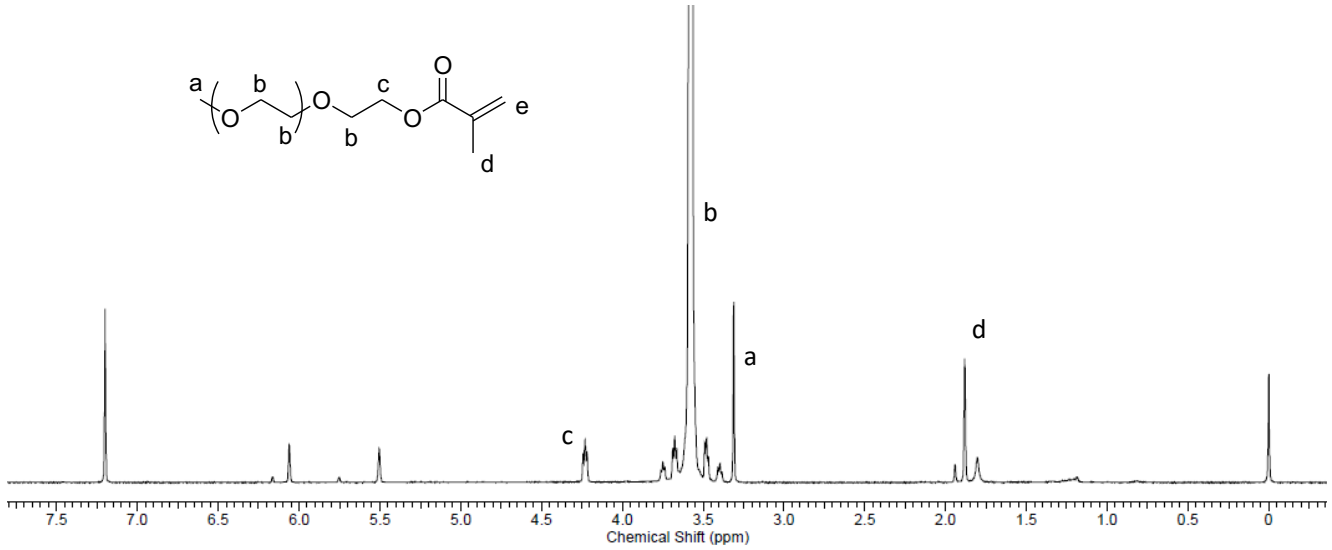
[236] Odile Dechy-Cabaret, Blanca Martin-Vaca, and Didier Bourissou, 'Controlled Ring-Opening Polymerization of Lactide and Glycolide', *Chemical Reviews*, 104.12 (2004), 6147–76.

[237] Anna Carlmark, Emma Larsson, and Eva Malmström, 'Grafting of Cellulose by Ring-Opening Polymerisation – A Review', *European Polymer Journal*, 48.10 (2012), 1646–59.

[238] Pierre-Emmanuel Dufils, 'Intermolecular Radical Addition of Alkoxyamines onto Olefins: An Easy Access to Advanced Macromolecular Architectures Precursors', *Polymer*, 48.18 (2007), 5219–25.

[239] Jonas Warneke, 'Methacryloyl Chloride Dimers: From Structure Elucidation to a Manifold of Chemical Transformations', *Tetrahedron*, 70.37 (2014), 6515–21.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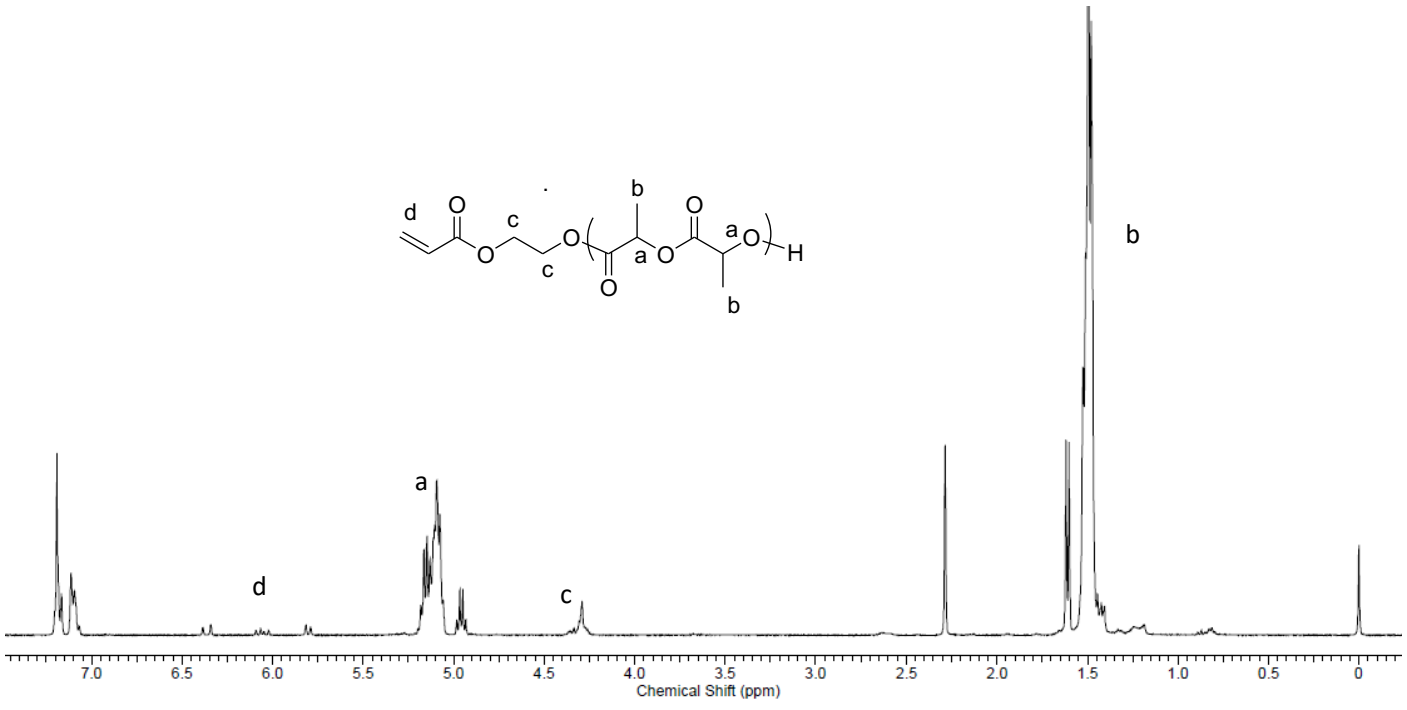
Chemical Shift (ppm)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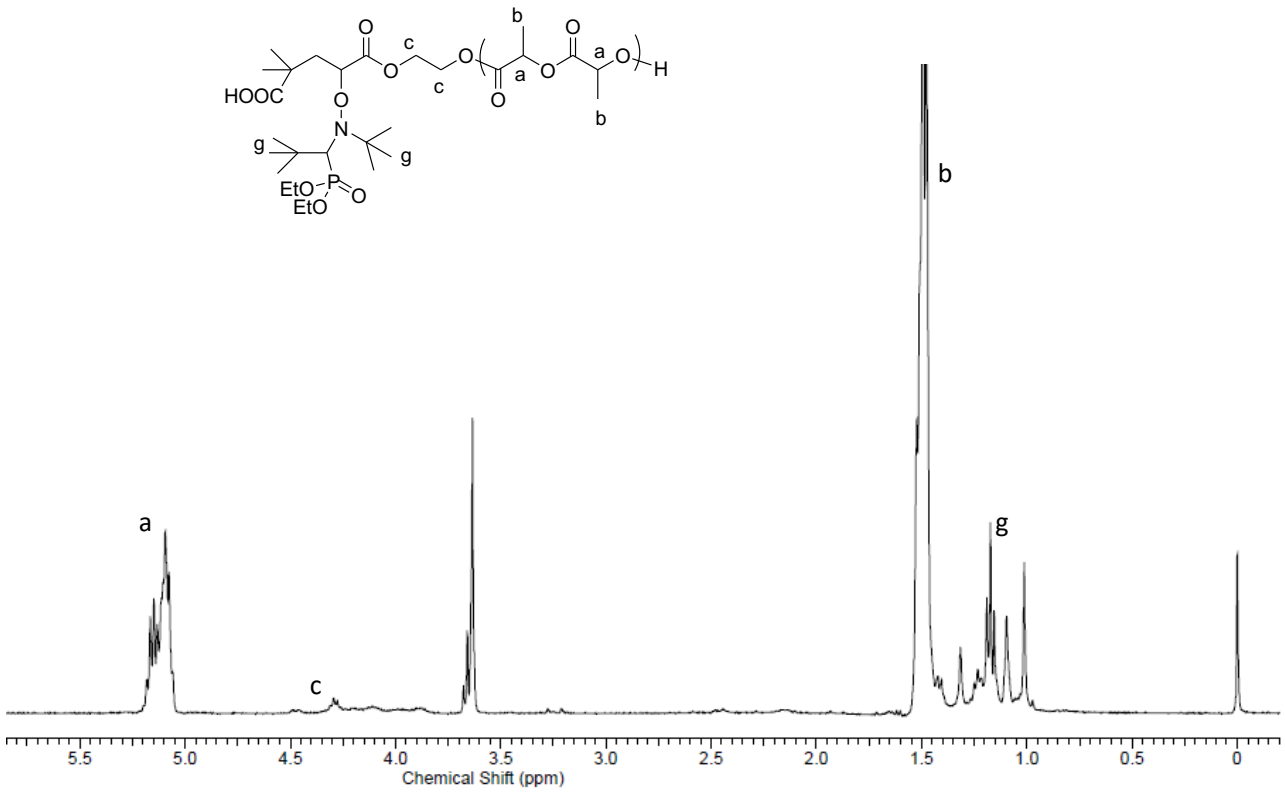
B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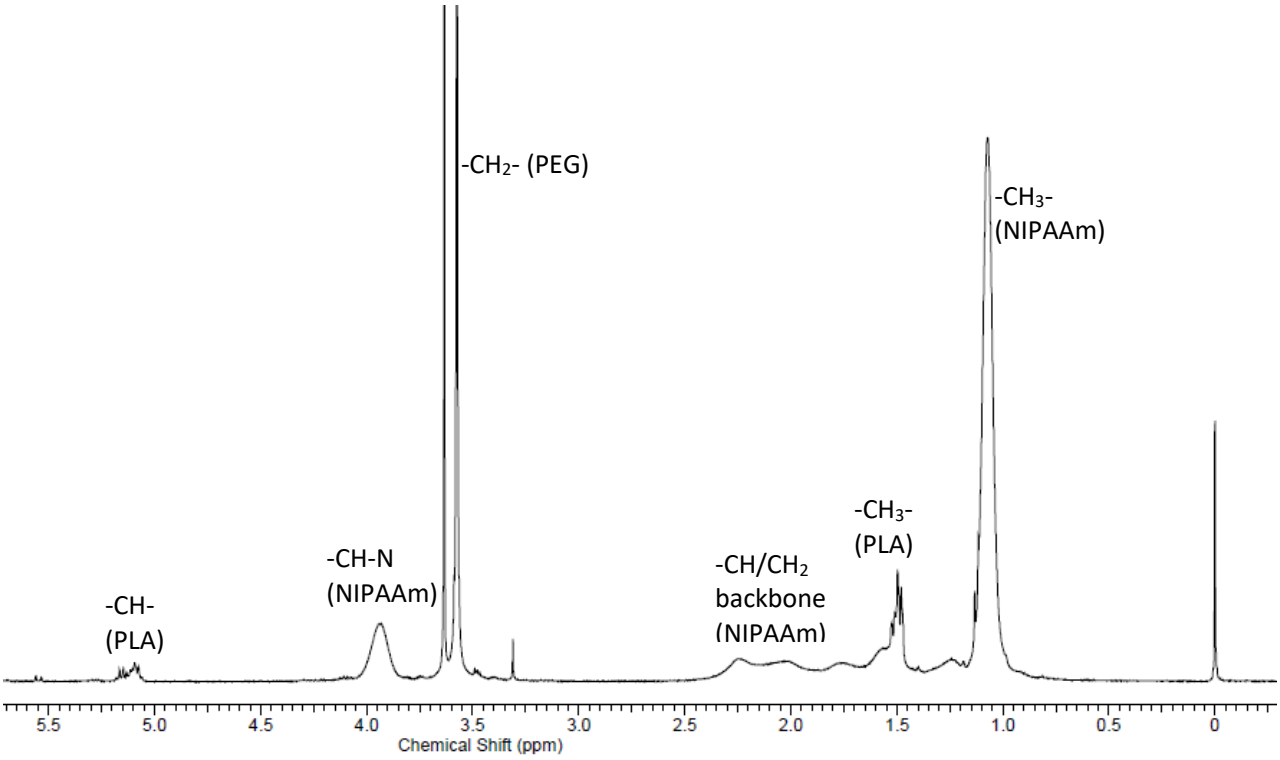
C


-CH-
(PLA)
-CH-N
(NIPAAm)
-CH2- (PEG)
-CH/CH2
backbone
(NIPAAm)
-CH3-
(PLA)
-CH3-
(NIPAAm)
Chemical Shift (ppm)

D

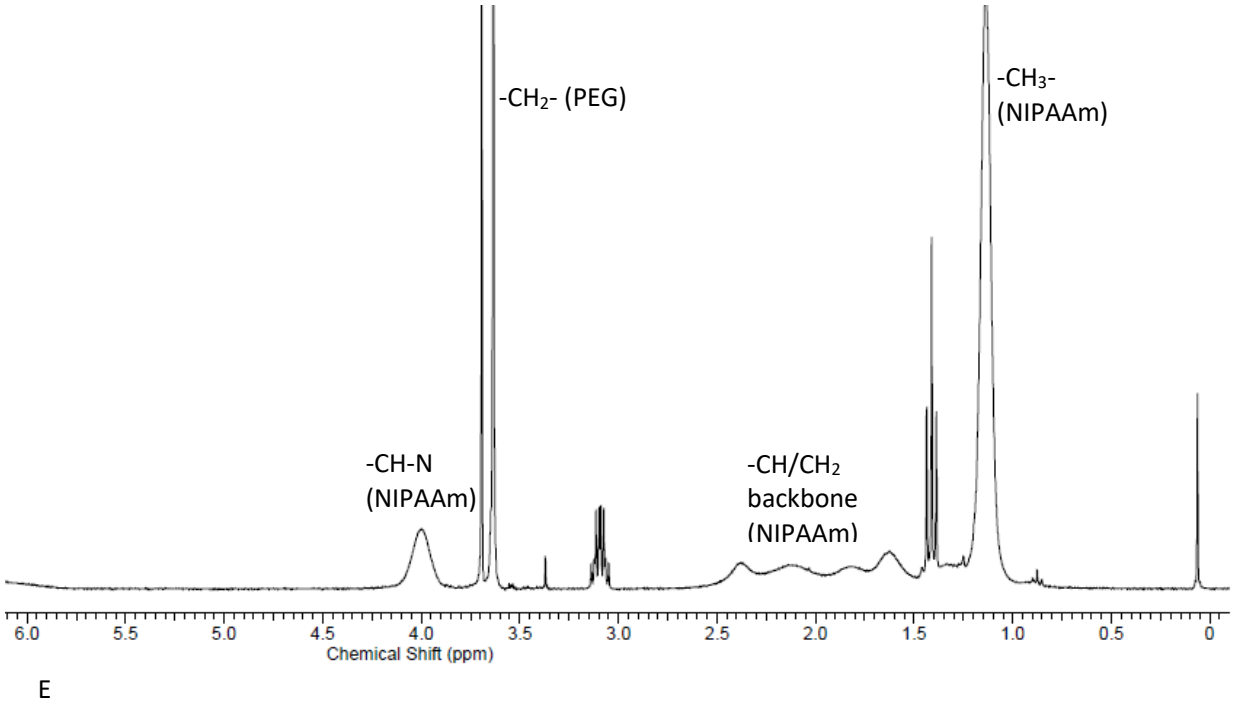
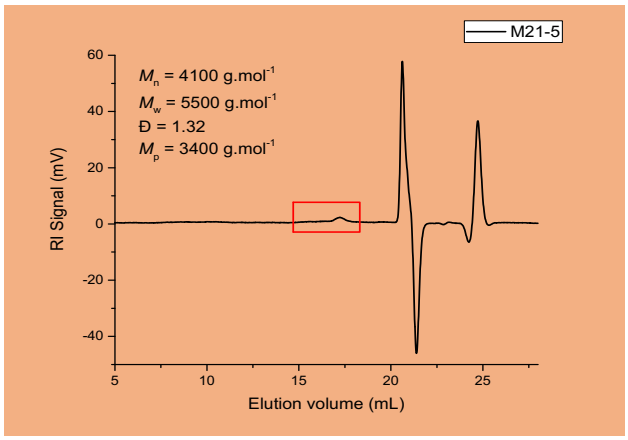


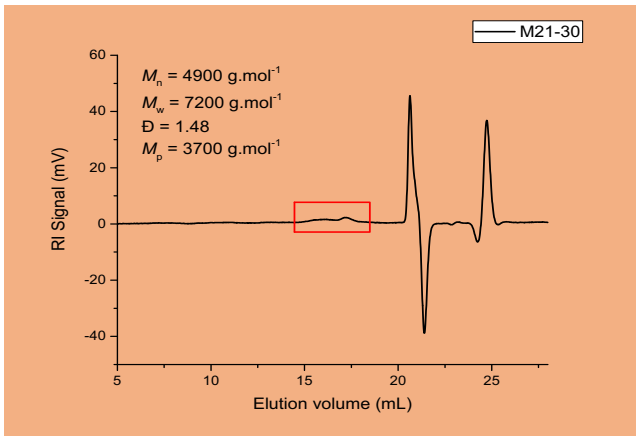
Fig. 18. ^{1}H NMR spectra of PEGMA (A), PLA-HEA (B), PLA-SG1 (C), PLA-b-P(NIPAAm-co-PEGMA) (D), (PEGMA) P(NIPAAm-co-PEGMA) (E).

^{1}H NMR spectra confirms the formation of the respective compounds. In fig. 18, the vinyl functions from methacryloyl chloride are observed at 5.5 and 6.0 ppm and CH_2 protons of PEG at 3.65 ppm (Figure 18a). This confirms the formation of PEGMA monomer, with integration confirming 100% functionalization yield. Fig. 18b shows the vinyl protons from HEA between 5.5 and 6.5 ppm and -CH- of PLA at 5.25, also confirming the formation of PLA-HEA. In Fig. 18c, t-butyl protons are observed around 1.0 ppm showing the incorporation of SG1 onto PLA. The -CH_3- at 1.0 ppm, -CH- at 4.0 in fig. 18d represent PNIPAAm, -CH_2- at 3.65 represents PEGMA meanwhile -CH- at 5.25 represents PLA confirming the formation of PLA-b-P (NIPAAm-co-PEGMA) copolymer. On the other hand, for P(NIPAAm-co-PEGMA), we observed the PNIPAAm and PEGMA peaks but not that of PLA (5.25 ppm) as expected (fig. 5). From the NMR calculations, the total molecular weight of the copolymer is 39058 g/mol of which constitutes 10% PLA (4000 g/mol) while PNIPAAm and PEGMA make up 72% and 18% respectively.

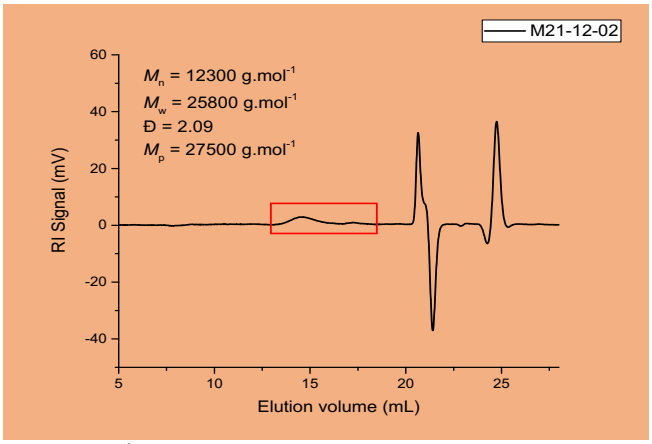
The SEC DMF analyses confirms the formation of the copolymers as early as 5 min from the start of the reaction. We can observe the growth in polymer as the reaction proceeds (fig. 19 and 20). The analyses also show that the NMP process is controlled since the polidespersity index (PDI) increases due to the simultaneous increase in both molecular weight and molecular number of the polymer as the reaction proceeds (fig. 19).



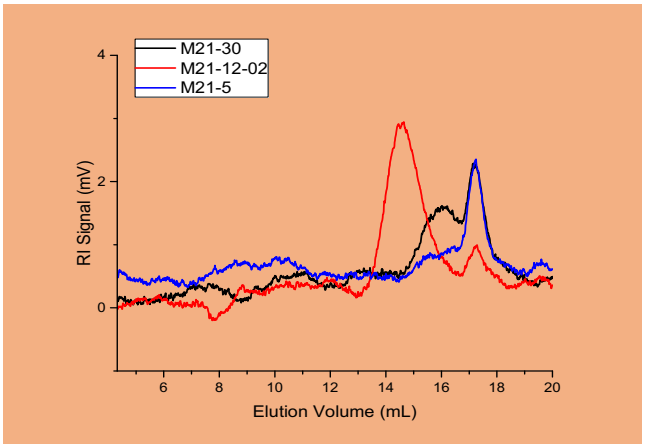
a. 5 min



b. 30 min

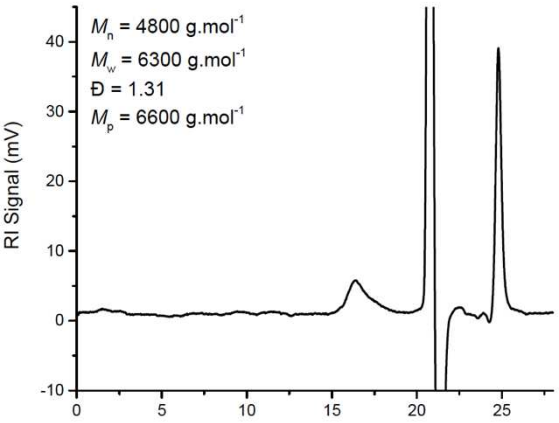


c. 18 h

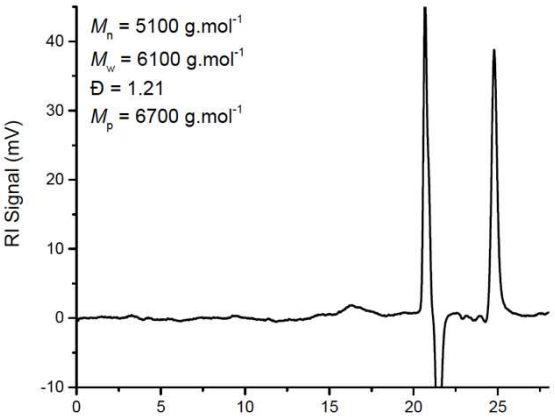


d. 5 min, 30 min and 18 h.

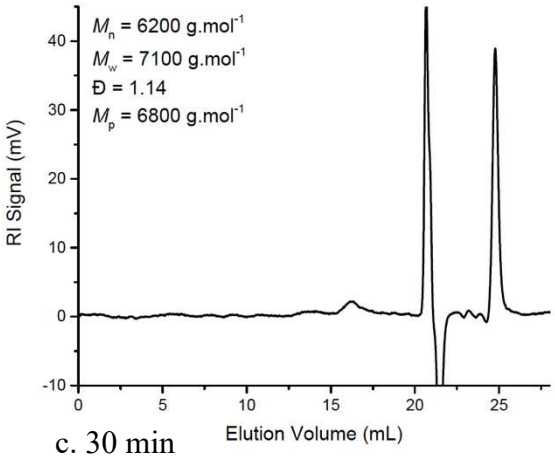
Fig. 19. SEC DMF analyses of the formation of PLA-b-P(NIPAAm-co-PEGMA) through time from 5min(a), 30 min(b), 18 h(c) and a relay of all three samples(d).



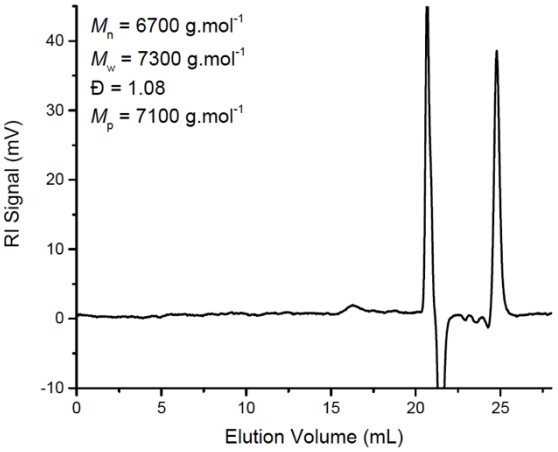
a. 5 min



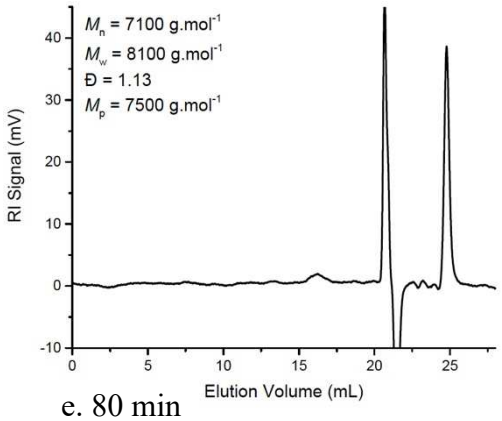
b. 15 min



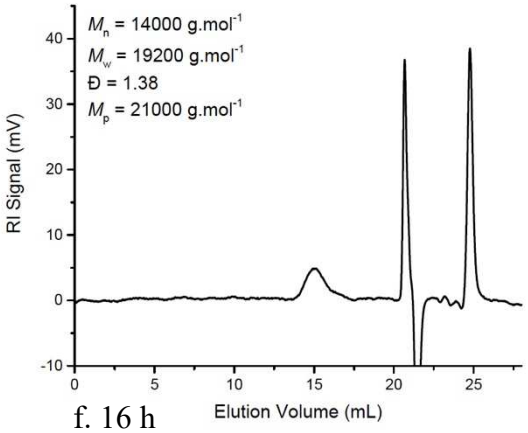
c. 30 min



d. 50 min



e. 80 min



f. 16 h

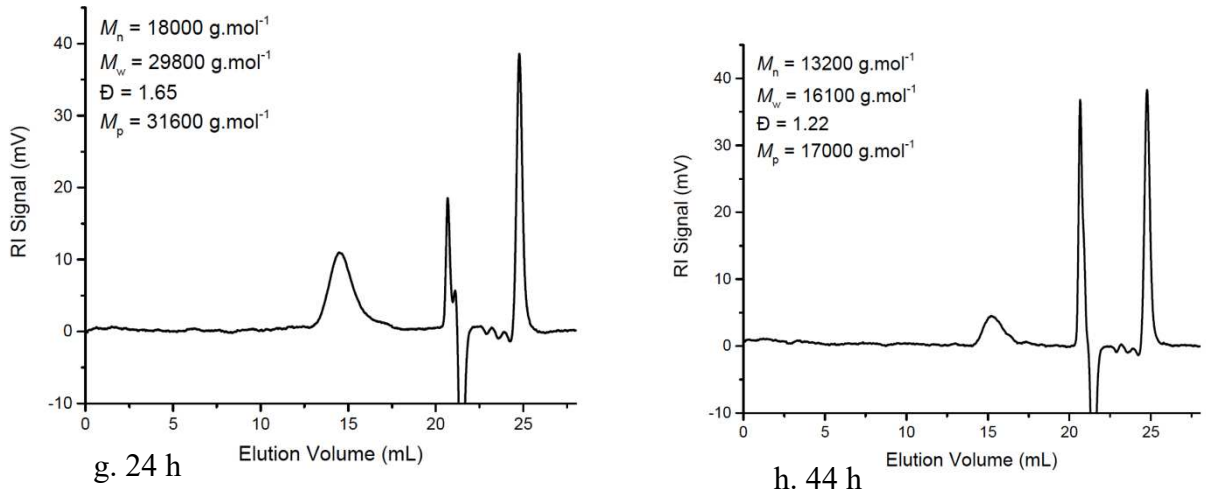


Fig. 20. SEC DMF analyses of the formation of PLA-b-PNIPAAm after 5 min (a), 15 min (b), 30 min (c), 50 min (d), 80 min (e), 16 h (f), 24 h (g) and 44 h (h).

6.4.2. Dynamic light scattering (DLS) analyses

Aqueous solution behavior of copolymer with PLA, (PLA-b-P(NIPAAm-co-PEGMA)) and without PLA, (P(NIPAAm-co-PEGMA)) was investigated by DLS (0.1 w% of copolymer in filtered PBS (i.e. 1 mg/mL)) (Fig 21A). Below transition temperature of PNIPAAm (i.e. when it is water-soluble), the behavior was different for the two copolymers. Indeed P(NIPAAm-co-PEGMA), since entirely water soluble, exhibited size of about 15 nm (representative of individual chains in solution) while PLA-P(NIPAAm-co-PEGMA) polymer self-assembled in micelles of about 80 nm due to its amphiphilic character (PLA hydrophobic segment as core). Upon heating above 30°C, the size of the PLA-P(NIPPAm-co-PEGMA) micelles decreased to about 60 nm, because NIPAAm becomes hydrophobic and tends to move into the core of the micelles. As for P(NIPAAm-co-PEGMA), micelles are formed upon heating above 30°C (after a brief destabilization phase) as the copolymer becomes amphiphilic, due to PNIPAAm becoming hydrophobic.

The existence of micellar state for PLA-b-P(NIPAAm-co-PEGMA) at room temperature was confirmed by hydrophobic dye (cyanine 5.5 carboxyl) solubilization. As shown in Fig 21B, PBS solution of the copolymer (0.1 %) mixed with the dye (3.6 µg/mL) led to solubilization of the dye (through micellar encapsulation) giving to nice blue color, while the dye remained

insoluble in the control P(NIPAAm-co-PEGMA) copolymer (precipitated dye at the bottom of the tube). Together with DLS, this clearly showed that PLA in the copolymer induces amphiphilic character for PLA-b-P(NIPAAm-co-PEGMA) and thus micelle formation.

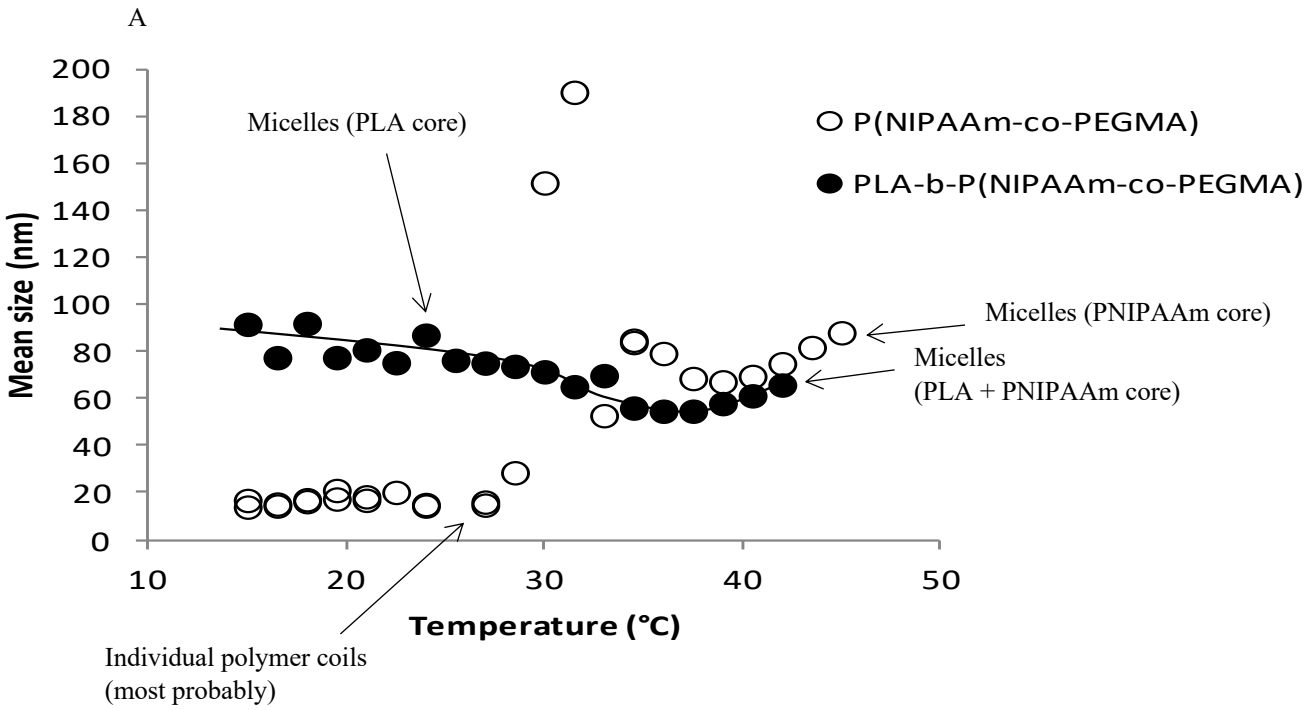


Fig. 21. A: Sol-gel transition of PLA-b-P(NIPAAm-co-PEGMA) and P(NIPAAm-co-PEGMA) copolymers, B: photographs of solutions (0.1% PBS) of PLA-b-P(NIPAAm-co-PEGMA) (right) and P(NIPAAm-co-PEGMA) (left) incubated with cyanine 5.5 carboxyl hydrophobic dye at room temperature.

6.4.3. Sol-gel phase transition analyses

The sol-gel transition of PLA-P(NIPAAm-co-PEGMA) shows the thermoresponsive and thermoreversible nature of the copolymer. We can observe how the turbidity of the copolymer

increases due to increase in temperature from 10°C-50°C (fig 22). The sol-gel transition occurs between 30°C and 37°C due to the explanation above (4.2). Such property is highly suitable for further in vivo injectability as gelation occurs below 37°C (physiological temperature but not below 30°C, meaning that the copolymer solution can be conveniently handled in liquid state at room temperature. The fact the copolymer maintains its gel state without the occurrence of syneresis at 50°C also indicates that the copolymer possesses some significant mechanical properties.

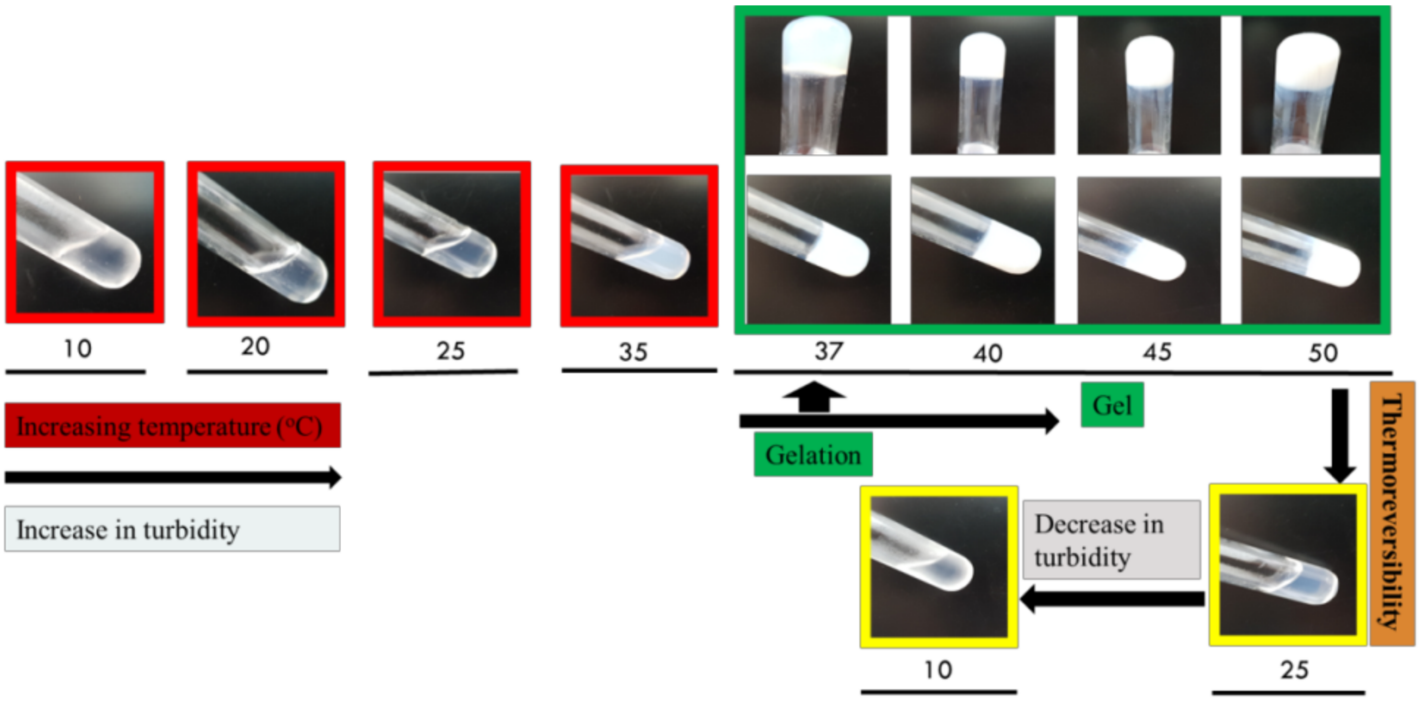


Fig. 22. Phase transition of PLA-b-P(NIPAAm-co-PEGMA) (15wt%) in phosphate buffer saline solution.

Interestingly, in the case of P(NIPAAm-co-PEGMA), the gel formation occurred at higher temperature (45°C, Fig 23). Thus, PLA was responsible for lowering the gelation temperature of PLA-b-P(NIPAAm-co-PEGMA). This lower gelation temperature for PLA-P(NIPAAm-co-PEGMA) can be attributed to the pre-existence of micelles at room temperature. This also shows that P(NIPAAm-co-PEGMA) is not suitable for bio-applications within

physiological conditions because of its high gelation temperature but it can be employed in other applications which do not require physiological conditions.

The copolymers also demonstrated thermoreversibility as the gel turned into a solution again at ambient temperature.

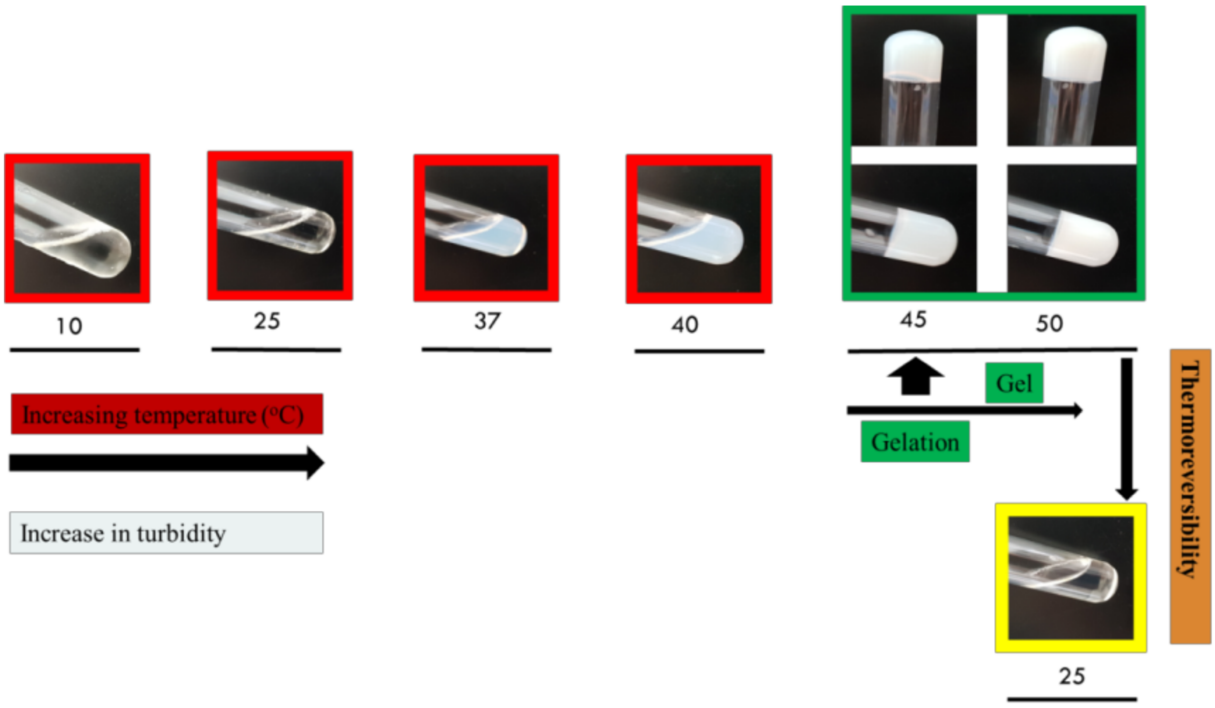


Fig. 23. Phase transition of PNIPAAm-co-PEGMA (15wt%) in phosphate buffer saline solution.

6.4.4. Degradation analysis

Both 15wt% and 20wt% copolymer gel in PBS showed signs of degradation after 4 h. The figure below shows observable changes in the degradation of the copolymer as its time in aqueous media at 37°C increases. Eventually, after 48 h, the copolymer had completely transformed from a gel to a liquid state indicating that the PLA has been partially or completely degraded leading to the disruption of PLA-PNIPAAm based amphiphilic copolymer.

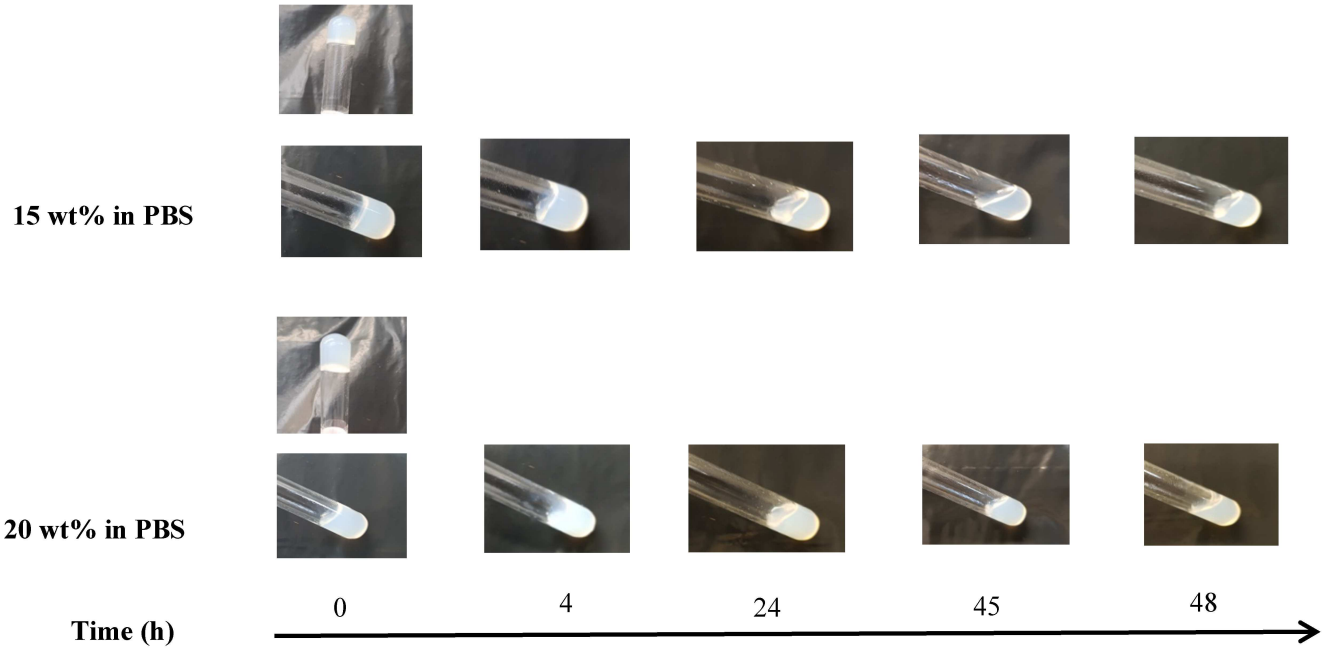


Fig. 24. Degradation of PLA-b-P(NIPAAm-co-PEGMA) at 37°C.

After 48 h in aqueous medium at 37°C, the gelation temperature of the copolymer was increased to 45°C. This strongly supports the degradation of PLA and disruption of the amphiphilic block copolymer, leading to a behaviour close to the P(NIPAAm-co-PEGMA) copolymer.

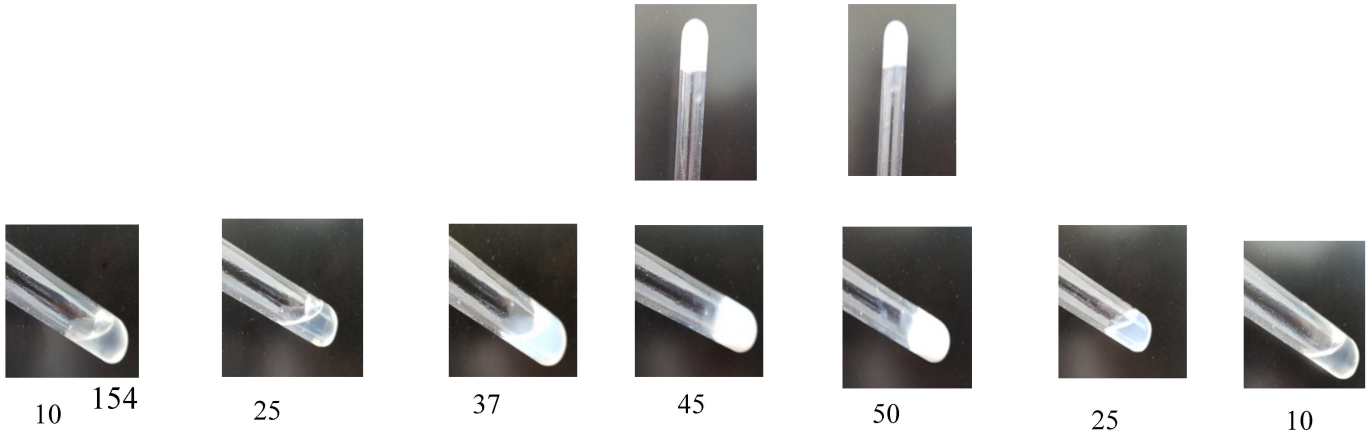


Fig. 25. Phase transition of PLA-b-P(NIPAAm-co-PEGMA)- 15 wt% in PBS after the degradation of PLA (48 h) from 10°C.

The cleavage of PLA (fig. 26) in the copolymer was confirmed by ^{1}H NMR and showed to occur by hydrolysis. The PLA breaks down to oligomers of oligolactide with COOH and OH end-groups (d and e protons) while releasing water. Since the copolymer has a block-graft structure of PLA-b-P(NIPAAm-co-PEGMA), it is my hypothesis that the breakdown of the hydrophobic PLA will cause the grafted PNIPPAm-co-PEGMA to become hydrophilic because of the hydrophilic nature of the PEGMA. This will enable the whole graft polymer to go into solution making it easy for renal excretion, thereby preventing PNIPAAm from remaining in the body system and poisoning some organs after application of the hydrogel. Nonetheless, the fast degradation of the hydrogel might be a drawback to its application in the treatment of ischemic stroke. This might be so, because the loaded drug on the hydrogel may require more than 48 h to properly treat the stroke. Using slower degrading monomers than lactide can be a perspective to slow down the degradation process.

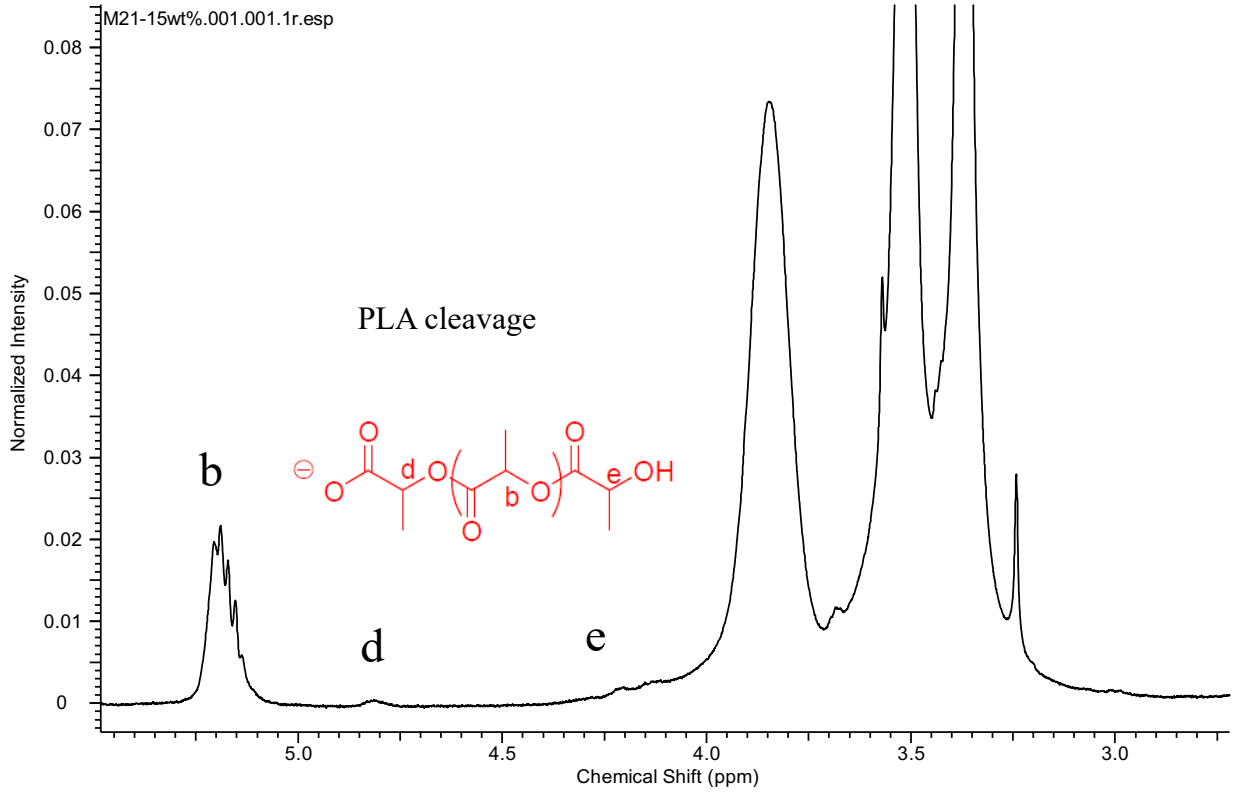


Fig. 26. ^{1}H NMR analysis of degraded hydrogel after drying.

6.5. Conclusions

In conclusion, a block-graft polymer, PLA-b-P(NIPAAm-co-PEGMA) was successfully synthesized using ROP, IRA and NMP polymerization techniques. The copolymer was degraded by hydrolysis and is most probably biodegradable since PLA is known to be biodegradable. The copolymer hydrogel is also thermosensitive and undergoes a sol-gel transition between 20°C and 37°C which is very useful within physiological conditions. This work has been presented as a poster at EuCHeMS conference on Organic Free Radicals (ECOFR 2018) June 17-20, 2018 - Marseille (France): **Nitroxide Mediated Polymerization and Addition for Synthesis of Novel PNIPAAm-based Biodegradable Copolymers** and also at XI Congresso Nazionale AICIng, Università di Bologna- Complesso di San Giovanni in

Monte, 9-12 Settembre 2018: **Sintesi di nuovi Copolmeri Biodegradabili a base Acrilica per mezzo della Polimerizzazione Radicalica Mediata da Nitrossidi (NMP).** There is a very high probability that the copolymer is biocompatible because its components have been used to synthesize other biocompatible polymers in the past. With all the above-mentioned properties, the biomaterial can be employed in drug delivery and tissue engineering in general and for the treatment and recovery of ischemic stroke. However, further research is necessary to examine and confirm its applications. The fact that, the hydrogel of the polymer does not break and possesses good water retentive properties even at 50°C confirms its good mechanical strength. The properties can be tuned to suit other applications such as cleaning of paintings and artworks and also in the treatment of concrete.

Polymer hydrogels of this nature should not only be applied in biomedical engineering, but applications in other fields cultural heritage, building & construction and architecture could be exploited. My future research comprises of testing the mechanical properties and the biocompatibility of the hydrogel and perform a clinical assay of its application in the treatment of ischemic stroke in order to complete the project started at Aix-Marseille Université. I would like to further exploit the application the hydrogel in cultural heritage and building & construction.