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ENZYMATIC SYNTHESIS OF A DIENE ESTER MONOMER FROM 10-UNDECENOIC ACID AND ITS APPLICATION IN THIOL-ENE POLYMERIZATION

Tese submetida ao Programa de Pós-Graduação em Engenharia Química da Universidade Federal de Santa Catarina.

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Florianópolis
2018

Ficha de identificação da obra elaborada pelo autor através do Programa de Geração Automática da Biblioteca Universitária da UFSC.

Romera, Cristian de Oliveira

ENZYMATIC SYNTHESIS OF A DIENE ESTER MONOMER FROM 10-UNDECENOIC ACID AND ITS APPLICATION IN THIOL-ENE POLYMERIZATION / Cristian de Oliveira Romera ; Orientador, Pedro Henrique Hermes de Araújo ; coorientadora, Claudia Sayer.

135 p.

Tese (doutorado) - Universidade Federal de Santa Catarina, Centro Tecnológico, Programa de Pós-Graduação em Engenharia Química, Florianópolis, 2018.

Inclui referências.

1. Engenharia Química. 2. Síntese enzimática. 3. ácido 10-undecenoico. 4. polimerização tiol-eno. 5. adição de Michael. I. De Araujo, Pedro Henrique II. Sayer, Claudia. III. Universidade Federal de Santa Catarina. Programa de Pós-Graduação em Química. IV. Titulo.

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Esta Tese de Doutorado foi julgada adequada para obtenção do Título de Doutor em Engenharia Química, área de concentração em Desenvolvimento de Processos Químicos e Biotecnológicos, e aprovada em sua forma final pelo Programa de Pós-Graduação em Engenharia Química.

Florianópolis, 31 de julho de 2018.

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Este trabalho é dedicado aos meus queridos pais.

AGRADECIMENTOS

Primeiramente agradeço aos meus pais, Nilson e Laura, por todo apoio emocional, pela dedicação e preocupação com a minha formação tanto moral quanto profissional, por sempre confiarem no meu senso de responsabilidade e por serem meus suportes.

À minha namorada, Caroline, por todo carinho, amor e paciência ao me apoiar em todas as etapas do presente trabalho durante esses 4 anos, sendo parceira em me acompanhar no laboratório em fins de semana e em horários alternativos, sempre me dando muita força, principalmente nos muitos momentos desafiadores do estudo.

Aos meus orientadores, Prof. Pedro e Profª. Claudia, pela confiança em mim depositada, sempre demonstrando amizade e compreensão, além da orientação e suporte constantes ao longo deste trabalho.

Aos membros da banca tanto de Qualificação quanto de Doutorado, Prof. Bruno, Prof.ª Débora, Dr.ª Priscilla e Prof. Adriano pelas contribuições e por aceitarem o convite para avaliar esse trabalho.

Aos colegas do Laboratório de Controle e Processos de Polimerização (LCP), pela amizade e companheirismo. Em especial aos integrantes do grupo de Miniemulsão pela troca de experiências e discussões científicas produtivas, ajudando direta ou indiretamente no presente trabalho.

A todos os meus amigos pelo carinho e preocupação que sempre tiveram.

Aos doutorandos Thiago Ouriques e Juliana Zanatta pela disponibilidade em fazer as inestimáveis análises de ^{1}H NMR, além da Central de Análises da Química por oferecer o equipamento e as instalações.

À doutoranda Alessandra Meneses pela disponibilidade em realizar as análises de TEM, e ao Laboratório Central de Microscopia Eletrônica (LCME - UFSC) por disponibilizar o espaço e os equipamentos.

Ao Leandro da Central de Análises do Departamento de Engenharia Química e Engenharia de Alimentos (UFSC) pela disponibilidade em realizar as análises de DSC.

Ao Programa de Pós-Graduação em Engenharia Química da UFSC pela oportunidade de estar em um curso de excelência.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelo apoio financeiro que permitiu o aperfeiçoamento de mais um pesquisador.

"A book is made from a tree. It is an assemblage of flat, flexible parts (still called 'leaves') imprinted with dark pigmented squiggles. One glance at it and you hear the voice of another person — perhaps someone dead for thousands of years. Across the millennia, the author is speaking, clearly and silently, inside your head, directly to you. Writing is perhaps the greatest of human inventions, binding together people, citizens of distant epochs, who never knew one another. Books break the shackles of time — proof that humans can work magic."

— Carl Sagan

ABSTRACT

Vegetable oils are increasingly in evidence as these renewable and versatile materials can replace petroleum derivatives for the synthesis of new compounds. Castor oil is mainly composed of ricinoleic acid, its structure is composed of an 18-carbon chain containing a double bond between carbons 9 and 10 and a hydroxyl group attached to carbon 12. This unique structure allows the production of derivatives as the 10-undecenoic acid, which presents terminal unsaturation, whose structure allows that through esterification reactions, this compound becomes a building block for monomers with renewable characteristics. In order to better preserve the double bonds, techniques using mild esterification conditions are quite attractive, highlighting Steglich and enzymatic (through lipases as biocatalysts) esterification for the construction of dienes in order to synthesize monomers for polymerization and cross-linkers. By these techniques it was possible to synthesize a diene ester derived from 10-undecenoic acid and 2-hydroxyethyl methacrylate with conversions about 90% and high purity in the presence of 1 wt% of Novozym 435 lipase at 50 °C in solvent-free system, maintaining these conversions even after 10 cycles. This ester was used as a monomer for thiol-ene polymerizations in bulk and miniemulsion (particle diameters ranged from 150 to 240 nm), reaching molar weights of up to 8.8 and 9.1 kDa, respectively. The surfactants chosen for the stabilization of the miniemulsions were sodium dodecyl sulfate and Lutensol AT50. Another option was to use this material as a cross-linker in free-radical polymerization, obtaining polymers with a gel content of about 70%. Due to its asymmetry, this ester can be reacted with a diamine, such as hexamethylenediamine, in order to use the selectivity of this reaction with the double bond originating from the methacrylate for the construction of a new monomer, this time symmetrical, with conversion of 80%. The thiol-ene polymerization in bulk of this compound resulted in the molar weight of 11 kDa, so that this polymer retains the functionality of the amine and thiol bonds.

Keywords: Enzymatic synthesis; 10-undecenoic acid; thiol-ene polymerization; miniemulsion; Michael addition.

RESUMO EXPANDIDO

Os óleos vegetais estão cada vez mais em evidência já que esses materiais renováveis e versáteis podem substituir derivados de petróleo para a síntese de novos compostos. Dentre esses óleos, o óleo de mamona é constituído principalmente por ácido ricinoleico, cuja estrutura singular é composta por uma cadeia de 18 carbonos, contendo uma ligação dupla entre os carbonos 9 e 10 e um grupo hidroxila ligado ao carbono 12. Essa estrutura única permite a obtenção de derivados como o ácido 10-undecenoico, que apresenta insaturação terminal, cuja estrutura permite que através de reações de esterificação, esse composto seja um bloco de construção para monômeros com características renováveis. Dentre as técnicas de síntese utilizadas atualmente, a aplicação da biotecnologia, com ênfase na utilização de enzimas como biocatalisadores de esterificação e transesterificação, tem um papel fundamental uma vez que dá origem a processos mais ambientalmente amigáveis. Em relação à utilização desses monômeros, a polimerização via tiol-eno em miniemulsão possui apelo ambiental uma vez que substitui a implementação de solventes orgânicos por sistemas aquosos de dispersão, propiciando também a obtenção de nanopartículas ou nanocápsulas de poli(tioéter-ésteres). Esses materiais são passíveis de sofrer hidrólise através das ligações éster, então podem apresentar caráter de biodegradabilidade em determinados sistemas. Com o objetivo de melhor preservar as duplas ligações, técnicas usando condições amenas de esterificação são bastante atrativas, destacando-se a esterificação de Steglich e a enzimática (através de lipases como biocatalisadores) para a construção de dienos com o objetivo de sintetizar monômeros para polimerização e agentes de reticulação. Através dessas técnicas foi possível observar que somente o éster diênico derivado do ácido 10-undecenoico e do metacrilato de 2-hidroxietila foi passível de ser obtido pelas duas técnicas, destacando-se a técnica de esterificação enzimática, que embora não tenha sido eficiente em converter o ácido sórbico em éster, permitiu a obtenção do derivado do ácido 10-undecenoico com conversões próximas a 90% na presença de somente 1% em massa da lipase Novozym 435 à 50 °C em sistema livre de solvente, mantendo essas conversões mesmo após 10 ciclos. Essa metodologia propiciou uma melhor purificação do monômero obtido, uma vez que apresentou conversões maiores e menor incidência de subprodutos, ao contrário da técnica de esterificação de Steglich, impactando positivamente nas posteriores aplicações desse material em reações de polimerização. Desse modo, o éster foi utilizado como monômero para polimerizações via tiol-eno em massa e miniemul-

são (os diâmetros de partícula variaram de 150 a 240 nm), atingindo massas molares de até 13.6 e 18.0 kDa, respectivamente, utilizando a mesma relação para o desbalanceamento estequiométrico entre monômero e ditiol de 1,0:0,9, não observando a presença de reações duplas residuais após essas reações, mesmo considerando que esse monômero continha diferentes ligações duplas, o que por sua vez origina diferente reatividade para cada uma delas. Os surfactantes escolhidos para a estabilização das miniemulsões foram o dodecil sulfato de sódio e o Lutensol AT50 com o objetivo de testar dois mecanismos diferentes de estabilização coloidal, a cobertura eletrostática e a proteção estérica, respectivamente. Esse monômero também foi utilizado como agente de reticulação em polimerização via radicais livres, obtendo polímeros com teor de gel de aproximadamente 70% em massa, e as partículas obtidas por meio dessa técnica se mostraram mais rígidas e estáveis quando analisadas por meio de microscopia de transmissão eletrônica, não observando degradação pela incidência do laser. Devido à sua assimetria e presença do grupo metacrilato, esse éster teve potencial para reagir com uma diamina, como a hexametilenodiamina, com o objetivo de usar a seletividade dessa reação com a dupla ligação originária do metacrilato para a construção de um novo monômero, dessa vez simétrico, com conversão de 80%. A polimerização em massa via tiol-eno desse composto resultou em massa molar de 11 kDa em estequiometria de 1,0:1,0 em relação à monômero:ditiol, de modo que esse polímero deteve a funcionalidade das ligações amina e tiol, além da presença do grupo éster já observado nas reações de síntese enzimática, o que conferiu a ele novas propriedades termoquímicas. Os resultados mostram o potencial desse monômero para a sua aplicação em diferentes reações químicas, incluindo a polimerização direta e reações de síntese adicionais utilizando sua estrutura assimétrica, além disso, o trabalho permitiu a aplicação de vários conceitos de química verde em todas as etapas do estudo.

Palavras-chave: Síntese enzimática; ácido 10-undecenoico; polimerização tiol-eno; miniemulsão; adição de Michael.

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LIST OF ABBREVIATIONS AND ACRONYMS

^{1}H NMR	Hydrogen Nuclear Magnetic Resonance
1,4-BDT	1,4-Butanedithiol
AIBN	α,α′-Azoisobutyronitrile
CALB	*Candida antarctica* lipase B
$CDCl_3$	Deuterated chloroform
DAG	Diacylglycerol
DCC	N,N'-dicyclohexylcarbodiimide
DCU	N,N'-dicyclohexylurea
DLS	Dynamic Light Scattering
DMAP	4-dimethylaminopyridine
DSC	Differential Scanning Calorimeter
GPC	Gel Permeation Chromatography
HEMA	2-hydroxyethyl methacrylate
HMD	Hexamethylenediamine
HPLC	High Pressure Liquid Chromatography
Lut50	Lutensol AT50
MAG	Monoacylglycerol
MHS	Monomer from HEMA and sorbic acid
MHU	Monomer from HEMA and 10-undecenoic acid
MHU-HMD	Monomer from Michael addition of MHU and HMD
PDI	Polydispersity index (for DLS)
PHU-HMD	Polymer from thiol-ene reaction of MHU-HMD and 1,4-BDT
SDS	Sodium Dodecyl Sulfate
TEM	Transmission electron microscopy
THF	Tetrahydrofuran

LIST OF SYMBOLS

[C]	Concentration ($g \cdot mL^{-1}$)
Ap_n	Area of peak n in HPLC analysis ($mV \cdot min$)
C	Conversion (%)
Dp_0	Droplet diameter (nm)
Dp	Particle diameter (nm)
dn/dc	Refractive increment ($mL \cdot g^{-1}$)
Đ	Polydispersity index (M_w/M_n)
I	Initiator
K	Optical contrast factor ($cm^2 \cdot mol^{-1} \cdot g^{-2}$)
k_d	Rate coefficient of initiator decomposition (s^{-1})
k_i	Rate coefficient of initiation ($cm^3 \cdot mol^{-1} \cdot s^{-1}$)
k_p	Propagation rate coefficient ($cm^3 \cdot mol^{-1} \cdot s^{-1}$)
k_{tc}	Rate coefficient of termination by combination ($cm^3 \cdot mol^{-1} \cdot s^{-1}$)
k_{td}	Rate coefficient of termination by disproportionation ($cm^3 \cdot mol^{-1} \cdot s^{-1}$)
k_{tm}	Rate coefficient for chain transfer to monomer ($cm^3 \cdot mol^{-1} \cdot s^{-1}$)
k_{tp}	Rate coefficient for chain transfer to polymer ($cm^3 \cdot mol^{-1} \cdot s^{-1}$)
M_n	Number average molar weight (kDa)
mol%	Mol-mol percentage
M_w	Weight average molar weight (kDa)
MW_{MHU}	Molar weight of MHU ($g \cdot mol^{-1}$)
MW_{UA}	Molar weight of 10-undecenoic acid ($g \cdot mol^{-1}$)
P_1•	Primary radical
P_n•	Active polymer chain
PDI	Polydispersity index of DLS
R	Rayleigh ratio (cm^{-1})
R•	Active radical
T	Temperature (ºC)
T_m	Melting temperature (°C)
V	Volume (mL)
$V_{dilution}$	Volume used in dilution (mL)
w_{HEMA}	Weight of HEMA (g)
w_{MHU}	Weight of MHU (g)
w_{sample}	Weight of sample (g)
wt%	Weight-weight percentage
w_{UA}	Weight of 10-undecenoic acid (g)

x_{MHU}	Weight fraction of MHU
Λ_n	Inactive polymer species of chain length n
δ	Chemical shift (ppm)

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CHAPTER I

1 INTRODUCTION

The substitution of petroleum derivatives for renewable materials has been the focus of several lines of research in order to achieve a more sustainable development, as result of the encouragement of the use of natural resources and biotechnology due to Agenda 21 of Rio 92 (EISSEN *et al.*, 2002 ; MEIER; METZGER; SCHUBERT, 2007).

Vegetable oils are outstanding as raw materials due to their ability to generate new monomers and polymers even though after few modification reactions, presenting high availability and industrial viability, showing importance for the development of new materials (ESPINOSA; MEIER, 2011). Thus, triglycerides have been used in the production of different polymers such as polyesters, polyurethanes, polyamides, polyesteramides, acrylic and epoxy resins, being versatile precursors (GÜNER, YAĞCI & ERCIYES, 2006).

Among these plant oils, castor oil has been used almost exclusively in industrial and pharmacological applications, mainly due to the high concentration of ricinoleic acid in its composition (90%), making it the only source of hydroxylated fatty acid (SEVERINO *et al.*, 2012). This property causes this oil to be a natural polyol, conferring resistance to oxidation (PATEL *et al.*, 2016). However, the great advantage of this characteristic is the ability to obtain several unsaturated compounds through chemical modifications, such as 10-undecenoic acid, methyl-10-undecenoate, 10-undecenol and 10-undecenoyl chloride (ESPINOSA; MEIER, 2011).

From the Brazilian socio-economic point of view, castor bean is produced throughout the national territory, but the semi-arid region of the Northeastern Region has an advantage, since the cost of production is lower since this plant has resistance to drought and presents ease of management, which could enable the income generation through family farming in this particular region (CUNHA; ROCHA; OLIVEIRA, 2017).

In terms of biotechnology, enzymes, mainly lipases, are being used as biocatalysts in various chemical modification processes, with advantages such as reduction in the use of hazardous compounds, reduction in the generation of by-products and application in mild temperature conditions, making products easier to purify and reducing energy expenditure during synthesis (BIERMANN *et al.*, 2011; VOULGARIS *et al.*, 2015).

The synthesis of dienes based on 10-undecenoic acid has been performed through different chemical pathways (WARWEL *et al.*, 2001;

FOKOU; MEIER, 2010; TÜRÜNÇ; MEIER, 2011; CARDOSO *et al.*, 2014; MACHADO *et al.*, 2017; CARDOSO *et al.*, 2018). In contrast, some studies have reported the enzymatic synthesis of unsaturated compounds acting as monomers in free-radical polymerizations (PATEL; RITTER, 1991; GEYER *et al.*, 1995; KITAGAWA; TOKIWA, 1998; KITAGAWA *et al.*, 2000; ZHANG *et al.*, 2002; KLOOSTERMAN *et al.*, 2014; QIN *et al.*, 2014). However, the enzyme catalyzed synthesis of a diene ester monomer was never performed before.

Terminal dienes are important building blocks in a wide list of polymerization reactions including metathesis using rutenium-based catalyst (FOKOU; MEIER, 2010; TÜRÜNÇ; MEIER, 2011; CARDOSO *et al.*, 2014), thiol-ene with different dithiols (LOBRY *et al.*, 2014; JASINSKI *et al.*, 2014; AMATO *et al.*, 2015; AMATO *et al.*, 2016; MACHADO *et al.*, 2017; MENESES *et al.*, 2017; CARDOSO *et al.*, 2018) and Michael addition applying various diamines (MOSZNER; MÖLKEL; RHEINBERG, 1996; KLEE *et al.*, 1999; MÜH *et al.*, 2001; MATHIAS *et al.*, 2004; WEICKMANN *et al.*, 2010; BILGICI *et al.*, 2011; GONZÁLEZ *et al.*, 2015; NAVARRO *et al.*, 2017). These reactions depend on functionalized groups near double bonds, which affects their reactivity and selectivity. In addition, the latter two reactions, besides providing the polymerization of the dienes, are responsible for giving new properties to the polymer chains, since they insert the heteroatoms of sulfur and nitrogen, respectively. Another interesting function of diene molecule is its application as cross-linker agent in copolymerization reactions, which is similar compared to step polymerization with tri or tetra functional reagents (SU, 2013).

In terms of polymerization processes, polymer nanoparticles derived from monomers with very low solubility in water have been obtained by miniemulsion polymerization, usually via free-radical mechanisms, where monomer nanodroplets act as nanoreactors, being the locus of reaction, while the continuous aqueous media acts to improve heat transfer and to replace organic solvents as continuous medium (ANTONIETTI & LANDFESTER, 2002; ASUA, 2002; SCHORK *et al.*, 2005).

Therefore, this study aims to utilize enzymatic esterification process to produce a new diene ester monomer from 10-undecenoic acid and 2-hydroxyethyl methacrylate. Afterwards, the diene ester monomer is capable to be polymerized by thiol-ene reaction in order to obtain a new linear poly(thioether-ester) or acting as a cross-linker agent by free-radical polymerization. In order to avoid the use of any organic solvent, the polymerization reactions are supposed to be carried out in bulk and aqueous miniemulsion. This work has as a concern to use the philosophy of

green chemistry in all possible steps, including the use of a renewable raw material and environmentally friendly synthesis and polymerization procedures. Furthermore, there is an additional challenge in this polymerization reaction since this molecule is asymmetrical, presenting two types of double bonds (from olefin and methacrylate), which grants different reactivities.

1.1 OBJECTIVES

The objective of this research is to develop a green synthesis process to produce a renewable and asymmetrical diene ester monomer from the enzymatic esterification between 10-undecenoic acid and 2-hydroxyethyl methacrylate and its posterior application in thiol-ene polymerization and as a cross-linker agent in free-radical polymerization in miniemulsion and bulk systems.

The following items are specific objectives of this study for each result chapter:

CHAPTER III

- Synthesis of unsaturated esters through Steglich esterification of sorbic acid and 10-undecenoic acid with 2-hydroxyethyl methacrylate using N,N'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as catalyst;
- Synthesis of unsaturated esters through esterification of sorbic acid and 10-undecenoic acid with 2-hydroxyethyl methacrylate using Novozym 435 as biocatalyst;
- Evaluation of the amount of solvent and enzyme on the kinetics of enzymatic esterification reactions;
- Evaluation of immobilized enzymes Novozym 435 and NS 88011 reuse considering 10 cycles using optimal conditions previously determined;
- Scale-up of enzymatic esterification.

CHAPTER IV

- Thiol-ene polymerization of the diene ester monomer in bulk and miniemulsion and evaluation of the effect of the amount of initiator, reaction temperature and thiol:ene molar ratio;
- Free-radical polymerization of the diene ester monomer in bulk and miniemulsion;

- Modification of asymmetrical monomer through Michael addition to build an amino-thioether-ester symmetrical terminal diene monomer;
- Thiol-ene polymerization of the amino-thioether-ester symmetrical terminal diene monomer in bulk;
- Characterization of polymers in terms of structure by gel permeation chromatography, high performance liquid chromatography, gel content and ^{1}H nuclear magnetic resonance, morphology of the nanoparticles by dynamic light scattering and transmission electron microscopy and polymer thermal properties by differential scanning calorimetry.

CHAPTER II

2 LITERATURE REVIEW

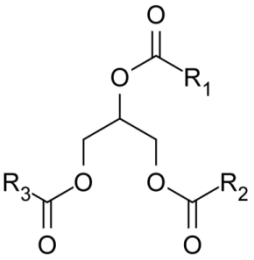
This chapter presents a review about the themes discussed in this thesis. Firstly, the natural and synthetic raw materials are introduced as potential precursors in esterification reactions in order to apply in production of new monomers. Then, Steglich and enzymatic esterification techniques are presented as viable methodologies. Thiol-ene, free-radical and Michael addition reactions are presented as polymerization techniques, describing conditions and applications. Lastly, miniemulsion polymerization media is addressed with its main technical features.

2.1 RENEWABLE RAW MATERIALS

2.1.1 Plant Oils

Plant oil is an expression to designate a triglyceride, i.e., a glycerol triester of long chain fatty acids, which is an organic fraction of plants, insoluble in water (hydrophobic) and liquid at room temperature. (GÜNER; YAĞCI; ERCIYES, 2006; MEIER; METZGER; SCHUBERT, 2007). Figure 2.1 presents a generic structure of a triglyceride.

Figure 2.1: Structure of a generic triglyceride, which R_1, R_2 and R_3 are carbon chains of fatty acids.

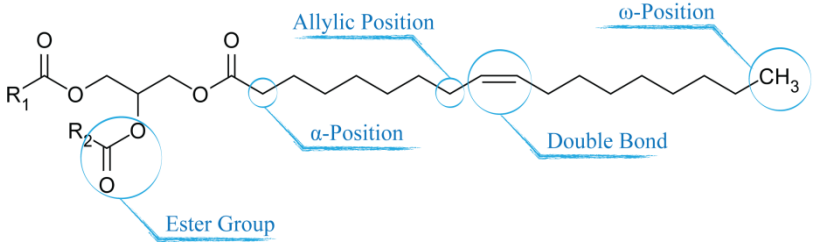


Source: Author.

Fatty acids represent about 95% of plant oils and they are responsible to confer different chemical and physical properties of such oils, which are affected by the presence of double bonds, degree of unsaturation and the length of the fatty acid chains (GÜNER; YAĞCI; ERCIYES, 2006; MEIER; METZGER; SCHUBERT, 2007). Table 2.1 presents fatty

acid composition of some plant oils, while Figure 2.2 shows active sites that can be found in common triglycerides.

Figure 2.2: Reactive sites present in a generic triglyceride.



Source: Adapted from Mutlu (2012).

Plant oils are commonly related to the obtainment of polymers, including castor, linseed, palm, rapeseed and soybean oils (GÜNER; YAĞCI; ERCIYES, 2006). Observing Table 2.1, these oils present a large contribution of unsaturated fatty acids, which make plant oils important raw materials for the synthesis of monomers or for the direct polymerization due to their chemical versatility and availability (ESPINOSA; MEIER, 2011).

Table 2.1: Major commodity triglyceride composition in terms of fatty acids (wt%) which C is the number of carbon atoms and DB is the number of double bonds (MUTLU, 2012; ORSAVOVA *et al.*, 2015).

Fatty acid (C:DB)	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
Castor*	0.9	0.8	2.9	4.5	0.6
Corn	10.9	2.0	25.4	59.6	1.2
Cotton seed	21.6	2.6	18.6	54.4	0.7
Linseed	5.5	3.5	19.1	15.3	56.6
Olive	13.7	2.5	71.1	10.0	0.6
Palm	44.4	4.1	39.3	10.0	0.4
Rapseed**	4.6	1.7	63.3	19.6	1.2
Soybean	11.0	4.0	23.4	53.2	7.8
Sunflower	6.1	3.9	42.6	46.4	1.0

*Castor oil contains about 90% of ricinoleic acid.
**Rapeseed oil contains 9% of gondoic acid (20:1).

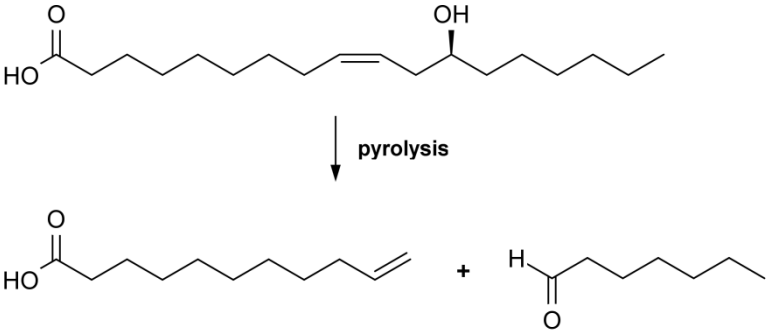
Furthermore, a wide list of polymers obtained by these natural oils includes oxypolymerized oils, polyesters, polyurethanes, polyamides, acrylic resigns, epoxy resins and polyesteramides could present advantages in comparison with those based on fossil resources including biodegradability, raw material accessibility and lower cost (GÜNER; YAĞCI; ERCIYES, 2006).

2.1.1.1 Castor oil and its derivatives

Among plant oils, castor oil has been highlighted in the last few years because of this potential use in chemical industry, since this resource is constituted by about 90% of ricinoleic acid, a versatile and interesting fatty acid that presents a 18-carbon backbone, with two sites to modify, a *cis* double bond (between carbons 9 and 10) and a hydroxyl group on the 12 carbon atom (ACHAYA, 1971; OGUNNIYI, 2006; MUTLU, 2012).

When submitted to pyrolysis (Figure 2.3) at high temperature (>400 °C), this particular ricinoleate molecule, forms10-undecenoic acid (or undecylenic acid) and heptaldehyde, being the first one an important raw material (MUTLU, 2012).

Figure 2.3: Pyrolysis reaction of ricinoleic acid.



Source: Based on Mutlu (2012).

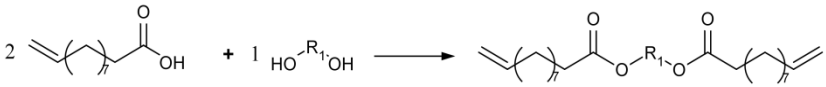
10-Undecenoic acid is able to be applied to obtain interesting building blocks as methyl-10-undecenoate, 10-undecenol and 10-undecenoyl chloride (ESPINOSA; MEIER, 2011). Besides, it may be used in organic chemistry to form other complex building blocks.

This acid has been shown versatile applications as in synthesis of *t*-butyl-2-methyl-1-iodo-undecanoate through methylation and iodination

(BAÁN *et al.*, 1986), to investigate its effect in metathesis of methyl 10-undecenoate (BALCAR; DOSEDLOVÁ; MATYSKA, 1987) in electrochemical polymerization into electrolysis cells (GOZLAN; ZILKHA, 1989), in synthesis of 3-methylcyclopentadecanone (MHASKAR; SUBBARAO, 1992), in formation of bisphenyl 10-undecenoate and biphenyl 10-undecenoamide (DEAK; VOGL; KILIMAN, 2001), in copolymerization with ethylene (SANTOS *et al.*, 2001), in deposition self-assembled layers on H-terminated Si surfaces (LI *et al.*, 2004), in self-metathesis to obtain dicarboxylic acids (NGO; JONES; FOGLIA, 2006), in enzymatic esterification with *oligo*-ricinoleic acid derivatives (HAYES *et al.*, 2012), in synthesis with polyols to apply as lubrificant base stocks (PADMAJA *et al.*, 2012), in reaction with *trans*-3-hexene by Tandem olefin cross-metathesis/epoxidation (DENARD *et al.*, 2014), in synthesis of epithio undecanoates (GEETHANJALI et al., 2014), as precursor in synthesis of dendrimers (KREYE *et al.*, 2014), in synthesis of 10-undecenoylthiolactonamide (GOETHALS *et al.*, 2014), as precursor of (R)-9-hydroxy-10-undecenoic acid (KATO *et al.*, 2014), in thiol addition of thiols in its methyl ester (PANG *et al.*, 2014; HU *et al.*, 2015), in obtainment of 10-undecenoic acid-capped Yb^{3+}/Er^{3+}-doped $NaYF_4$ nanoparticles (MEESARAGANDIA; MAHALINGAM, 2015), to prepare amino acid-based undecanoic acid derivative (GOPAL *et al.*, 2016), using its methyl ester in reaction in methanol and carbon monoxide under high pressure (LEMBERG; SADOWSKI, 2016), as precursor to synthesize C_{22}-dimer acid (YASA *et al.*, 2017), in synthesis with phenolic acids (NARRA *et al.*, 2017) and in esterification with supercritical methanol (NARAYAN *et al.*, 2017).

This compound and its derivatives also are useful for the synthesis of dienes to apply in polymerization reactions. Figure 2.4 illustrates a generic diene from 10-undecenoic acid.

Figure 2.4: Generic diene from esterification of 10-undecenoic acid and a diol.



Source: Author.

Warwel *et al.* (2001) have worked with transesterification of 5 ω-unsaturated fatty acid methyl esters (from 5-hexenoic, 6-heptenoic, 9-decenoic, 10-undecenoic and 13-tetradecenoic acids) with 3 diols, including

ethylene glycol, 1,4-butanediol and 1,4-bis(hydroxymethyl)cycloxane. After copolymerization of 10-undecenoic based ester with ethylene, applying homogeneous palladium catalyst, M_w of 178.7 kDa was achieved.

Fokou and Meier (2010) have synthesized 1,3-propylene diundec-10-enoate from 10-undecenoic acid and 1,3-propanediol (2.2:1) using triazabicyclodecene (TBD) as catalyst at 100 °C during 25 h. This ester was submitted to metathesis reactions in order to polymerize it.

Türünç and Meier (2011) have obtained undec-10-enoic anhydride in order to test its reactivity via metathesis and thiol-ene polymerizations. Polymers resulted in M_n up to 11.9 kDa.

Cardoso *et al.* (2014) have synthesized 1,3-propylene diundec-10-enoate through esterification of 10-undecenoic acid and 1,3-propanediol (2.45:1) at 135 °C in toluene. Resulted ester was submitted to metathesis polymerization in order to obtain a renewable polyester through bulk and miniemulsion with weight average molar weight (M_w) up to 9.4 and 26.4 kDa, respectively. Posteriorly, Cardoso *et al.* (2018) have applied the same ester in thiol-ene polymerizations with 1,4-butanedithiol, obtaining M_w up to 38.3 kDa in bulk and 33.9 kDa in miniemulsion.

Machado *et al.* (2017) working with thiol-ene polymerization of dianhydro-D-glucityl diundec-10-enoate, an ester from 10-undecenoic acid and isosorbide (2:1), have obtained M_w up to 21.4 kDa in bulk, and up to 38.3 kDa in miniemulsion after thiol-ene polymerization with the same thiol.

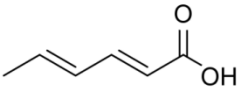
2.1.2 Sorbic Acid and Its Salts

Sorbic acid is a natural carboxylic acid endowed by trans-trans conjugated double bonds, presenting a high reactive carboxylic group liable to formation of salts and esters. Its reactivity in food systems can influence its antimicrobial activity such as the safety in products (SOFOS; BUSTAS, 1993). Sorbic acid concentrations in bacterial inhibition range from 10 to 10000 µg/mL, being *Lactobacillus* and *Clostridium* the most resistant genera to this compound (SOFOS *et al*., 1986). Figure 2.5 shows molecular structure of sorbic acid.

Bell, Etchells and Borg (1959) have compared the presence of sorbic acid with control (no sorbic acid) in different pH (6.8 to 3.5) against bacteria, yeasts and fungi. In pH 3.5 no bacteria had grown up, which is related to the dissociate curve, since dissociation percentage is about 0% in this pH, instead of pH 6.8, which this percentage is about 95%, showing that non-dissociated form was more effective in antimicrobial activity.

Eklund (1983) has presented similar behavior when sorbic acid was applied to *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Bacillus cereus*, observing an increase of Minimum Inhibitory Concentration (MIC) with an enhance of pH, however, dissociated form also showed antimicrobial effects. Zare *et al.* (2014) applied sorbic acid and potassium sorbate against *S. aureus* and *E. coli* in pH 7 and 5, noticing a decrease of MIC with the reduction of pH, for both acid and its salt.

Figure 2.5: Molecular structure of sorbic acid.



Source: Author.

In terms of applications, Bell and De Lacy (1987) have incorporated sorbic acid as emulsified antioxidant in pasteurized cured meat products, which 0.125% was the minimum effective concentration. As antimicrobial agent, this compound was incorporated in cheese samples (ROBERTS, 2002), cellulose acetate film to preserve pastry (SILVEIRA *et al.*, 2007[a]; SILVEIRA *et al.*, 2007[b]), polyvinyl acetate coating in contact with Gouda cheese and pork (HAUSER; WUNDERLICH, 2011) and polyvinyl alcohol as an antimicrobial package (DOBRE *et al.*, 2012; JIPA *et al.*, 2012).

Huiying *et al.* (2011) have proposed alternative elucidation about the way weak organic acids, as sorbic and benzoic acids, affect bacteria besides of membrane permeation and cytoplasmic acidification mechanisms, attributing antimicrobial effect also to the attenuation of anaerobic glycolysis causing ATP depletion.

As harmful effect, Namiki *et al.* (1980) have related that 2-methyl-1,4-dinitropyrrole, a product resulting from sodium nitrite and sorbic acid (4:1) reaction, exhibits mutagenic effect associated to carcinogenic activity. However, authors have argued that this reaction could be inhibited by lots of food constituents. Another concern is that whereas sorbic acid and potassium sorbate do not present mutagenic effect, sodium sorbate has acted as a carcinogen after testing it in Chinese hamster cells, with an induction of chromosome aberrations higher than 94% in concentration of 800 mg/L (HASEGAWA *et al.*, 1984).

2.2 2-HYDROXYETHYL METHACRYLATE (HEMA)

Poly(2-hydroxyethyl methacrylate) has shown high cyto-and hemocompatibility (GOLDBERG, 2008; TOMIĆ *et al.*, 2010).

Draghoslav Lim and Otto Wichterle (1960) were the first researchers to obtain poly(2-hydroxyethyl methacrylate) applying this material in biological uses including in manufacture of contact lenses.

Its polymer is also capable to produce hydrogels because of hydroxyl group presents in HEMA chain, and this property may be used in mechanical reinforcement (KHAN; HASSAN; DRZAL, 2005), skin and wound medical care (TOMIĆ *et al.*, 2010), drug release (ANANTHOJI, 2012), obtainment of shape-memory polymers (LIU *et al.*, 2013) and preservation of reserve lipids (OLIVEIRA, 2015).

2.3 ESTERIFICATION TECHNIQUES IN MILD CONDITIONS

2.3.1 Steglich Esterification

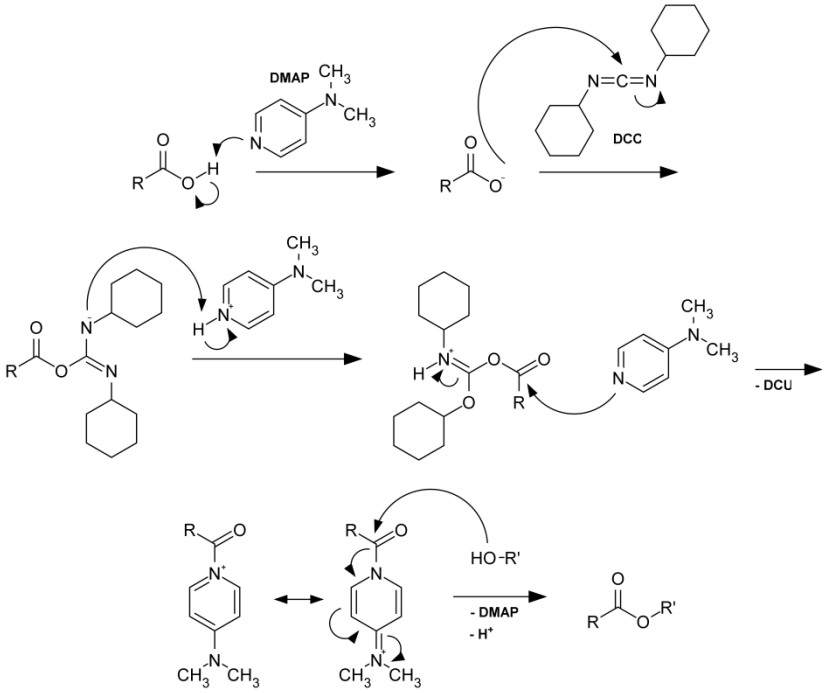
Neises and Steglich (1978) have elaborated a method to esterificate different carboxylic acids using N,N'-dicyclohexylcarbodiimide (DCC) to prepare carboxylates in presence of 4-dimethylaminopyridine (DMAP) as catalyst, producing esters and thioesters at room temperature. Figure 2.6 shows the mechanism of the Steglich esterification.

In this procedure, the strong nucleophile DMAP is responsible to accelerate DCC reaction with the carboxylic acid, which allows the formation of an *O*-acylisourea intermediate. This compound presents similar reactivity to carboxylic acid anhydride, making easier the addition of an alcohol (or thiol) to the activated carboxylic acid. Desirable ester is obtained from this mechanism, while dicyclohexylurea (DCU) is a precipitated product that could be separated through filtration. At least for tested carboxylic acids, this methodology has worked with good yields and even the formation of side products was suppressed.

Aprotic solvents as dichlorometane, diethyl ether, tetrahydrofuran and acetonitrile can be applied in this procedure (NEISES; STEGLICH, 1985).

Cova *et al.* (2013) have applied Steglich esterification to react Rhodamine B with HEMA, obtaining a fluorescent macromonomer, in order to polymerize it with methyl methacrylate, synthesizing biocompatible nanoparticles for tracking of stem cells.

Figure 2.6: Mechanism of Steglich Esterification.



Source: Based on Organic Chemistry Portal (2017).

Gilles *et al.* (2015) have worked with juglone (extracted from the husk of walnut fruit) esterification, applying long-chain fatty acids as lauric, palmitic and stearic acids. An alternative procedure was involved, once juglone was treated with $CeCl_2$, allowing a modification of its structure during Steglich esterification.

2.3.2 Enzymatic Esterification

Chemical esterifications usually need the application of strong acids or bases to be accelerated, which could originate complex and expensive processes to purify products or even make impossible complete separation of these compounds (FERREIRA-DIAS; FONSECA, 1995; SILVA; JESUS, 2003).

As biocatalysts, the use of enzymes in chemical reactions has shown many advantages as versatility, substrate selectivity, reduction of dangerous compounds and mild conditions (VOULGARIS *et al.*, 2015).

Among existing enzymes, lipases have been widely applied as biocatalysts, since they exhibit high stability and activity, in addition, they also show stereo-, chemo- and regioselectivity, even in non-aqueous media. Biocatalysts are usually very expensive, however, initial high cost could be compensated by an easier purification, moreover, these reactions often occur in mild temperatures, which may evolve reduction in energy costs (BIERMANN *et al.*, 2011).

These enzymes are efficient catalysts to the hydrolysis of esters, however, they are also able to perform the reverse process in the absence of water and in the presence of organic solvents, in other words, promoting esterification. Furthermore, the use of immobilized enzymes has been grown since the support increases the stability of the enzyme in reaction media, besides of allowing an easier separation and recover in final process, if compared with enzymes without supports (VALIVETY *et al.*, 1991; PAIVA; BALCÃO; MALCATA, 2000).

2.3.2.1 Enzymatic reactions of unsaturated precursors

Lipases are commonly applied in synthesis of natural lipids attached to nutritional demands, in production of detergents and in cosmetic industry to obtain aroma compounds (HOUDE; KADEMI; LEBLANC, 2004; KOURIST; HOLLMANN; NGUYEN, 2014). Despite large number of publications attributing the application of enzymes has published in the last decades, only examples related to the production of esters from unsaturated acids (or esters) are highlighted as following.

Pavel and Ritter (1991) have performed esterification reactions between 11-methacryloylaminoundecanoic acid with monoalcohols (isobutyl alcohol, ciclohexanol, DL-menthol, cholesterol and testosterone), using lipase from *Candida cylindracea* in cyclohexane/water for up to 8 days at 42 °C. Due to lower sterical hindrance among tested alcohols, isobutyl alcohol allowed the best result in terms of conversion (about 90% after 1 day). In a second approach, the same enzyme was applied to esterificate 11-methacryloylaminoundecanoic with 12-hydroxylauric acid for 8 days at 40-45 °C. This polymerization gave a M_n of about 1.2 kDa.

Berger, Laumen and Schneider (1992) studying lipases from *Rhizomucor miehei*, *Rhizopus delemar* and *Chromobacterium viscosum*, have realized synthesis of diacylglycerols (DAG) from vinyl acids, natural fatty acids and glycerol. Glycerol was adsorbed in a support to create an artificial interface between hydrophobic media and hydrophilic glycerol, which allowed the conversion of this compound in DAG. In a parallel study, Berger and Schneider (1992) have applied the same technique

to produce monoacylglycerols (MAG). All synthesis reactions were subjected at room temperature during 96 h.

Bornscheuer and Yamane (1995) have elaborated a methodology to obtain MAG from glycerol and different fatty acid vinyl esters applying lipase from *Pseudomonas cepacia*. These reactions carried out in bulk or *n*-pentane at 25 °C during up to 20 h, using vinyl palmitate, vinyl stearate, vinyl laurate and vinyl decanoate as acyl donors. Despite of excess of glycerol, there was high amount of TAG and DAG in these reactions, depending on presence of solvent and vinyl ester precursor.

Chen, Dordick and Rethwisch (1995) have synthesized α-methyl galactoside 6-acrylate by transesterification of α-methyl galactoside with vinyl acrylate catalyzed through lipase P (*Pseudomonas cepacia*) in anhydrous piridine at room temperature during 2 days. Conversion of 60% was obtained by this procedure, and unsaturated product was capable to suffer cross-linking after chemical intervention.

Geyer and Kleem (1995) have obtained an unsaturated ester from esterification between methyl α-D-glucopyranoside and 11-methacryloylaminoundecanoic acid, using lipase from *Candida antarctica* for 24 h at 75 °C. This ester was able to polymerize in presence of AIBN.

Kitagawa and Tokiwa (1998) have synthesized 6-*O*-vinylsebacyl-D-glucose from D-glucose and divinylsebacate for 7 days at 30 °C using diverse lipases (porcine pancreas, *Rhizopus delmer*, *Pseudomonas* sp., *Candida antarctica* and *Alcaligenes* sp.) in pyridine. Lipase from *Alcaligenes* sp. presented the best conversion (99%). This compound was polymerized using AIBN as initiator and M_n of 11 kDa was obtained.

Nakagawa *et al.* (1998) have examined reaction parameters in synthesis of geranyl acetate from geraniol and citronellol using vinyl esters as acyl donors and lipase of *Trichosporon fermetans* (adsorbed in celite) as biocatalyst. Transesterification reactions were performed at 30-40 °C, reaching a stable conversion (97.5%) after 5 h with 3% of water (v/v).

Uyama, Yaguchi and Kobayashi (1999) working in enzymatic synthesis of polyesters from dicarboxylic acid divinyl esters and α,ω-glycols, have analyzed reactions under presence of lipases derived from *Candida antarctica*, *Candida cylindracea*, *Mucor miehei*, *Pseudomonas cepacia*, *Pseudomonas fluorescens* and porcine pancreas. During polymerization reaction of divinyl isophthalate and 1,6-hexanediol, *Candida antarctica* lipase showed the highest catalytic activity, reaching an yield of 74% and Mn of 5.5 kDa at 60 °C for 48 h.

Kitagawa *et al.* (1999) have tested diverse hexoses including D-glucose, D-mannose, D-galactose and α-methyl D-galactoside in trans-

esterification reactions with divinyladipate in dimethylformamide. Biocatalyst was protease from *Streptomyces* sp. and reactions carried out during 7 days at 35 °C. Conversions ranged from 21 to 74%. In other study, Kitagawa *et al.* (2000) have applied the same methodology in order to observe the effect of temperature during conversion of reaction between D-glucose and divinyladipate. Conversions increased with enhance of temperature from 30 to 50 °C, reaching 90% at 50 °C, however, with the increase of temperature from 50 to 70 °C, there was a reduce to 50%.

Kitagawa *et al.* (2000) have also performed the synthesis of 5'-*O*-vinyladipoyl trymidine through transesterification of trymidine with divinyladipate at 30 °C during 7 days in dimethylformamide with biocatalyst protease from *Streptomyces* sp. Conversion of this reaction reached about 90% after 4 days. As a vinyl ester, this compound was capable to polymerize in presence of initiator AIBN, obtaining M_w of 69 kDa.

Park and Chang (2000) have described the synthesis of sucrose acrylate esters from sucrose and vinyl acrylate using protease from *Bacillus licheniformis* (Optimase M-440), as biocatalyst and pyridine as solvent. Transesterification reactions carried out at 30 °C resulting in conversion about 90% after 24 h. Product was a mixture of sucrose mono and diacrylate, being a distribution of 70 and 30%, respectively, after 5 days of reaction.

Xie and Hsieh (2001) have applied different enzymes in order to transesterificate cellulose solids with vinyl acrylate to obtain cellulose derivative containing double bonds, which was capable to suffer posterior graft copolymerization. The enzymes were Chymotrypsin type II (bovine pancrease), subtilisin Carsberg (*Bacillus licheniformis*), Proteases (*Streptomyces caespitosus* and Papaya) and Lipases (porcine pancrease and *Candida rugosa*), being first ones more efficient, obtaining 80 and 65% of transfer efficiency, respectively. However, subtilisin Carsberg was the only tested enzyme able to transesterificate cellulose with vinyl acrylate at 37 °C during 5 days and using pyridine as solvent, showing regioselectivity on primary hydroxyl group.

Ferreira *et al.* (2002) have worked with transesterification of inulin with vinyl acrylate applying a protease from *Bacillus subtilis* (Proleather FG-F). Reactions in dimethylformamide after 96 h allowed conversions greater than 57% at 50 °C.

Li and Rethwisch (2002) have performed scale-up of synthesis of α-methyl glucoside acrylate through transesterification between α-methyl glucoside and vinyl acrylate (1:2). This reaction was carried out at 60 °C, using Novozym 435 as catalyst and acetone as solvent during up to 24 h.

Conversions reached 100% when molecular sieves were applied, while conversion of 65% was obtained without water removal.

Zhang *et al.* (2002) have reported the synthesis of 6-*O*-vinylacetyl glucose from esterification between vinylacetic acid and glucose for 24 h at 50 °C, working with Chirazyme L-2 C2 (immobilized lipase from *Candida antarctica*). Among tested solvents (acetonitrile, acetone, 2-methyl-2-propanol and 2-methyl-2-butanol), acetonitrile allowed the best conversion which was 35%. An addition of 100 $mg.mL^{-1}$of molecular sieves enabled the increase of conversion to 74%. This unsaturated ester was submitted to polymerization reaction with AIBN, which resulted in M_w of 5 kDa.

Armesto *et al.* (2003) have worked in synthesis of 4-*O*-cinnamoyl derivatives of quinic and shikimic acids applying *Candida antarctica* lipase A and B. During transesterification, vinyl esters reached good yields when producing hydrocinnamic esters when lipase A was used at 40 °C for up to 25 h. However, lipase B allowed hydrolysis of vinyl esters, harming the yield during transesterification processes.

Kim *et al.* (2004) have performed biocatalytic synthesis of β-methylglucoside methacrylate and β-methylglucoside acrylate from vinyl methacrylate and vinyl acrylate, respectively. Novozym 435 was applied as biocatalyst, while 1,4-dioxane, acetonitrile, acetone, *t*-butanol and *t*-amyl alcohol were used as solvents. In addition, 1, 3 and 5 w/v% of enzyme were tested up to 48 h. Optimum conditions were obtained and *t*-butanol, 5 w/v% lipase and molar ratio of 1:3 were chosen to perform reactions at 50 °C. Both vinyl esters allowed reactions to reach 100% of conversion after 24 h, however, observed initial rate of vinyl acrylate was faster.

Wongsakul, H-Kittikun and Bornscheuer (2004) have produced 1,3-diacylglicerides using lipases arising from *Rhizopus oryzae*, *Burkholderia cepacia, Rhizomucor miehei, Candida antarctica* lipase B (CALB) and porcine pancreatic lipase. A vinyl ester and a saturated ester were precursors as acyl donors. In case of DAG synthesis, the best conditions included the use of CALB as biocatalyst, vinyl ester as acyl donor at 0 ºC during 4 h.

Wu *et al.* (2004) have tested transesterification of 5 different mono and disaccharides with divinyl dicarboxylates. Reaction between D-glucose with divinyl hexanedioate in pyridine at 50 °C for 24 h using protease from *Bacillus subtilis* presented a conversion of 65%.When divinyl butanedioate was applied, however, this conversion reached 100%.

Afach, Kawanami and Izumori (2005) have performed the synthesis of D-allose fatty acid ester reacting vinyl esters (vinyl caprylate, vinyl

caprate and vinyl laurate) with D-allose at 45 °C dissolved in acetonitrile, 3-methyl-3-pentanol or acetone, during 2 days. Novozym 435 (CALB), Amano AK (*Pseudomonas fluorescence*), Amano PS (*Burkholderia cepacia*) and porcine pancreatic lipase were applied as biocatalyst. The reaction between D-allose with vinyl caprylate in 3-methyl-3-pentanol using previous lipases resulted in conversions of 9, 0, 15 and 38%, respectively. The best condition involved the same reaction in acetonitrile applying Novozym 435, obtaining a conversion of 90%.

Wang *et al.* (2005) have investigated transesterification reaction between disaccharide sucrose with divinyl hexanedioate. Anhydrous piridine were applied as solvent and the reactions were performed during 3 days, which results in a conversion of 47% using protease from *Basillus subtilis* (18 wt%).

Jeong *et al.* (2006) have tested acylation of 1,4-sorbitan using methacrylic acid or vinyl methacrylate in molar ration 1:3 at 50 °C during 30 h, applying Novozym 435. Conversions of 57 and 88% were obtained for both acyl donors, respectively.

Lee, Widjaja and Ju (2006) have applied a methodology in order to produce ethyl ferulate (from ferulic acid and ethanol) and octyl methoxycinnamate (from *p*-methoxycinnamic acid and 2-ethyl hexanol), which are unsaturated esters. In both synthesis, Novozym 435 was used as biocatalyst in isooctane (best solvent compared to acetone, *t*-butanol, benzene, hexane and heptane). Ethyl ferulate was synthesized at 75 °C, while octyl methoxycinnamate at 80 °C, resulting in conversions of 87 and 90%, respectively.

Rustoy *et al.* (2007) have shown application of Novozym 435 as biocatalyst in obtainment of poly(N-(2-hydroxyethyl)acrylamide) from reaction between ethyl acrylate with ethanolamine at 25 °C for 48 h. Molar ratio of 1:0.5 (ethyl acrylate:ethanolamine) allowed higher M_w (1.9 kDa).

Tokiwa *et al.* (2007) have synthesized arbutin undecylenic acid ester from undecylenic acid vinyl ester and arbutin at 40 °C in dimethylformamide and water during 7 days using a protease f*rom Bacillus subtilis* (Bioprase) with conversion of 55%. This ester was 30% more efficient to inhibit melanoma cells than arbutin after 6 days.

Sen *et al.* (2008) have performed transesterification reaction between hydroxyl-terminated polyisobutylenes (polyisobutylene or Glissopal 2300) and vinyl methacrylate for 24 h at 50 °C, adding hexane as solvent and Novozym 435 as biocatalyst. This reaction was purged using nitrogen and resulted in 100% of conversion.

Burham *et al.* (2009) have proposed a methodology in order to synthesized palm-based ascorbyl esters, using Novozym 435 as biocatalyst during 16 h at 40 °C. Conversions of 70% were reached when a relation of 1:8 of ascorbic acid and palm oil were used, respectively.

Walsh *et al.* (2009) working with transesterification of vinyl laurate and lactose, have tested different enzymes and solvents (acetone, methylethylketone and 2-methyl-2-butanol) in order to produce lactose monolaurate. Best conditions involved application of lipases from *Thermomyces lanuginosus* (after 7 days at 55 °C) and *Pseudomonas cepacia* (after 14 days at 55 °C), in 2-methyl-2-butanol, which results in conversion of 52.4 and 56.6%, respectively.

Chiaradia *et al.* (2010) have synthesized eugenyl acetate from eugenol and acetic anhydride in solvent-free system at from 25 to 60 °C during 6 h. A molar ratio of 1:3 (eugenol:acetic anhydride) at 50% allowed conversion of 99% using Novozym 435 as biocatalyst (5.5 wt%).

Jadhav *et al.* (2010) have developed a methodology of single step to convert mannitol and sorbitol into dialkanoate derivatives from vinyl ester. Using Novozym 435, researchers have applied acetone as solvent and submitted these reactions at 45 ºC during 48 h.

Yang *et al.* (2010) working with polymerization of dicarboxylic acids with diols, have used first a methodology applying *Candida tropicalis* ATCC20962 in order to transform acids (oleic and erucic acid) in unsaturated diacids with posterior use of Novozym 435 (10 wt%) as catalyst to polymerize these compounds with diols (including 1,3-propanediol, 1,8-octanediol and 1,16-hexanedecanediol) in bulk and in diphenyl ether at 90 °C for 2 to 48 h. For α,ω-diacid from oleic acid, M_w of up to 45 kDa was found, while 29 kDa was obtained through α,ω-diacid from erucic acid.

Reyes-Duarte *et al.* (2011) have developed an efficient and inexpensive process in order to produce L-ascorbic acid fatty acid esters from fatty acids, alkyl and vinyl esters, using lipase Lipozyme TL IM, from *Thermomyces lanuginosus*, as alternative to Novozym 435. These reactions were performed at 40 °C during up to 168 h. Fatty acid ascorbyl ester conversion of 20, 20 and 100% were obtained considering the use of ethyl palmitate, palmitic acid and vinyl palmitate as acyl donors, respectively. Comparing Lipozyme and Novozym in this study, when triolein was used as acyl donor, conversions were 71 and 82%, respectively.

Galonde *et al.* (2013) have synthesized mannosyl myristate from D-mannose and vinyl myristate, adding 5w/v% of Novozym 435 in presence of three different ionic liquids or *t*-butanol as solvent at 60 °C. Comparing the first cycle, *t*-butanol allowed a conversion of 60%, while the

best ionic liquid resulted in a conversion of 70%. A loss of 57% in relation with enzyme activity were verify after 5 cycles with alcohol as solvent.

Kempen *et.al* (2013) have performed a comparison with esterification and transterification in order to obtain oligofructose lauric acid mono-esters through reactions at 60 °C during 63 h with Novozym 435 (1%). The use of lauric acid (esterification) allowed a conversion of 36% after 63 h, while the reaction with vinyl laurate (transterification) gave a conversion of 38% after 39 h. In this study, when no molecular sieves was used, conversions after 16, 39 and 63 h were 25, 35 and 28%, respectively, indicating hydrolysis reaction in presence of by-product water.

Moreno-Perez *et al.* (2013) have applied Novozym 435 in order to obtain ascorbyl oleate reacting ascorbic acid with oleic acid in tert-amyl alcohol. Molecular sieves were used to reduce water concentration, while the reaction was carried out during 48 h. Conversions of ascobyl oleate were up to 80% at 45 °C.

Kloosterman *et al.* (2014) have obtained glucosyl acrylate monomers from D-glucose with 2-hydroxyethyl acrylate, HEMA or 4-hydroxybutyl acrylate. β-glucosidase (from almond) were chosen as biocatalyst under co-solvents 1,4-dioxane and water at 50 °C for 24 h. Conversions were found lower than 50%. All hydroxyl functional acrylates were able to produce monomers when reacted with D-glucose, besides, they also were capable to copolymerize with vinyl monomers, resulting in M_w up to 2524 kDa.

Qin *et al.* (2014) have synthesized an unsaturated ester from hyaluronic acid (11.4 kDa) and vinyl esters through application of CALB. Synthesis was performed using a solution of 1 wt% of hyaluronic acid (1 g) and divinyl adipate (0.32 or 0.96g) in anhydrous DMSO at 50 °C during 24, 48, 72 or 96 h, using around 9 wt% of lipase. Produced ester was photopolymerized to obtain cross-linked hydrogels.

Jiang *et al.* (2015) have elaborated a methodology to perform polycondensation reactions using dimethyl itaconate, 1,4-butanediol and diacid ethyl esters, with Novozym 435 (10 wt%) as biocatalyst. Dimethyl itaconate is an ester derived from itaconic acid, a dicarboxylic acid containing a double bond in its molecule. These enzymatic polymerizations were carried out in two stages, being the first one the application of nitrogen atmosphere during 2 h at 80 °C, then reduced pressure of 2 mmHg was established during 94 h. M_w revealed results up to 94 kDa. Besides, enzyme showed preference for diacid ethyl esters with higher carbon-chains.

Pellis *et al.* (2015) have performed a polycondensation reaction between dimethyl itaconate and 1,4-butanediol (1:1.1) for 5 h at 40 °C, using reduced pressure (70 mbar) using Novozym 435 (10 wt%).

Taresco *et al.* (2016) have polymerize glycerol and divinyl adipate for 24 h in tetrahydrofuran, applying Novozym 435. Different temperatures were tested (40, 50, 60 and 70 °C), interestingly the best M_n was found at 50 °C (13.0 kDa).

Zhao *et al.* (2016) have compared different ionic liquids in enzymatic synthesis using lipases Novozym 435 (45 °C) and Lipozyme TLIM (40 °C) for 2 h, from reactions between methyl glucoside with lauric acid and glucose with vinyl laurate, respectively. This study resulted in conversions up to 30.89% and 26.84% for Novozyme 435 and Lipozyme TLIM, respectively.

Prabhakar *et al.* (2017) have synthesized monoacyl and diacyl trehalose from trehalose and different unsaturated fatty acids as linolenic, oleic and erucic acids. In this two step methodology, firstly unsaturated acids were converted into 2,2,2-trifluoroethyl esters using 2,2,2-trifluoroethanol through enzymatic esterification with immobilized CALB as biocatalyst and *t*-butanol as solvent, obtaining a conversion about 80% when microwave oven was used to control temperature (51 °C) for 1 h. The second step was transesterification of these esters with trehalose, using same conditions. Ranging equivalent of ester from 1.1 to 4.0 (in relation to trehalose) results in a increase of conversion from mono (73%) to diacyl (85%) trehalose.

Siebenhaller *et al.* (2017) have performed hydrolysis of beechwood carbohydrates in order to obtain saccharides, which were purified and dried to mixture with choline chloride. These mixtures were applied in transesterification reactions of glucose and vinyl-octanoate resulting inglucose-4-*O*-octanoatefor 72 h at 50 °C, using Novozym 435 as biocatalyst.

Perin and Felisberti (2018) have synthesized a serie of unsaturated esters from D-frutose and D-glucose applying Novozym 435 and Protyn NY 100 (protease) as biocatalysts for 24 h at 50 °C (in *t*-butanol) and 45 °C (in dimethyl formamide), respectively. 2,2,2-trifluoroethyl methacrylate, vinyl methacrylate, methacrylic acid and ethylene glycol dimethacrylate were applied as acyl donors. Higher conversions were obtained when Novozym 435, D-frutose and 2,2,2-trifluoroethyl methacrylate were chosen, which resulted in 99% of conversion based on formation of mono and dimethacrylates carbohydrate esters.

The application of vinyl ester donors is interesting since they increase the reaction rate, however in some cases there is the production of

aldehyde (ANTONOPOULOU *et al.*, 2016). In addition, despite of vinyl esters had shown high reactivity in order to perform enzymatictransesterification synthesis, the final product was often saturated when a vinyl ester, instead of a divinyl ester, was used.

In terms of enzymatic reaction conditions, CALB appears more versatile, being used in lots of works, specially its immobilized form (Novozym 435), which is easily removed from reaction media through filtration. Temperature of 50 °C was also frequently observed.

The choice of solvent is more complicated, since it depends greatly on enzyme, reaction temperature, reagents (solubility and chemical interaction) and products, being necessary to evaluate each case separately.

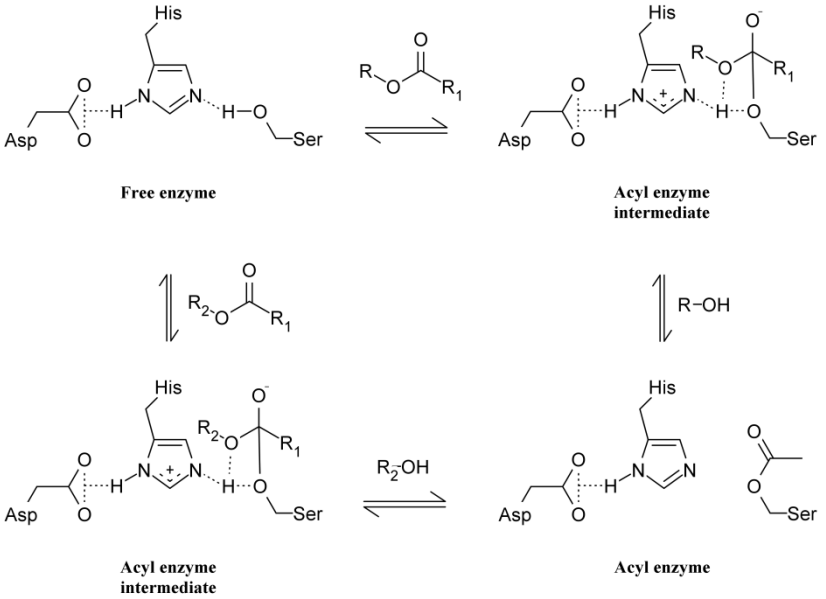
2.3.2.2 CALB-catalyzed mechanism

Candida antarctica lipase B (CALB) is very active in presence of a wide range of esters, amides and thiols (ANDERSON; LARSSON; KIRK, 1998), allowing a broad list of applications, including esterification, transesterification, aminolysis, aza-Michael reaction and aldol condensation (QUIROS *et al.*, 1993; GOTOR-FERNÁNDEZ; BUSTO; GOTOR, 2006; LE JOUBIOUX *et al.*, 2011; LE JOUBIOUX *et al.*, 2013). Immobilized form of this enzyme (Novozym 435) have been broadly studied in the last decade as biocatalyst to synthesize diverse esters.

In terms of mechanism, the catalytic reaction is given by a Ser-His-Asp triad. Figure 2.7 shows the mechanism of esterification/hydrolysis when CALB is used as biocatalyst, which R can be H or a carbon-chain.

Lipases are more active in water systems, since they were designed to perform hydrolysis more efficiently than other reactions. In this kind of reaction, carbonyl carbon from ester bond performs a nucleophilic attack in active site serine, which leads the formation of an acyl-enzyme intermediate and an alcohol. The acyl-enzyme intermediate reacts with water in a hydrolysis reaction forming a carboxylic acid (ADLERCREUTZ, 2013).

Figure 2.7: Mechanism of enzymatic esterification/hydrolysis using CALB as biocatalyst.



Source: Based on Anderson, Larsson and Kirk (1998).

In addition, esters are not the only compounds that can be acted as acyl donors. Free carboxylic acid also acts as acyl donors, which allows esterification (reverse hydrolysis) reactions to occur. Drawing a parallel, a mechanism of esterification involves a nucleophilic attack of serine by carbonyl carbon from acid, forming an acyl-enzyme intermediate. In hydrolysis mechanism, water is responsible for deacylate acyl-enzyme intermediate, however, in an organic media, alcohol can act in order to deacylate this intermediate, which leads the production of ester and water (ADLERCREUTZ, 2013).

The selectivity of substrate is related to physical restrictions and hydrophobic behavior of the active site area. Serine, active site of CALB, is located at the bottom of a narrow and deep pocket which explains why B-lipase is less active in the presence of large triglycerides in comparison to A-lipase. While the walls of the pocket are covered by hydrophobic amino acids, there is three hydrophilic amino acids in the proximity (UPPENBERG *et al.*, 1995; ANDERSON; LARSSON; KIRK, 1998).

2.3.2.3 Effect of different parameters in activity of lipases

Some factors can interfere in catalytic activity of enzymes as amount of water, type of solvent, temperature, pH and substrates.

Active conformation of enzyme is best kept under presence of low polarity solvents, since many enzymes lose part of their activities in polar solvents media due to the removal of critical or essential water (ZAKS; KLIBANOV, 1988; FABER; RIVA, 1992; GROSS; KUMAR; KALRA, 2001).

Lipases often express high catalytic activity at low amount of water, in addition, nucleophiles can compete with water in these conditions for deacylation of the acyl-enzyme, which is related to the fact lipases are proper to perform transferase-type reaction, as transesterification (MA; PERSSON; ADLERCREUTZ, 2002).

Branco *et al.* (2009), working with molecular dynamics simulation in order to investigate mechanism of hydration in CALB, have observed that when water was at low amount, single molecules of this compound occupied water binding sites on the hydrophilic enzyme surface, meanwhile, hydrophobic surface was not covered by water network, which results in more flexibility (or mobility) without loss of esterification activity (HALLING, 2004).

In terms of thermal stability, optimal temperature range is often 40-60 °C, although CALB is able to start denaturation process in a range of 50-60 °C, being depended on pH (ANDERSON; LARSSON; KIRK, 1998; LEE; WIDJAJA; JU, 2006).

Active sites (functional groups) from enzymes are affected by pH, since ionization causes influence in reaction rate. Regardless of pH concept is different in organic media, the phenomena of ionization is still important. However, there is no evidence for an optimum pH value in esterification reactions (STERGIOU *et al.*, 2013).

A pK_a dependence was observed when Nordblad and Adlercreutz (2008), working with acrylic acid (pK_a = 4.26) as acyl donor, observed that activity of Novozym 435 was quite low. This observation was corroborated by Hollmann *et al.* (2009), when they worked with esterification of 1-octanol with various acids, testing the influence of its pK_a in activity of lipase, which resulted in an inactivation of CALB when it was submitted to acids with pK_a smaller than 4.8 in general. All tested acids (and its pK_a) are shown in Table 2.2.

Besides of media conditions, lipases are more selective for certain compounds. For example, as previously discussed, vinyl esters, as acyl

donors, allow increase of the reaction rate (ANTONOPOULOU *et al.*, 2016).

Table 2.2: Acids and its pK_a applied in esterification with 1-octanol (HOLLMAN *et al.*, 2009).

Carboxylic acid	pK_a	Activity ($U \cdot g^{-1}$)
2-bromo butyric acid	2.90	72
2-bromo hexanoic acid	2.90	34
2-bromo propionic acid	2.97	<20
2-chloro butyric acid	2.80	<20
2-chloro propionic acid	2.90	<20
2-hydroxy hexanoic acid	3.90	64
2-methyl butyric acid	4.82	116
2-methyl hexanoic acid	4.90	720
2-methyl propionic acid	4.84	613
2-methyl valeric acid	4.90	270
3-methyl valeric acid	4.90	640
4-methyl valeric acid	4.90	3900
2-oxo butyric acid	2.50	<20
Acetic acid	4.86	36
Acrylic acid	4.26	<20
Butyric acid	4.82	>5000
Hexanoic acid	4.90	>5000
Lactic acid	3.76	<20
Malic acid	3.41	<20
Propionic acid	4.87	940
Tartaric acid	3.03	<20
Valeric acid	4.84	>5000

Each enzyme and substrate are affected in a particular way by these parameters, so there is a challenge in applying this techinique depending on the analyzed system.

2.4 POLYMERIZATION TECHNIQUES

2.4.1 Thiol-ene Polymerization

In 1905, Postner showed that a thiol and an ene could react with each other or in presence of an acid, however, a thiol-ene polymerization was just presented in 1926, when Braun and Murjahn observed that an allyl mercaptan was able to form a gel upon heating. Kharasch *et al.* (1938) elucidated the mechanism of thiol-ene as a free-radical mediated polymerization (KHARASCH; READ; MAYO, 1938; CLAUDINO, 2011).

2.4.1.1 Thiol-ene Mechanism

In terms of thermodynamics, the reaction that results in an addition of the thiol group across the olefin double bond (hydrothiolation) is exothermic, which enthalpies range from -10.5 to -22.6 kcal mol^{-1}. The ideal thiol-ene reactions are observed when vinyl ethers are applied since they present electron-rich double bonds (HOYLE, BOWMAN, 2010).

Table 2.3 represents the mechanism involved in a photopolymerization in thiol-ene reaction. The first step represents the photolysis of initiator, then primary radicals from this reaction have the function of abstraction of the hydrogen from thiol group, involving the formation of thiyl radicals (2), which reacts with the ene double bond (3). From this reaction, a secondary radical is formed and it can react with hydrogen of another thiol group (4), characterizing a chain transfer step and propitiating that a new thiyl radical appears to repeat the process. The termination could occurs through the combination of two primary radicals, two secondary radicals or a primary radical with a secondary radical (HOYLE *et al.*, 2003; ORTIZ; BLANDÓN; SANTOS, 2012). Figure 2.8 shows a simplified scheme of step-growth thiol-ene polymerization.

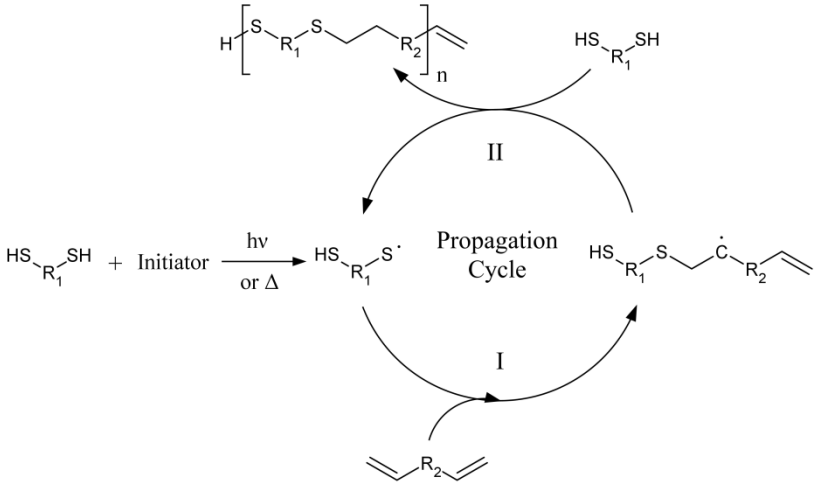
The reactivity of both thiol and ene affects kinetic parameters, since the rate of thiyl propagation is strictly related to electron density while the rate of chain transfer is controlled by carbon radical stability, in other words, there is a dependency of the presence of abstractable hydrogen atoms and the reactivity of the double bond. (CRAMER *et al.*, 2003). Through experiments with different compounds, Hoyle, Lee and Roper

(2004) have elaborated an ene reactivity order, presenting norbonene and vinyl ether the most reactive double bonds, otherwise, methacrylate, styrene and conjugated dienes have presented the last ones reactivities.

Table 2.3: Mechanism of thiol-ene photopolymerization.

$I \xrightarrow{h\nu} 2\,I\bullet$	(1)
$I\bullet + RSH \longrightarrow IH + RS\bullet$	(2)
$RS\bullet + H_2C{=}CHR' \longrightarrow RSCH_2C\bullet HR'$	(3)
$RSCH_2C\bullet HR + RSH \longrightarrow RSCH_2CH_2HR' + RS\bullet$	(4)

Figure 2.8: Simplified scheme of step-growth thiol-ene polymerization, where I is addition of thiyl radical to double bond, while II is the chain-transfer to thiol group.



Source: Based on Claudino (2011).

The mechanism of this addition is affected by stereochemistry even as reversible reactions of thiyl radical when it occurs in internal double bonds, causing *cis-trans* isomerization reactions, which reduces conversions and reaction rates (WALLING; HELMREICH, 1959; FERRERI *et al.*, 1999; LOWE, 2014). Hoyle, Lee and Roper (2004), performing a copolymerization with a monofunctional thiol, have shown that 1-hexene is

8 and 18 times more reactive than *trans*-2-hexene and *trans*-3-hexene, respectively, which was related to a steric hindrance.

In addition, thiol-ene reactions present little inhibition during polymerization in presence of oxygen, since peroxy radicals formed from the reaction between oxygen and carbon are capable to abstract hydrogen from thiol, continuing the free-radical mechanism. However, when it occurs in reactions using an ene as acrylate or methacrylate, an alkyl peroxy radical is formed, which will instantly terminate since it has low reactivity (HOYLE *et al.*, 2003).

2.4.2 Free-Radical Polymerization

Table 2.4 shows the mechanism that occurs in free-radical reactions, being quite widespread in literature. These reactions have three steps to occur, which the first one is the initiation, characterized by the thermal decomposition of initiator (*I*) in active radicals (*R*•), being added to monomer (*M*), forming a primary radical (P_1•).The propagation step occurs by successive addition of molecules to radical, resulting in chain-growth polymerization, but keeping active radicals (P_{n+1}•). Chain termination is the last step of free radical reactions, which a combination of two active polymeric radicals (P_n• and P_m•) or a disproportionation of them will result an interruption of chain-growth by the formation of inactive chains (Λ_n e Λ_m).

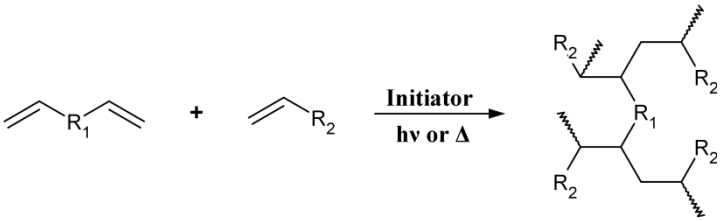
At the same time to the main mechanism, chain transfer effects could occur, causing the deactivation of the chain-growth due to the transference from active radical to another molecule, occasioning the growth of a new chain or attaching as branch (LOUIE; CARRATT; SOONG, 1985; COSTA, 2013).

The copolymerization between an alkene and a diene, which contains two double bonds, allows the obtainment of cross-linked polymers in free-radical polymerization. This reaction is similar if compared to the step polymerization where tetra functional reagents are applied. In addition, the cross-linking reaction depends on the relative reactivities of the two double bonds of the diene, and its extent are related to the reactivity of the two double bonds of the diene and the amount of diene relative to the alkene (SU, 2013). Figure 2.9 shows a simplified scheme of a cross-linking reaction between a diene and an alkene.

Table 2.4: Free radical mechanism, which n,m ≥ 1.

Initiation	$I \xrightarrow{k_d} 2R\bullet$ $R\bullet + M \xrightarrow{k_i} P_1\bullet$
Propagation	$P_n\bullet + M \xrightarrow{k_p} P_{n+1}\bullet$
Chain transfer to monomer	$P_n\bullet + M \xrightarrow{k_{tm}} \Lambda_n + P_1\bullet$
Chain transfer to polymer	$P_n\bullet + \Lambda_m \xrightarrow{k_{tp}} \Lambda_n + P_m\bullet$
Chain disproportionation	$P_n\bullet + P_m\bullet \xrightarrow{k_{tc}} \Lambda_{n+m}$ $P_n\bullet + P_m\bullet \xrightarrow{k_{td}} \Lambda_n + \Lambda_m$

Figure 2.9: Simplified scheme of cross-linking free-radical polymerization of a diene and an alkene. R_1 and R_2 represent carbon-chains or functionalized groups.



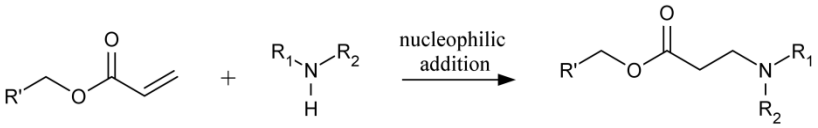
Source: Author.

2.4.3 Michael Addition

Arthur Michael (1853-1942) defined Michael addition as a reaction base-catalyzed by nucleophiles in order to add to carbon-carbon multiple bonds from activated olefins and alkynes (MATHER *et al.*, 2006). Michael donors, nucleophiles, could be amines, thiols and phosphines, while Michael acceptors include acrylates, acrylonitrile, acrylamides, maleimides, alkyl methacrylates, cyanoacrylates and vinyl sulfones (WU *et al.*, 2004; MATHER *et al.*, 2006).

In aza-Michael reaction, a version of Michael addition where the heteroatom nitrogen is Michael donor, amine can be used as both base and nucleophile, being unnecessary the use of an additional base in order to catalyze this type of reaction (MATHER *et al.*, 2006). This system is interesting in organic chemistry since it allows the synthesis of diverse bioactive compounds and polymerizable building blocks (GENEST *et al.*, 2017). A simplified scheme of Michael addition of amine in acrylate group is shown in Figure 2.10.

Figure 2.10: Simplified scheme of Michael addition of amine in acrylate group. R_1 could be a hydrogen (primary amine) or carbon-chain (secondary amine), R' and R_2 represent carbon-chains.



Source: Based on Escalante, Carrillo-Morales and Linzaga (2008).

In addition, these reactions are conducted in mild conditions, present high functional tolerance, resulting in high conversions and propitious reaction rates, besides of aza-Michael addition fulfills almost all criteria to be a green chemistry reaction (MATHER *et al.*, 2006; GENEST *et al.*, 2017). For instance, Abbotto *et al.* (2003),working with reaction between 2-(2-aminoethylcarbamoyloxy)-ethyl derivative with ethylenediamine in anhydrous acetonitrile and under nitrogen atmosphere, have carried out this reaction for 2 h at 0 °C, obtaining a conversion of 74%.

Wu *et al.* (2004) studying the polymerization of diacrylates with trifunctional amines, have observed that reactivity of amines changed with the running of polymerization. An acceptable reactivity sequence is defined as 2° amine > 1° amine, however, after reaction, formed 2° amines had a reactivity smaller than 1° amines. Researchers have attributed this behavior to steric hindrance of carbon-chain, which causes difficulty in interacting nucleophile with double bond. In addition, they have obtained conversions from 75 to 86%, during at least 248 h.

Despite of direct polymerization, aza-Michael addition may be used with the aim of synthesizing functionalized compounds (as monomers) through reaction between a diamine with 2 equivalent (or more) of activated alkenes.

Ichinobe (2003) have described a Michael addition between a dibehenyl itaconate (from esterification between itaconic acid and behenyl alcohol) and a modified two-(bis-(3-aminopropyl)-terminated silicone in xylene for 5 h at 130 °C. Conversion of 95% was obtained from this procedure.

In terms of selectivity, Michael addition is directly dependent on the type of functionalized group attached to carbon-carbon double bonds (NAVARRO *et al.*, 2017). Domingo, Pérez and Contreras (2004) have screened 39 compounds endowed with double bonds to examine the way functionalized groups modify electronegativity of Michael acceptors. Comparing acrolein with methyl vinyl ketone and methyl acrylate, a substitution of H by methyl and methoxy group, respectively, caused an electrophilic deactivation.

An electrophilic deactivation also was observed when methyl groups were add to double bond, making it internal double bonds, comparing with methyl acrylate (DOMINGO; PÉREZ; CONTRERAS, 2004). Nevertheless, there is report of Michael addition reaction with methyl crotonate (containing internal double bond) and benzylamine in methanol at 150 °C for 180 min under microwave irradiation, with conversion up to 98% (ESCALANTE; CARRILLO-MORALES; LINZAGA, 2008).

Reactivity of unsaturated esters for amine reaction decreases following the order acrylates > maleates > fumarates >methacrylates. Considering these differences in selectivity in relation to functionalized groups and steric hindrances, these reactions present regioselectivity to asymmetric divinylic compounds.

Moszner, Mölkel and Rheinberger (1996) have applied 1,3-propanediamine with aim of performing a Michael addition with 4 equivalent of 2-(acryloyloxy)ethyl methacrylate, obtaining a methacrylate terminal dendrimer. The reaction was carried out at 60 °C in methanol for 48 h, resulting in 90% of conversion.

Klee *et al.* (1999) have used ethylene glycol acrylate methacrylate to perform Michael addition with different amines in methanol, using butylated hydroxytoluene as catalyst, at 23 °C for 24 h, to obtain branched methacrylates. Conversions ranged from 34 to 57%.

Müh *et al.* (2001) have synthesized ethylene glycol acrylate methacrylate and ethylene glycol bisacrylate in order to perform Michael addition with diverse alkoxysilylamines, obtaining polymers from bisacrylate, while methacrylate double bonds were conserved as terminal groups.

Mathias *et al.* (2004) have performed Michael addition of ethanolamine, diethylene glycol amine, triethylene glycol amine, tetradecyla-

mine and adamantanamine with 3-(acryloyloxy)-2-hydroxypropyl methacrylate at room temperature for 24 h, obtaining conversions from 80 to 87%. They also synthesized a tetramethacrylate monomer from reaction between hexamethylenediamine and 4 equivalent of 3-(acryloyloxy)-2-hydroxypropyl methacrylate in chloroform at room temperature for 1 week.

Weickmann *et al.* (2010) have obtained methacrylate-functionalized tertiary alkyl amine via Michael Addition of stearyl amine and ethylene glycol acrylate methacrylate (1:2) after 4 days at 23 °C, with conversion up to 83%.

Bilgici *et al.* (2011) have synthesized secondary amines from reaction between diethyl aminomethylphosphonate or diethyl 2-aminoethylphosphonate and 3-(acryloyloxy)-2-hydroxypropyl methacrylate at room temperature for 24 h in order to apply in photopolymerization.

Redondo *et al.* (2014) have worked with reaction of 2-(acryloyloxy)ethyl methacrylate with N,N,N',N'-tetraethyl-diethylenetriaminein chloroform at 40 °C for 48 h. Interestingly, the resulted ester exhibited an increase in sensibility to hydrolysis.

González *et al.* (2015) have observed that when 1,6-hexanediol diacrylate was substituted by triethylene glycol dimethacrylate in reaction with poly(ethyleneimine) and bisphenol A glycerolate diacrylate through Michael addition, conversion reduced from 87 to 9%, considering acrylate and methacrylate conversion, respectively. They attributed this fact to the reduction of electrophilic behavior because of the steric hindrance caused by methyl group. However, it is important to emphasize that methacrylate was competing with acrylate from bisphenol A glycerolate diacrylate in this system.

Navarro *et al.* (2017) have tested two asymmetric cross-linkers 2-(acryloyloxy)ethyl methacrylate and 2-(acrylaminoethyl) methacrylate and one symmetric 2-(acrylamino)ethyl acrylate. These compounds were submitted to Michael addition in the presence of dibutylamine or diethylamine in chloroform at 40 °C for 3 h (acetic acid was applied as catalyst in some reactions). 2-(acryloyloxy)ethyl methacrylate when reacted with dibutylamine resulted in a selective reaction between double bond from acrylate and amine, while double bond of methacrylate were preserved.

2.5 MINIEMULSION

Miniemulsion is defined as an aqueous dispersion whose droplets are metastable within a size range from 50 to 500 nm, usually prepared in

a system containing an organic monomer, water, a surfactant and a co-stabilizer (LANDFESTER *et al.*, 1999).

As opposed to the emulsion, where monomer needs to diffuse into the aqueous media to the interior of micelles (since the surfactant is above the critical micelle concentration), in a stable miniemulsion there is no mass transfer initially, because monomer nanodroplets will have similar sizes after a controlled procedure of dispersion and will be stabilized against coalescence through surfactant and against diffusional degradation due to the co-stabilizer (ASUA, 2002).

In miniemulsion systems there is preferably a droplet nucleation instead of micellar nucleation, since it is not desirable that micelles are presented in the system (ASUA, 2002; SCHORK *et al.*, 2005). The droplet nucleation suggests that droplets formed during the emulsification are polymerized, i. e., it is expected that each droplet is nucleated (ANTONIETTI & LANDFESTER, 2002). In this case, these nanodroplets can be treated as nanoreactors, since each of them is a locus of polymerization, and it is one of the most interesting characteristics of this technique (ANTONIETTI & LANDFESTER, 2002).

The miniemulsion process is quite versatile, allowing nanoparticles to be obtained through various polymerization methods such as anionic, cationic, free-radical and polycondensation (LANDFESTER, MUSYANOVYCH & MAILÄNDER, 2010). In addition, this process is used in several applications such as the production of low viscosity latexes, the control of radical polymerization in dispersed media, catalytic polymerizations, encapsulation of inorganic solids, incorporation of hydrophobic monomers, formation of hybrid polymer particles, among others (ASUA, 2002).

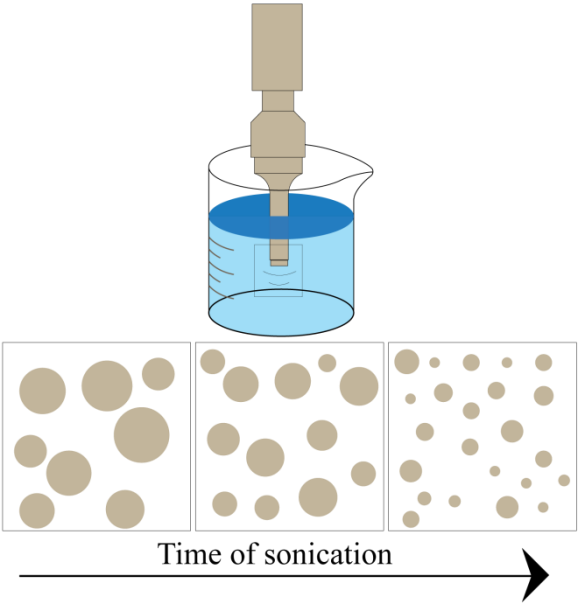
Although bulk reactions are simpler than aqueous dispersed systems, in free-radical polymerizations the monomer is usually liquid while the polymer is solid, which may cause engineering problems, since it could become difficult to remove the product from reactors.

Further, such reactions are often exothermic, causing increase in viscosity and consequently offering resistance to thermal exchange. The interest in the miniemulsion is linked to a number of advantages that this method of polymerization presents, among them, a better heat transfer between the heating source and reaction media and the use of water instead of an organic solvent (ASUA, 2002; SCHORK *et al.*, 2005)

2.5.1 Droplet Size Distribution Control

The disruption of monomeric droplets is a consequence of the application of energy to the system, generating turbulence and cavitation in the dispersion, producing shear and collision forces between droplets, which reduce droplet size and increase surface area (ASUA, 2002). Figure 2.11 shows the effect of an ultrasound probe on the breakage of droplets.

Figure 2.11: Scheme of dispersion by sonication.



Source: Based on Asua (2002).

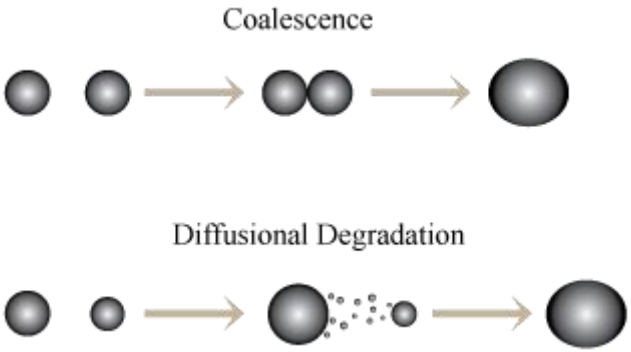
In order to reduce the surface tension (caused by the increase in surface area) it is necessary to add a surfactant to the system, with the purpose of avoiding coalescence, one of the phenomena that causes destabilization by the agglutination of droplets through Brownian motion and van der Waals forces (ASUA, 2002).

The other phenomena causing loss of stability is the degradation due to monomer diffusion, since smaller droplets have a higher chemical potential, which represents the driving force, causing diffusion in the direction from smaller to larger droplets. This transfer phenomenon is known as Diffusional Degradation or Ostwald Degradation, and can be

mitigated by the use of a co-stabilizer, usually a long carbon-chain organic compound (ASUA, 2002).

Figure 2.12 illustrates the mechanism associated with modification of droplet size.

Figure 2.12: Degradation mechanisms of monomer droplets.



Source: Author.

Thus the droplet size distribution is influenced by the dispersing system, as well as the variables linked to the equipment such as time and operating power. In addition, the choice of suitable co-stabilizer and surfactant is essential to control the thermodynamic phenomena associated with destabilization.

2.6 THIOL-ENE POLYMERIZATION IN MINIEMULSION

The application of thiol-ene polymerization in miniemulsion is quite recent, few studies have been reported so far in this research field. An overview of key findings and developments in this area are presented below.

Lobry *et al.* (2014) have performed for the first time thiol-ene polymerization in miniemulsion. Applying diallyl adipate as diene and ethylene glycol dithiol as thiol, they obtained nanoparticles with diameter of 155 nm, stabilized with surfactant sodium dodecyl sulfate (SDS) and costabilizer hexadecane, endowed with M_n of 30 kDa (soluble fraction). In this study, glass transition temperature of this poly(thioether) was -63°C, while presented a melting temperature of 18 °C.

Jasinski *et al.* (2014) have reported the preparation of polymer nanoparticles from thiol-ene miniemulsion polymerization. In these reactions, they applied diallyl adipate as diene, ethylene glycol dithiol as thiol, hexadecane as costabilizer and SDS as surfactant, obtaining particle diameters of 130 nm. Molar weights (M_w) ranged from 14 to 23 kDa with polydispersity indexes (Đ) of 2.5 by photopolymerization using α-hydroxyketone as initiator.

Amato *et al.* (2015) have related thiol-ene miniemulsion photopolymerization of 1,3,5-triallyl-1,3,5-triazine-2,4,6, a triene, with pentaerythritol pentaerythritoltetra(3-mercaptopropionate), a tetrathiol. The surfactant was SDS, while hexadecane was applied as costabilizer. In order to avoid polymerization during sonication step, 4-*p*-methoxy phenol was used as inhibitor. These methodologies allowed the obtainment of sub-100 nm cross-linked polythioether particles.

Amato *et al.* (2016) have applied encapsulation of carvacrol and thymol through thiol-ene polymerization between diallyl phthalate and pentaerythritoltetra(3-mercaptopropionate). These nanocapsules were used in order to destroy pathogens.

Machado *et al.* (2017) have used this technique to polymerize dianhydro-D-glucityl diundec-10-enoate, an ester from 10-undecenoic acid and isosorbide (2:1), have obtained M_w up to 38.3 kDa in miniemulsion after thiol-ene polymerization with 1,4-butanedithiol, initiated by AIBN. Three different surfactants were applied (SDS, Lutensol AT50 and Lutensol AT50) in this study, and nanoparticles with diameters from 194 to 218 nm have shown biocompatibility in murine fibroblast and uterine colon cancer. In posterior work, Meneses *et al.* (2017) have used this system to entrap clove oil, obtaining antioxidant activity.

Cardoso *et al.* (2018) have applied 1,3-propylene diundec-10-enoate in thiol-ene polymerization in miniemulsion with 1,4-butanedithiol, obtaining M_w up to 33.9 kDa in miniemulsion using AIBN as initiator. SDS and Lutensol AT80 were used as surfactants and nanoparticles from 114 to 173 nm were prepared. These nanoparticles also have shown biocompatibility in murine fibroblast and uterine colon cancer.

There are still many challenges in order to investigate more thiol-ene polymerization in miniemulsion. In this context, the application of a diene monomer from a bioprocess as enzymatic esterification was not performed yet. In addition, the diene monomers described above are symmetrical, therefore, the investigation of a compound having double bonds whose reactivities are different is required.

CHAPTER III

3 COMPARISON BETWEEN CHEMICAL AND ENZYMATIC PROCEDURES IN ORDER TO OBTAIN DIENE ESTERS FROM SORBIC AND 10-UNDECENOIC ACIDS

3.1 INTRODUCTION

This chapter refers to the study of comparison between sorbic and 10-undecenoic acid when applied in Steglich and enzymatic esterification reactions with HEMA with the objective of production of diene esters through mild condition techniques, preserving double bonds to test the application of these materials in different polymerization strategies.

3.2 EXPERIMENTAL PROCEDURE

3.2.1 Materials

Chemical precursors to perform esterification reactions were sorbic acid (Vetec), 10-undecenoic acid (Sigma-Aldrich) and 2-hydroxyethyl methacrylate (HEMA, Sigma-Aldrich).

While, as catalyst, coupling agent and solvent in chemical Steglich esterification, 4-dimethylaminopyridine (DMAP, Sigma-Aldrich), N,N'-dicyclohexylcarbodiimide (DCC, Sigma-Aldrich) and acetonitrile (P. A., Sigma-Aldrich) were applied, respectively.

In enzymatic esterification reactions, lipases Novozym 435 (44.9 ± 1.9 $U \cdot g^{-1}$) and NS 88011 (46.6 ± 2.7 $U \cdot g^{-1}$) were used as biological catalysts (both from *Candida antarctica*), kindly donated by Novozymes®. Chloroform (P. A., Qhemis) was chosen as solvent in some reactions.

Applied solvents in purification process were *n*-hexane (P.A., Vetec), methylethylketone (P.A., Vetec), ethyl acetate (P.A., Vetec), ethyl ether (P.A., Alphatec) and distilled water. Sodium sulfate anhydrous (Vetec) was used to retain water, while silica gel for column chromatography 70-270 mesh (Macherey-Nagel) was applied to separate HEMA from monomer.

All materials were used as received.

3.2.2 Evaluation of auto polymerization of HEMA

A round bottom flask containing 25 g of HEMA and 0.11 g of *p*-toluenesulfonic acid was submitted to 60, 75 and 90 °C in a rotary evaporator, which simulates an usual esterification without adding carboxylic acid, in order to test a possible polymerization process during this procedure.

3.2.3 Synthesis using Steglich esterification procedures

The methodology applied in this study was adapted from Cova *et al.* (2013).

In a 150 mL round bottom flask containing 20 mL of acetonitrile, 0.0021 mol of sorbic acid (0.2341 g), or 10-undecenoic acid (0.3847 g), and 0.0025 mol of HEMA (0.3250 g) were mixed. Another mixture containing 0.0021 mol of DCC (0.4300 g) and 0.0001 mol of DMAP (0.0130 g) was prepared in 20 mL of acetonitrile, being transferred to a burette and it was dripped into the reactor containing reagents. These reactions were performed during up to 72 h at 40 °C into a water bath heated by magnetic stirrer IKA C-MAG HS 7.

3.2.4 Synthesis using Enzymatic esterification procedures

3.2.4.1 Comparison study

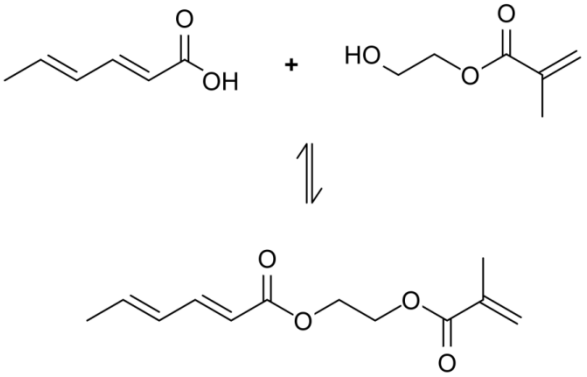
Exploratory reactions were performed in a 150 mL round bottom flask containing 100 mL of chloroform, 0.021 mol of sorbic acid (2.3406 g), or 10-undecenoic acid (3.8470 g), and 0.025 mol of HEMA (3.2601 g) were mixed. The amount of lipase Novozym 435 was 0.56 and 0.71 g (10 wt% related to substrates), respectively. These reactions were carried out during 72 h at a controlled temperature of 50 °C, under gentle magnetic stirring and maintained through water bath heated by magnetic stirrer IKA C-MAG HS 7.

A simplified scheme is presented in Figure 3.1 to illustrate the reaction performed to the obtainment of monomer from HEMA and sorbic acid (MHS), while another scheme is presented in Figure 3.2 to illustrate the reaction to produce monomer from HEMA and 10-undecenoic acid (MHU).

3.2.4.2 Synthesis reaction kinetics of MHU

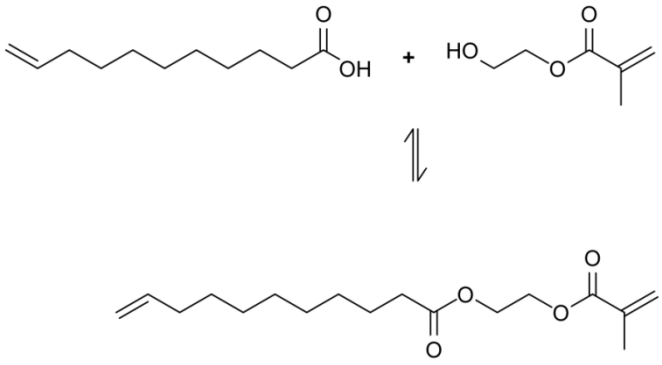
All duplicate chemical reaction kinetics were performed into a conical bottom vial of 3 mL (Supelco) with screw cap, containing 0.1956 g (0.0015 mol) of HEMA, 0.2308 g (0.0013 mol) of 10-undecenoic acid, varying the amount of enzyme in 1, 5 and 10 wt% (0.0043, 0.0213 and 0.0426 g, respectively), in bulk or solution (2.1 or 0.3 mL of chloroform), under gentle magnetic stirring and heating by magnetic stirrer IKA C-MAG HS 7 at 50 °C.

Figure 3.1: Scheme of esterification reaction between sorbic acid and HEMA to produce MHS.



Source: Author

Figure 3.2: Scheme of esterification reaction between 10-undecenoic acid and HEMA to produce MHU.



Source: Author

3.2.4.3 Reaction reuse of different lipases

Duplicate reactions also were performed into a conical bottom vial of 3 mL (Supelco) with screw cap, containing 0.1956 g (0.0015 mol) of HEMA, 0.2308 g (0.0013 mol) of 10-undecenoic acid. The amount of enzyme was 0.0043 g for Novozym 435 and NS 88011. These reactions were performed under gentle magnetic stirring and heating by magnetic stirrer IKA C-MAG HS 7 at 50 °C. The enzyme used was filtered and washed using chloroform and dried at room temperature.

3.2.4.4 Scale-up reaction

In order to scale up esterification reaction, a 150 mL round bottom flask containing 11.5410 g (0.063 mol) of 10-undecenoic acid and 9.7800 g (0.075 mol) of HEMA were mixed at 50 °C into a water bath heated by a magnetic stirrer IKA C-MAG HS 7. In comparison, the same amount of reagents was tested in a 150 mL erlenmeyer into a water bath heated at 50 °C in a shaker. The amount of lipase and time of reaction were defined after optimization during reaction kinetic steps.

3.2.5 Enzyme activity determination

The enzymatic activity was obtained through hydrolysis of *p*-nitrophenyl palmitate by spectroscopy UV-vis, measuring the increase of absorbance at 410 nm caused since *p*-nitrophenol is produced during *p*-nitrophenyl palmitate reaction in solution of 0.5 % (v/v) of ethanol (CHIOU; WU, 2004).

3.2.6 Nuclear Magnetic Resonance (^{1}H NMR)

^{1}H NMR was recorded in $CDCl_3$ on Bruker AVANCE DPX spectromoter operating at 200 MHz. Chemical shifts (δ) are reported in parts per million related to the internal standard tetramethylsilane (δ = 0.00 ppm).

3.2.7 High Performance Liquid Chromatography (HPLC)

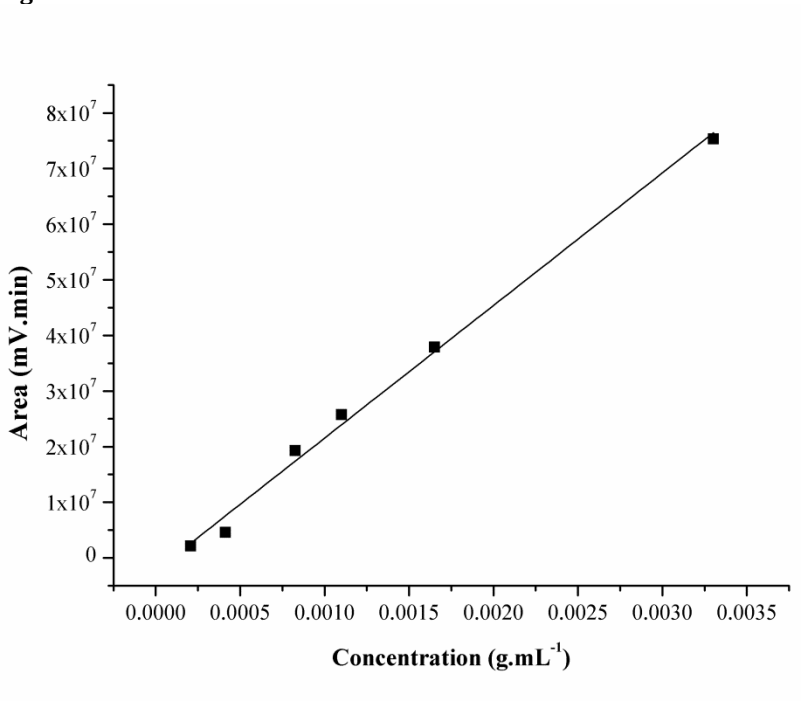
Liquid chromatography analyses were carried out using LC-20A (Shimadzu) equipped with a precolumn SUPELCOSIL LC-18 SUPELGUARD (5 µm, 20×4 mm) and a column SUPELCOSIL LC-18 (5 µm, 250×4.6 mm). Applied eluent was a mixture containing water (30%) and

acetonitrile (70%) with flow rate of 1.0 mL·min^{-1} (27 °C). UV detector was SPD-20A, applying wavelength of 205 nm.

3.2.8 Conversion

In order to quantify esterification and polymerization reactions involving MHU, a calibration curve was made from purified monomer ester (purity of 93%). Six samples with concentration ranging from $2.1 \cdot 10^{-4}$ to $3.3 \cdot 10^{-3}$ g·mL^{-1} were analyzed through HPLC in duplicate, obtaining area (mV.min) and using these results to plot calibration curve showed in Figure 3.3. Samples with about 0.02 g are diluted in 15 mL of acetonitrile in order to obtain area of peak from HPLC analysis using software LC Solution.

Figure 3.3: Calibration curve of MHU.



An equation of straight line (5) was obtained, with an adjusted R-squared of 0.9938, where Ap_{MHU} is area of HPLC peak related to ester (MHU) and [C] is the concentration (g·mL^{-1}). Thus, it was possible to

calculate weight fraction of MHU (x_{MHU}) from equation 6, where $V_{dilution}$ is volume used in dilution and w_{sample} is weight of diluted sample. Using the initial weight of reagents (w_{HEMA} and w_{UA}) is possible to calculate weight of monomer (w_{MHU}) using equation (7).

$$[C] = \frac{Ap_{MHU} + 2299430.70}{23842322026.95} \left(\mathrm{g \cdot mL^{-1}}\right) \quad (5)$$

$$x_{MHU} = \frac{[C] \times V_{dilution}}{w_{sample}} \quad (6)$$

$$w_{MHU} = x_{MHU} \times (w_{HEMA} + w_{UA}) \text{ (g)} \quad (7)$$

Since 10-undecenoic acid is the limiting reactant, calculation of conversion (C) depends on the relation of w_{MHU} and weight if all 10-undecenoic acid was consumed, thus equation (8) presents calculation of conversion, where MW_{MHU} and MW_{UA} is the molar weight of MHU and 10-undecenoic acid, respectively.

$$C = \frac{w_{MHU}}{\frac{w_{UA} \times MW_{MHU}}{MW_{UA}}} \times 100(\%) \quad (8)$$

3.2.9 Statistical Analysis

Software OriginPro 8 (OriginLab Corporation) was used in order to perform statistical analysis for some experimental data, applying a confidence level of 95% ($p < 0.05$) in two-way analysis of variance (ANOVA).

3.3 RESULTS AND DISCUSSION

3.3.1 Thermal Stability of HEMA

Usual procedures of esterification need temperatures higher than 100 °C and strong acids (or bases) as catalysts to perform. In order to evaluate the thermal stability of HEMA under usual esterification conditions, applying *p*-toluenesulfonic acid (PTSA) as catalyst, this precursor was submitted at 60, 75 and 90 °C during 3 h without a carboxylic acid. Table 3.1 shows results from GPC analysis.

There is an increase of M_n with the enhance of temperature. Although polymers obtained after reactions at 75 and 90 °C were not soluble in THF, these materials became more viscous and then solid, which indicated a polymerization reaction.

This effect could be associated with the polymerization of methacrylate group in presence of an acid (HADDLETON *et al.*, 1998). There are reports of HEMA polymerization in this temperature range. Seidel and Malmonge (2000), aiming to study polyHEMA hydrogels, have performed a polymerization at 75 °C during 2 h applying benzoyl peroxide as initiator. Vargün *et al.* (2010), working with copolymerization between methyl methacrylate and HEMA, have carried out the reaction at 65 °C during 2 h using ammonium persulfate initiator.

Table 3.1: Distribution of molecular weight of HEMA in different temperatures.

Sample	Temperature (°C)	M_n(kDa)	Đ
HEMA/PTSA	60	0.31	1.1
HEMA/PTSA	75	*	*
HEMA/PTSA	90	*	*
HEMA	-	0.13	-

* This sample was not completely soluble in THF.

Considering these preliminary results, the utilization of an usual esterification was discarded, due to the possible homopolymerization of HEMA in high temperatures. Aiming to perform the reaction in mildconditions, Steglich and enzymatic esterification have appeared as alternative procedures.

3.3.2 Different methodologies to perform esterification of sorbic and 10-undecenoic acids with HEMA

3.3.2.1 Steglich Esterification Methodology

Water is a common by-product in usual esterification reactions, being often necessary its removal from the system since these reactions in-

volve chemical equilibrium. In the case of the Steglich esterification, instead of water production, this reaction allows the formation of dicyclohexylurea (DCU) in stoichiometric amount to the desirable ester, which is a precipitated product that could be separated by filtration.

In order to test this methodology to produce diene esters monomers, two biobased unsaturated acids, sorbic and 10-undecenoic acids, were chosen to perform esterification reactions with HEMA.

After 48 and 72 h of reaction, samples of non-purified products were withdrawn using a syringe, prepared and analyzed through HPLC technique to verify if the reaction was effective to produce the expected ester. In order to allow the identification of the peak of product during characterization of samples, standard samples containing acid (sorbic or 10-undecenoic acid), HEMA and DCC were prepared to observe exactly in which time each compound appears in spectrum.

Table 3.2 shows results of normalized area of peak in terms of total area after integration of each peak in Figures 3.4 and 3.5, related to HPLC analysis.

Table 3.2: Normalized area (in relation with total area) of HPLC peaks obtained from software LC Solution.

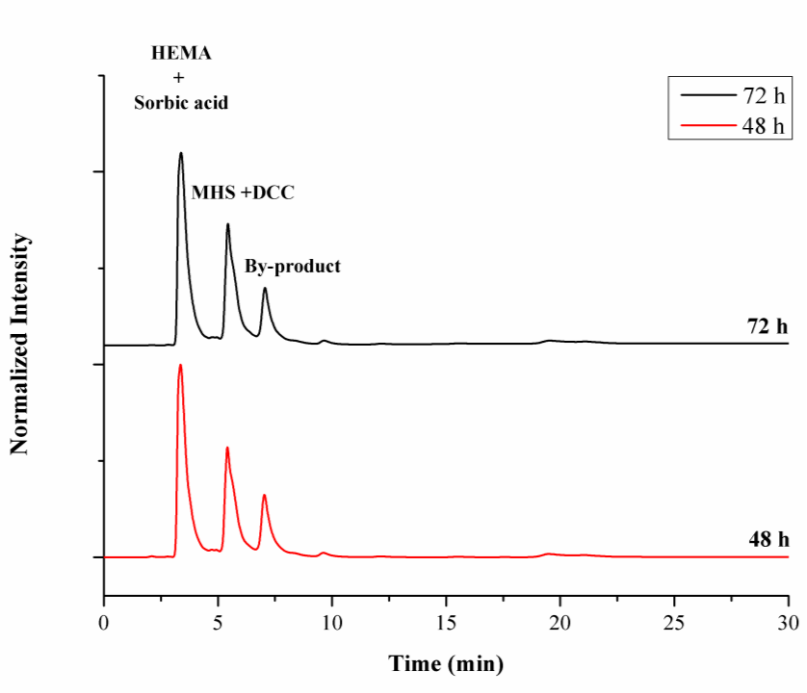
Area	Sorbic acid			10-Undecenoic acid		
	Time	48 h	72 h	Time	48 h	72 h
Ap_1	3.4 min	0.550	0.560	3.3 min	0.363	0.354
Ap_2	5.4 min	0.305	0.322	5.4 min	0.042	0.038
Ap_3	7.1 min	0.145	0.118	18.3 min	0.327	0.333
Ap_4	-	-	-	24.8 min	0.268	0.275

Figure 3.4 shows spectra obtained by HPLC related to non-purified monomer MHS through Steglich Esterification. HEMA and sorbic acid appear in 3.3 and 3.5 min, respectively, however, when analyzed in the same sample, the separation of these compounds in the spectrum becomes difficult.

In Steglich esterification mechanism (Figure 2.6), the reaction between DCC and carboxylic acid generates a by-product, in this case, DCC reacts with sorbic acid generating a by-product with molar weight of

317.44 g·mol^{-1} that is probably observed in 7.1 min, while ester 2-(sorboyloxy)ethyl methacrylate (MHS) with molar weight of 224.26 g·mol^{-1} appears in 5.4 min.

Figure 3.4: Spectra of HPLC related to Steglich esterification to produce MHS after 48 and 72 h of reaction.



The comparison of spectra shows that the product still contains a great fraction of non-reacted sorbic acid and HEMA. Moreover, a complex mixture of reagents and products is presented after reaction.

In terms of integration area, the relation of Ap_2 (MHS and DCC) and Ap_1 (HEMA and sorbic acid) remains almost the same after 48 and 72 h, 0.55 and 0.57, respectively. As long as HEMA and sorbic acid are consumed, Ap_1tends to reduce, while peak of DCC also should reduce by formation of DCU, resulting in an increase of MHS peak. Meantime, Ap_2 contains either MHS and DCC (206.32 g.mol^{-1}), hindering a comparison.

An interesting parameter to investigate this reaction is the relation between Ap_3 (by-product) and Ap_2. As long as MHS is produced, the peak

of by-product should reduce, which is observed through reduction of Ap_3/Ap_2 from 0.47 to 0.37, considering 48 and 72 h, respectively.

Conversion of this reaction can be calculated measuring the weight of DCU contained in filter paper, which is used to obtain the number of moles of DCU. The relation of number of moles between DCU and DCC (added in the beginning of the reaction) is applied to calculate conversion. After 72 h, a conversion of 74% was reached. Cova *et al.* (2013) working with Steglich esterification of Rhodamine B and HEMA in acetonitrile for 24 h, have observed a conversion of 86% using the same methodology to calculate this parameter. Gilles *et al.* (2015) have obtained a conversion of 10% after Steglich esterification between juglone and palmitic acid in THF during 72 h.

Figure 3.5 presents spectra obtained by HPLC related to non-purified monomer MHU through Steglich Esterification. As observed before, HEMA appears in 3.3 min, however, 10-undecenoic acid has a poor absorbance, then it is not shown in these spectra. Performing a HPLC analysis just for 10-undecenoic acid, this compound appeared in 7 min using the same conditions.

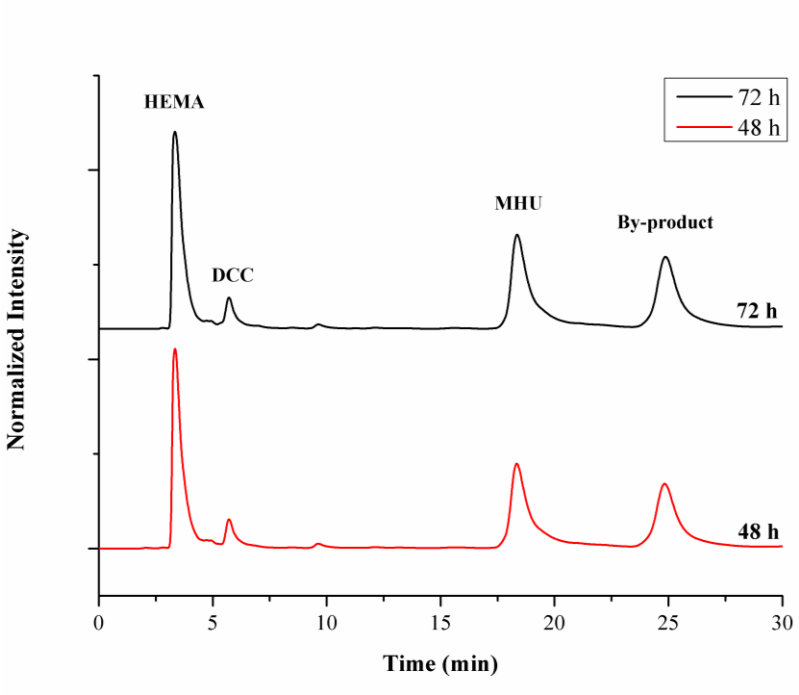
The by-product from reaction between DCC and 10-undecenoic acid (389.6 $g.mol^{-1}$) exhibited a peak in 24.8 min, while a peak in 18.3 min is associated with ester 2-(10-undecenoyloxy)ethyl methacrylate (MHU) with molar weight of 296.42 $g.mol^{-1}$.

The relation of Ap_3 (MHU) and Ap_1 (HEMA) increases as long as time is increasing, being 0.90 and 0.94 after 48 and 72 h, respectively, which is expected since Ap_1 tends to diminish for as much as reaction is happening, while Ap_3 is increasing with production of MHU. However, the relation between Ap_4 (by-product) and Ap_3 remains 0.82 after 48 h, which indicates that ester production reached an equilibrium.

Conversion can be calculated following previous procedure for reaction between HEMA and sorbic acid. This reaction allowed a conversion of 60%.

A feature of this reaction seems to be the presence of great fraction of non-reacted precursors and by-products, turning purification complex because of the presence of many compounds dispersed in synthesis media. Besides, both Steglich reagents are hazardous, while DCC is an allergen, DMAP is highly toxic since it is capable to be absorbed by skin.

Figure 3.5: Spectra of HPLC related to Steglich esterification to produce MHU after 48 and 72 h of reaction.



3.3.2.2 Enzymatic Esterification Methodology

Aiming to reduce the presence of by-products observed after Steglich esterification, enzymatic esterification was tested to try obtaining both diene esters (MHS and MHU).

The methodology was chosen and applied for both biobased acids in order to perform a fair comparison between sorbic acid and 10-undecenoic acid (solid and liquid at room temperature, respectively), then bulk reaction was discarded at the first moment.

Wu *et al.* (2004) applied several solvents in order to transesterificate D-glucose and divinyl butanedioate under presence of protease from *Bacillus subtilis*, including acetonitrile and chloroform, showing conversions of about 20 and 50%, respectively.

Duan *et al.* (2010) compared 11 different solvents in order to esterificate oleic acid with glycerol using Novozym 435 at 60 °C. The

choice of chloroform as solvent allowed conversion of 88%, which was similar to toluene (90%), tetrachloromethane (90%), cyclohexane (89%), *n*-hexane (90%), *n*-heptane (91%) and *n*-octane (91%), and better than acetone (60%), tetrahydrofuran (77%), *t*-butanol (84%) and 4-methyl-2-pentanone (85%).

Besides of the good performances in these studies, chloroform presents boiling point of 61.2 °C, which means a possibility to carry out reactions at low temperatures and its easy recovery by distillation. Purification step often involves washing the product with water to remove alcohol, then an organic solvent with some solubility are interesting. Sorbic acid is insoluble in several organic solvents, as *n*-hexane, *n*-heptane, *n*-octane and toluene, however is soluble in chloroform.

During literature review, CALB often appeared as an effective catalyst in esterification/transesterification reactions, and its immobilized form (Novozym 435) allowed enzyme recovery, facilitating the separation step. Although there are reports of application of Novozym 435 at high temperatures, 50 °C was chosen in order to prevent auto polymerization of HEMA during esterification according to previous experiments. In addition, there are reports of successful studies applying this temperature in reactions of unsaturated compounds with CALB (ZHANG *et al.*, 2002; KIM *et al.*, 2004; JEONG *et al.*, 2006; SEN *et al.*, 2008; CHIARADIA *et al.*, 2010; QIN *et al.*, 2014; TARESCO *et al.*, 2016; SIEBENHALLER *et al.*, 2017).

The molar ratio of biobased acid and alcohol was maintained to 1.0:1.2, since there is no removal of water during esterification, thus an excess of HEMA is necessary to get shift the reaction equilibrium.

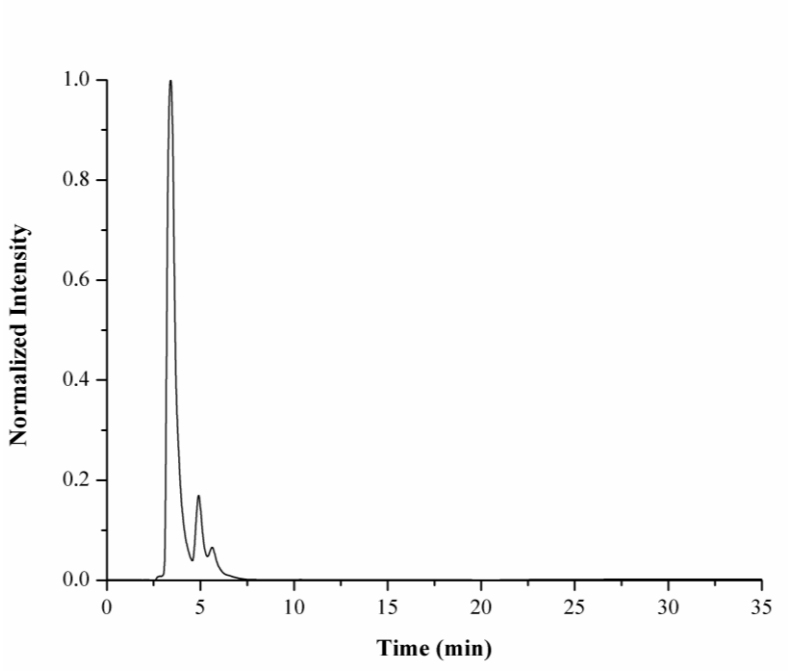
Figure 3.6 shows a HPLC spectrum of the enzymatic reaction between sorbic acid and HEMA after 72 h. HEMA and sorbic acid appears in 3.3 and 3.5 min, these compounds are difficult to separate as observed previously, while the second peak is related to solvent. Ester did not appear in this spectrum, which suggests that sorbic acid was not able to esterificate in these conditions.

Chu, Hawes and Lorigan (2009) compared the interaction of sorbic acid ($pK_a = 4.76$) and decanoic acid ($pK_a = 4.90$) with phospholipid membranes, observing that sorbic acid was located in surface between membrane and aqueous phase. On the contrary, decanoic acid migrated easily to the interior of hydrophobic regions of lipid membranes.

Activity of Novozym 435 was quite low when acrylic acid ($pK_a = 4.26$) was used as acyl donor (NORDBLAD; ADLERCREUTZ, 2008). In addition, Hollmann *et al.* (2009), working with esterification of 1-octanol and various acids, have observed an inactivation of CALB when

submitted to strong acids (pK_a< 4.8). An acceptable explanation is that short-chain carboxylic acids tend to dissociate more, which causes decrease of pH near the lipase and it can induce a reduction in catalytic activity.

Figure 3.6: Spectra of HPLC related to enzymatic esterification to produce MHS.



Sorbic acid presents a relatively polar behavior and its pK_a is equal to 4.76, which suggests that this compound could not be capable to interact properly with lipase in this system, preventing that esterification occurs.

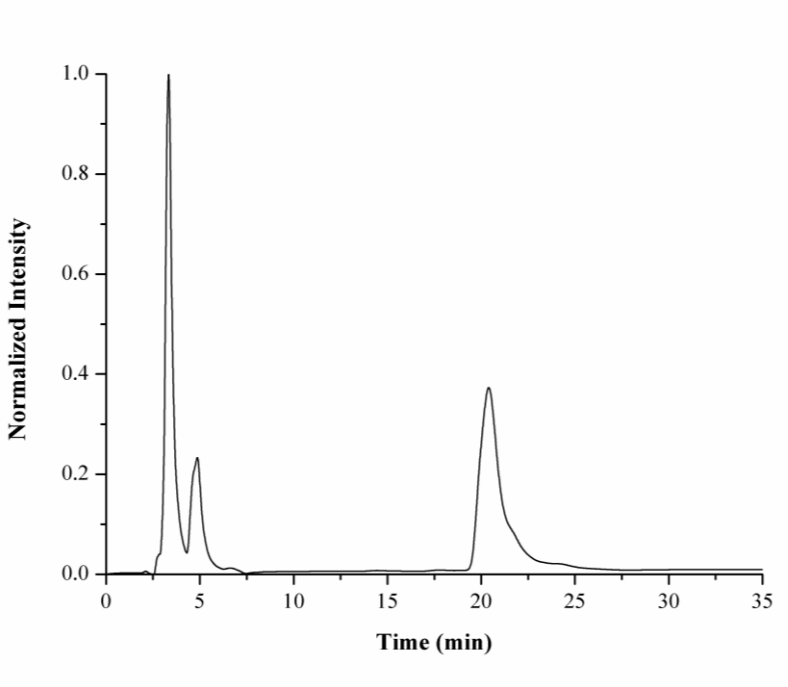
Figure 3.7 shows a spectra from sample after reaction between 10-undecenoic acid and HEMA has finished (72 h).

HEMA appears in 3.3 min, however, 10-undecenoic acid has a poor absorbance, as mentioned before. Peak in 5 min is related to solvent. Opposite to the behavior of the reaction with sorbic acid, in this case 10-undecenoic acid, being more hydrophobic, was able to undergo esterifi-

cation successfully, since ester is appearing in spectrum in 18.3 min. Conversion was obtained from HPLC spectrum, and it reached about 49% through calibration curve.

Graber *et al.* (2007), after testing six different solvents (2-pentanone, 3-pentanone, 2-methyl-2-pentanol, 3-methyl-3-pentanol, 2-methylpentane and 3-methylpentane) have shown that CALB is inhibited by ketone and tertiary alcohol, while hydrocarbons were not able to bind in the active site, allowing enzyme to interact with substrates.

Figure 3.7: Spectra of HPLC related to enzymatic esterification to produce MHU.



An inhibitory effect in Novozym 435 during reaction between sorbic acid and HEMA could be associated with solvent, however, when observing results of 10-undecenoic acid, chloroform allowed the production of ester.

Considering that 10-undecenoic acid was able to be converted into an ester, allied to the fact of this compound presents a pK_a of 5.02 (> 4.8), these observations corroborated the impossibility to convert sorbic acid

using procedure previously mentioned. Then, optimization of the esterification procedures were performed just with 10-undecenoic acid.

3.3.3 Evaluation of MHU enzymatic synthesis

3.3.3.1 Kinetics discussion

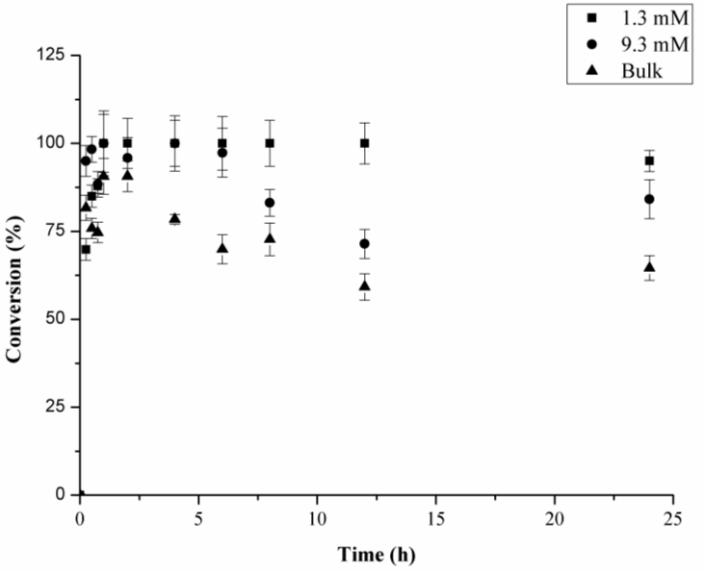
In order to evaluate kinetic behavior, different enzyme amounts were used to obtain the minimum enzyme concentration related to substrates, as well as minimum time able to reach about 100% of conversion. The presence of an organic solvent as chloroform (0.3 and 2.1 mL) was also analyzed, to verify if this compound could affect esterification reactions due to solubilization (positively) or enzyme inhibition (negatively). Temperature was maintained at 50 °C, since previous works have shown good activities at this temperature and above 50 °C, there is a risk of self polymerization of HEMA, reported in section 3.3.1.

Figure 3.8 shows the conversions using 10 wt% of enzyme (related to substrates). In this case, 2.1 mL of chloroform (1.3 mM of substrates) was able to better disperse substrates, in addition, produced water was partially solubilized in this system, reducing its concentration, which contributed with the maintenance of high conversions during long times.

High concentrations of enzyme are related to high activities for esterification, however, hydrolysis side reaction is also more propitious to occur since there is no molecular sieve to remove water. This could explain the reason for bulk and solution (9.3 mM of substrates) reactions to reduce their conversions, considering that short times (below 7 h) are insufficient to reach equilibrium, hydrolysis will increase in importance as a side reaction as the reaction time increases, specially in higher substrate concentrations (CHRISTMAN; LEWIS, 1921).

An ester could suffer hydrolysis depending on time, amount of water and presence of biocatalyst. Shimada *et al.* (1994) performed incubation of cells with cholesteryl ester and observed an increase in relation of free cholesterol and cholesteryl ester as time incubation was increased. They attributed this behavior to the hydrolysis of ester in presence of extralysosomal enzyme. Zhang *et al.* (2002) have observed a decrease in conversion after 5 days without using molecular sieves during esterification synthesis of 6-*O*-vinylacetyl glucose using Chirazyme L-2 C2 (CALB).

Figure 3.8: Kinetic study of MHU synthesis using 10 wt% of Novozym 435 at 50°C with different concentration of substrates (bulk, 1.3 and 9.3 mM).



The use of times up to 4 h to measure conversion for other enzyme percentages was possible, since enzyme 10 wt% was able to reach 100% of conversion quickly. Figures 3.9 and 3.10 show conversions for 5 and 1 wt% of enzyme, respectively. As long as enzyme percentage reduced, the time to achieve high conversions was longer, in other words, there was a reduction in reaction rate. Using enzyme 5 wt%, it was possible to note a reduction of conversion in bulk esterification after 2 h, most likely because of the hydrolysis side reaction observed previously.

Considering reactions with 1 wt% of enzyme, it is possible to observe that bulk and 9.3 mM reactions could reach about 100% of conversion in 2 h, while, 1.3 mM reactions reached conversion of 50% in the same time.

Lower concentration of enzyme could affect the possibility of an encounter between substrate molecules and biocatalyst in order to perform esterification reaction. Besides, low concentration of substrate could interfere in kinetic behavior.

Figure 3.9: Kinetic study of MHU synthesis using 5 wt% of Novozym 435 at 50 °C with different concentration of substrates (bulk, 1.3 and 9.3 mM).

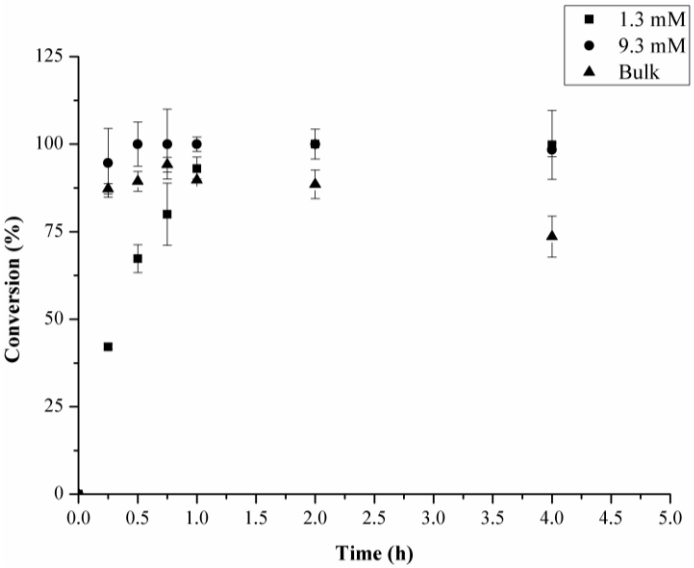
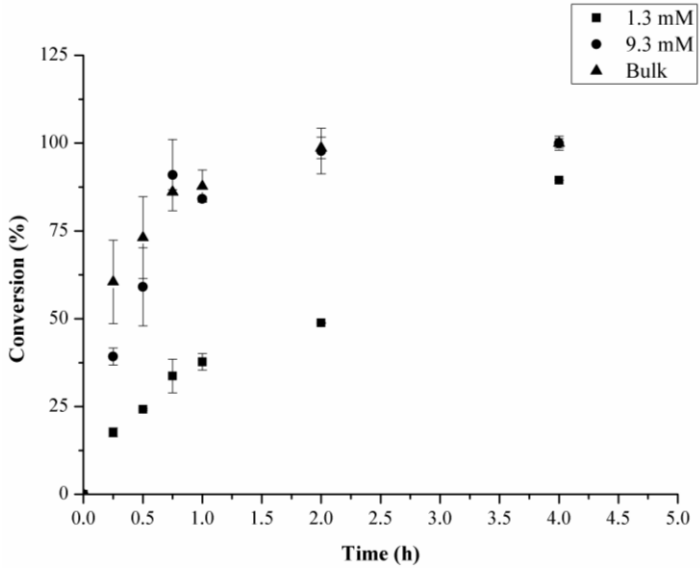


Figure 3.10: Kinetic study of MHU synthesis using 1 wt% of Novozym 435 at 50 °C with different concentration of substrates (bulk, 1.3 and 9.3 mM).



Wehtje and Adlercreutz (1997), working with esterification of decanoic acid with dodecanol in organic solvents (toluene and diisopropyl ether), have observed that lipases (from *Rhizopus arhizus* and*Candida rugosa*) presented optimal activity at low water activity (0.064) when low concentrations of substrates were used, reducing as the water activity was enhanced. Otherwise, at high concentration of substrate, enzyme activity enhanced with increase of water activity.

Low incidence of hydrolysis during reaction synthesis and less amount of biocatalyst, which reduces the cost of the process, allied to the purpose of avoiding the use of organic solvents, contributed to the selection of following conditions: 1 wt% of enzyme, without chloroform, applying 2 h as an optimum time to reach high conversions.

3.3.3.2 Reaction reuse of different lipases

Two immobilized lipases from *Candida antarctica* were used to compare their performances during the synthesis of MHU. Table 3.3 shows the characterization of enzymatic supports Novozym 435 and NS 88011.

Table 3.3:Characterization of immobilized enzymes applied in reuse studies (SÁ *et al.*, 2018).

Immobilized Enzyme	Novozym 435	NS 88011
Surface area (m^2/g)	78.7	15.2
Total pore volume (cc/g)	0.28	0.03
Average pore diameter (Å)	144.2	69.0

At first, these enzymes were applied using 5 wt% in relation to substrates to compare hydrolysis side reaction in these systems at 50 °C for 2 h. Table 3.4 presents all conversions of MHU obtained from HPLC analysis.

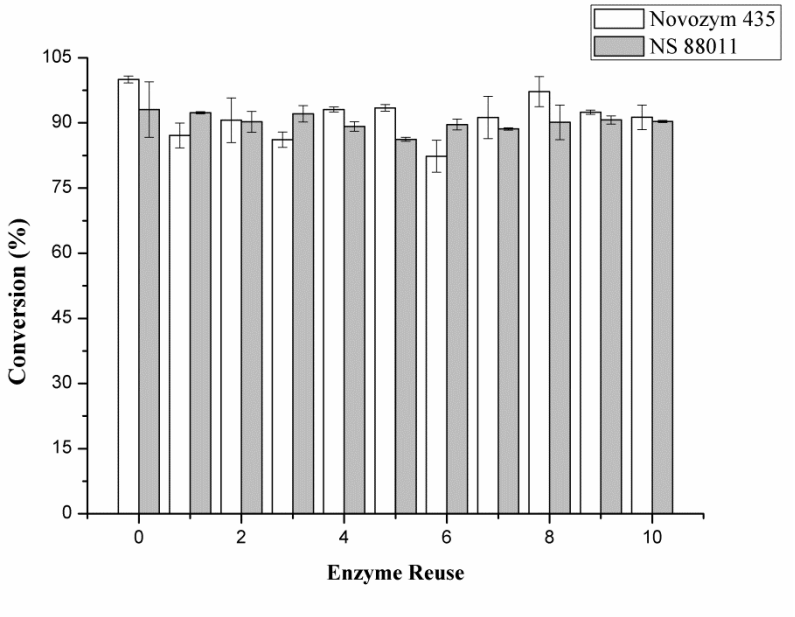
A higher incidence of hydrolysis is verified using Novozym 435 compared to NS 88011 in bulk reactions, which could be related with a higher activity of Novozym 435 in this esterification. Polloni *et al.* (2017), working with enzymatic polymerization of macrolactones at 60 °C, have observed that NS 88011 needed longer reaction times to achieve high yields.

Table 3.4: Conversion of MHU under two different supported enzyme systems using 5 wt% of lipases at 50 °C during 2 h.

Immobilized Enzyme	Concentration of substrates	Conversion (%)
Novozym 435	1.3 mM	100.0±2.0
	Bulk	88.5±4.0
NS 88011	1.3 mM	96.0±4.0
	Bulk	100.0±2.0

Applying optimum parameters obtained from kinetic section 3.3.3.1, reuse reactions were performed using two commercial enzymes, Novozym 435 and NS 88011, both CALB supported in two different materials. Figure 3.11 illustrates the behavior after 10 reuse reactions.

Figure 3.11: Comparison between reuse of Novozym 435 and NS 88011.



Two-way analysis of variance (ANOVA) at the 0.05 level did not show significant difference in "Enzyme Reuse" ($p = 0.05642$) neither in

"Type of Enzyme" (p = 0.15054), considering no interactions between factors.

When interactions are considered, however, at the same 0.05 level, once again "Type of Enzyme" did not show significant difference (p = 0.06664), meanwhile, "Enzyme Reuse" and the interaction between these factors became significant, which p was equal to 0.00606 and 0.00946, respectively.

Statistically, both enzymes are not significantly different, being the cycle of reuse the factor more important. In addition, Novozym 435 lipase reached the highest conversion, while it also showed high variations between reuses, ranging from 82.4 to 100%, while NS 88011 showed a variation from 86.5 to 93.1%. In general, even after 10 reuses, conversions achieved above 90%, which indicates good esterification activity for both enzymes.

Galonde *et al.* (2013), after applying 5 cycles of transesterification between D-mannose and vinyl myristate at 60 °C during 24 h using Novozym 435 as biocatalyst (5 wt%), have noted a reduction from 60 to 30% of conversion when solvent *t*-butanol was used. Foukis *et al.* (2017) have worked esterificating biobased acids using Novozym 435 aiming to develop a methodology of biofuel production, emphasizing reuse of the enzyme, since it was capable to maintain its esterification potential above of 90% after ten cycles. Sá *et al.* (2018) have applied Novozym 435 and NS 88011 in order to obtain benzyl propionate from benzyl alcohol and propionic acid, obtaining conversions of 25.6 and 14.3%, respectively.

The results involving the reuse of the enzymes become even more interesting since NS 88011 support is made from a low-cost hydrophobic polymeric resin developed by Novozymes® to be more cost competitive than Novozym 435 support (POLLONI *et al.*, 2017). In addition, the possibility of reuse of these biocatalysts makes the process as a whole less expensive.

3.3.3.3 Increase in production volume

Previous reactions were performed in 3 mL vials, then an increase in production volume was required to obtain an enough amount of monomer to apply in polymerization reactions and to test optimum parameters. As described in methodology, considering previous bulk reactions, the amount of substrate was increased from 0.43 g to 21.3 g, which indicates an increase of 50 times in monomer production volume.

Magnetic stirring and shaker were applied in order to maintain esterification reactions at 50 °C and gentle stirring. After 2 h, the enzyme

was removed by filtration and conversions of 88.2±0.7% and 83.5±1.7%, respectively, were obtained. These results shows magnetic stirring system offers a more homogeneous technique compared to shaker, which allowed the substrates come in contact with enzyme properly, resulting in higher conversion.

A conversion difference of about 10 % was observed when the sample from the magnetic stirring scale-up was analized comparing with a kinetic point (98.6±1.9 %) under the same conditions (bulk, lipase concentration of 1 wt% and 2 h of reaction), which could be attributed to differences in the diffusivity of the two systems.

3.3.3.4 Purification

It is important to highlight that conversion of 88% represents a monomer weight fraction of 0.77 in the reaction medium, since HEMA is used in excess, then a purification procedure is necessary to isolate MHU.

During purification steps for monomer obtained from exploratory studies, it was observed that ethyl acetate and methylethylketone were not good solvents to use in combination with hexane, since they were very difficult to remove from final product. Then, a solvent system with hexane:ethyl ether (2:1) was applied in monomer obtainment from scale-up section. Figure 3.12 shows ^{1}H NMR analysis for this monomer after purification.

Spectrum integration allows the calculation of purity. Integration of peak HEMA (δ = 3.81) leads 0.00877, however, it represents 2 atoms of H, so an unit for HEMA is calculated dividing that value by 2, obtaining 0.00439. On the other hand, peak 2, for instance, represents one atom of H from monomer, leading 0.02538. From these calculations, 1.00000 mol of monomer in this spectrum equals to 0.17277 mol of HEMA, considering that MW_{MHU} is 296.4 g·mol^{-1} and MW_{HEMA} is 130.14 g·mol^{-1}, monomer represents 93 wt% of analyzed sample.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.88-5.80 (m, 1 H, CH_2=C(CH_3)–COO–), 5.65-5.39 (ddt, *J* = 17.4, 10.3, 6.6 Hz, 1 H, –CH=CH_2), 5.34-5.25 (m, 1 H, CH_2=C(CH_3)–COO–), 4.78-4.56 (dd, *J* = 15.2, 8.8 Hz, 2 H, –CH=CH_2), 4.12-3.95 (m, 4 H, RCOO–CH_2–), 2.13-1.94 (td, J = 7.3, 4.9 Hz, 2 H, –CH_2–COO–), 1.83-1.71 (m, 2 H, –CH_2–CH=CH_2), 1.71-1.68 (s, 3 H, –CH_3), 1.44-1.21 (m, 2 H, –CH_2–CH_2–COO–), 1.17-0.83 (m, 10 H, CH_2) ppm.

Figure 3.12: ^{1}H NMR spectrum of purified MHU.

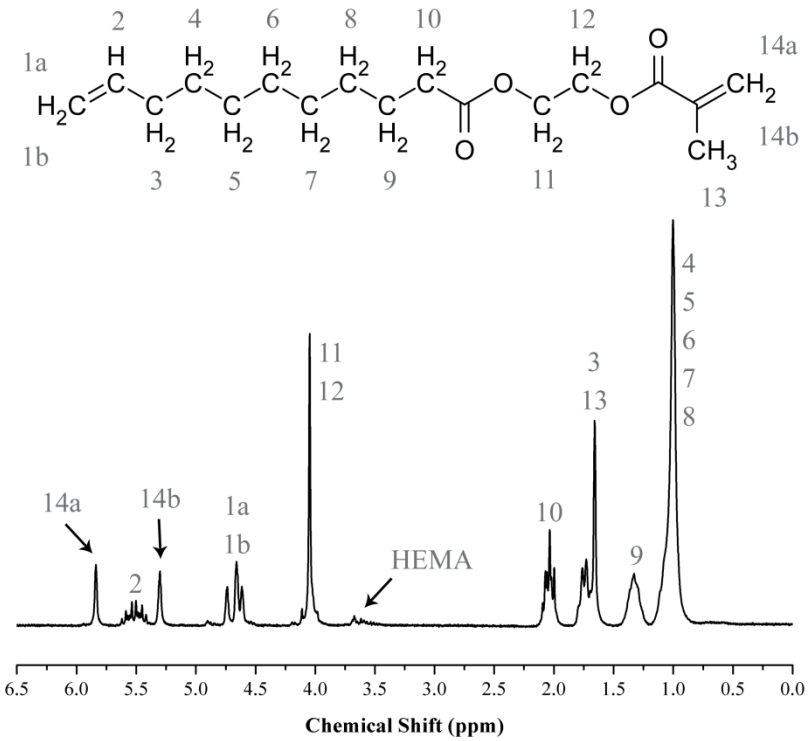
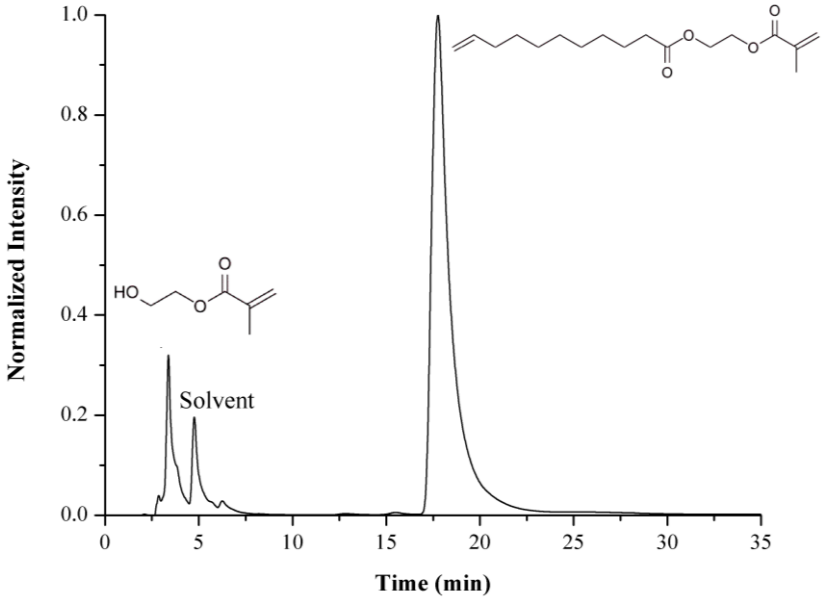


Figure 3.13 presents HPLC spectrum of purified MHU. This spectrum proves that a hydrophobic product was synthesized after enzymatic esterification, since it appeared at 18 min in a C18 column (hydrophobic). This result, in addition with ^{1}H NMR analysis, confirmed the presence of the ester.

Figure 3.13: HPLC of MHU after all purification steps.



3.4 FINAL CONSIDERATIONS

The comparison of tecniques using mild temperature conditions is interesting since it involves lower energy costs, besides of preserving the double bonds of unsaturated compounds. In this context, the synthesis of sorbic acid was only possible using Steglich procedure, however, purification step was difficult because of the presence of by-products and reagents with similar chemical behavior. Otherwise, 10-undecenoic acid was successfully converted into 2-(10-undecenoyloxy)ethyl methacrylate using both methodologies, with emphasis on enzymatic esterification since it was capable to obtain higher conversions and easier purification steps, besides of its environmentally friendly appeal, avoiding the use of hazadous materials and organic solvents.

However, the most interesting results were in relation to the enzyme reuse, in which enzymes Novozym 435 and NS 88011 maintained high conversions even after 10 cycles and the more economic enzymatic support (NS 88011) gave very promising results.

CHAPTER IV

4 THIOL-ENE AND FREE-RADICAL POLYMERIZATION OF DIENE ESTER MONOMER MHU

4.1 INTRODUCTION

This chapter shows the application of the 2-(10-undecenoyloxy)ethyl methacrylate (MHU) monomer in thiol-ene and free-radical polymerization, evaluating its behavior in bulk and miniemulsion systems, ranging initiator percentage and temperature, as well as surfactants in order to stabilize miniemulsion to obtain nanoparticles. This monomer was also reacted with diamine in order to use its asymmetry to obtain a symmetrical and functionalized monomer capable to polymerize by thiol-ene technique.

4.2 EXPERIMENTAL PROCEDURE

4.2.1 Materials

The initiator was α,α′-azoisobutyronitrile (AIBN, Vetec), while dithiol for thiol-ene polymerization was 1,4-butanedithiol (1,4-BDT, Sigma-Aldrich, 97%). Sodium dodecyl sulfate (SDS, Vetec) and Lutensol AT50 a poly(ethylene oxide) hexadecyl ether with 50 ethylene oxide units (Lut50, BASF) were applied as surfactants.

Chloroform (P. A., Qhemis) was chosen as solvent in Michael addition modification, while hexamethylenediamine (Sigma-Aldrich, 98%) was applied as nucleophile and catalyst of this reaction.

All materials were used as received, except hexamethylenediamine that was submitted at 60 °C during 24 h to remove water before reaction and AIBN, which was recrystallized. Distillated water was used in miniemulsion reactions.

4.2.2 Polymerization reactions

4.2.2.1 Polymerization of MHU

Bulk and miniemulsion polymerizations were performed into a conical bottom vial of 3 mL (Supelco) with screw cap, adding 0.5000 g of monomer for bulk and 2 mL of latex for miniemulsion polymerizations.

Nitrogen purging during 2 min was applied in systems before the beginning of polymerization. Reactions were carried out during 4 h under magnetic stirring and heating by magnetic stirrer IKA C-MAG HS 7 at 60, 70, 80 and 85 °C. Table 4.1 and 4.2 show formulations for bulk polymerizations of impure MHU (IMHU, 73%) and MHU after purification (93%), respectively, using 0.5000 g of monomer (IMHU or MHU).

Table 4.1: Bulk formulations for impure MHU polymerization.

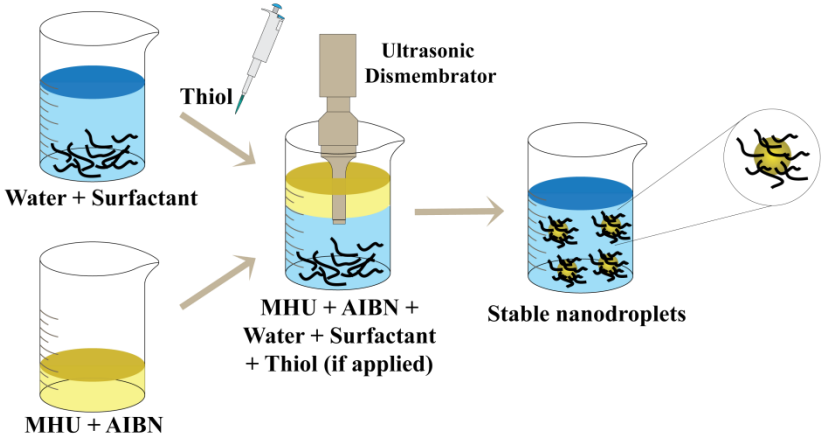
Reaction	Reagents (g)	
	1,4-BDT	AIBN
B01	0.2062	0.0014
B02	0.2062	0.0028
B03	0.2062	0.0056
B04	0.2062	0.0014
B05	0.2062	0.0028
B06	0.2062	0.0056
B07	0.2062	0.0014
B08	0.2062	0.0028
B09	0.2062	0.0056

Table 4.2: Bulk formulations for purified MHU polymerization.

Reaction	Reagents (g)	
	1,4-BDT	AIBN
B10	0.2062	0.0028
B11	0.2062	0.0028
B12	0.2062	0.0028
B13	0.2062	0.0014
B14	0.2062	0.0028
B15	0.2062	0.0056
B16	0.2062	0.0028
B17	0.2062	0.0056
B18	0.1824	0.0028
B19	0.1563	0.0028
B20	-	0.0028
B21	-	0.0056

A scheme of miniemulsion procedures is presented in Figure 4.1. In order to obtain miniemulsion systems, two pre-emulsions were prepared, the first one (1) mixing water and surfactant, while monomer and AIBN form the second one (2). After 20 min of magnetic stirring, 1 and 2 were put together and 1,4-BDT is added (for thiol-ene polymerizations), therefore the system is sonicated (Fisher Scientific, Ultrasonic Dismembrator 500, 400 W) for 2.5 min, using a 0.5" tip, with an amplitude of 60%. An ice bath was used to prevent the increase of temperature during the sonication.

Figure 4.1: Scheme of miniemulsion procedures for thiol-ene polymerizations.



Source: Author.

Surfactants were applied in concentrations of 9.0 μmol·cm^3 for SDS (2 wt% related to monomer) and Lut50 (20 wt%), and 13.5 μmol·cm^3 for Lut50 (30 wt%). Table 4.3 presents miniemulsion formulations applied in this study.

Table 4.3: Miniemulsion formulations for MHU polymerization.

Reaction	Reagents (g)				
	Water	**1,4-BDT**	**AIBN**	**SDS**	**Lut50**
M01	9.0000	0.3671	0.0111	-	0.2000
M02/04	9.0000	0.3671	0.0056	-	0.3000
M03/05	9.0000	0.3671	0.0111	-	0.3000
M06/08	9.0000	0.3500	0.0056	-	0.3000
M07/09	9.0000	0.3500	0.0111	-	0.3000
M10/12	9.0000	0.3500	0.0056	0.0235	-
M11/13	9.0000	0.3500	0.0111	0.0235	-
M14	9.0000	-	0.0056	-	0.3000
M15	9.0000	-	0.0111	-	0.3000

4.2.3 Modification of MHU through Michael Addition

In a 15 mL vial, 0.0017 mol of MHU (0.5000 g) was reacted with 0.0008 mol of hexamethylenediamine (0.0933 g) in 10 mL of chloroform, during 24 and 72 h, under magnetic stirring and heating by magnetic stirrer IKA C-MAG HS 7 at 50 °C.

4.2.4 Gel Permeation Chromatography (GPC)

Molar weight analyses were performed using gel permeation chromatography technique in LC-20A (Shimadzu) equipped with a precolumn PLgel MiniMIX (5 µm, 50×4 mm) and two columns PLgel MiniMIX (5 µm, 250×4,6 mm) in series. Applied eluent was tetrahydrofuran (THF) with flow rate of 0.3 $mL \cdot min^{-1}$ (40 °C) using refractive index detector RID-20A. In order to determine molar weight distribution, polystyrene standards ranging from 580 to 9,810,000 Da were used.

4.2.5 Nuclear Magnetic Resonance (^{1}H NMR)

^{1}H NMR was recorded in $CDCl_3$ on Bruker AVANCE DPX spectromoter operating at 200 MHz. Chemical shifts (δ) are reported in parts per million related to the internal standard tetramethylsilane (δ = 0.00 ppm).

4.2.6 Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS, Malvern Instruments, ZetasizerNano S) was used to obtain the intensity average diameters of droplets (Dp_0), particles (Dp) and polydispersity indexes (PDI). The latex samples were diluted approximately 1:15 with distilled water prior to DLS analysis.

DLS was also used to estimate M_w for insoluble polymers in THF. Five concentrations of polymer (2.96, 3.74, 5.93, 9.94 and 11.85 $g \cdot L^{-1}$) in chloroform were measured to obtain intensity (kcps), with these results Zetasizer software (Malvern) is capable to calculate Rayleigh ratios (R) for each concentration. Debye plot is obtained after plotting K[C]/R *versus* [C], where K is optimal contrast factor and [C] is concentration. The linear regression of these points gives a y-intercept that represents $1/M_w$ (Da^{-1}). Intensity of chloroform was measured through DLS resulting in 118.2 kcps, considering refractive index of 1.442.

4.2.7 Gel Content

Gel content was obtained by dilution of samples (0.02 g) in THF (4 mL) during 48 h and posterior filtration using a nylon filter (0.45 µm). The gel was retained by the filter (or in vial) and its weight measured and compared to the polymer content of the sample.

4.2.8 Differential Scanning Calorimetry (DSC)

Samples were dried at 60 °C for 24 h and analyzed (up to 9 mg) by a DSC 4000 Perkin Elmer, from -60 to 120 °C at a heating rate of 10 $°C \cdot min^{-1}$ under inert atmosphere (10 $mL \cdot min^{-1}$). The melting temperatures were obtained from the second heating run. Analyses were performed in the *Central de Análises* at the Department of Chemical and Food Engineering of the Federal University of Santa Catarina.

4.3 RESULTS AND DISCUSSION

4.3.1 Polymerization of MHU

4.3.1.1 Thiol-ene and free-radical bulk polymerization of MHU

Purification steps are often the most expensive and complex processes, then preliminary polymerization reactions with impure monomer

(IMHU), containing 73% of MHU, were performed in order to evaluate the necessity of posterior purification procedures. Table 4.4 shows thiol-ene polymerization reactions with different amounts of initiator applying three temperatures (60, 70 and 80 °C). Molar ratio of IMHU:1,4-BDT was maintained 1:1.

The reactions were performed during 4 h since times higher than 4 h do not seem to affect M_w. Machado *et al.* (2017) working with thiol-ene polymerization of dianhydro-D-glucityl diundec-10-enoate, an ester from 10-undecenoic acid and isosorbide, have tested these reactions for 4, 6 and 8 h at 70 °C and 1 mol% of AIBN, which gives M_w of 13.99, 15.00 and 14.16 kDa, respectively.

Table 4.4: Distribution of molar weight of impure monomer (IMHU) under different thiol-ene polymerization parameters in bulk system.

Reaction	Initiator (mol%*)	Temperature (°C)	M_w (kDa)	M_n (kDa)	Đ	N_2 purging
B01	0.5	60	3.3	1.6	2.0	YES
B02	1.0	60	2.9	1.6	1.8	YES
B03	2.0	60	3.4	1.6	2.1	YES
B04	0.5	70	3.9	1.7	2.3	YES
B05	1.0	70	3.6	1.7	2.1	YES
B06	2.0	70	4.8	1.9	2.6	YES
B07	0.5	80	3.4	1.7	2.1	YES
B08	1.0	80	2.8	1.5	1.9	YES
B09	2.0	80	4.0	1.7	2.4	YES

*Related to 1,4-BDT.

There is a high fraction of non-reacted HEMA after esterification reactions in IMHU, since the molar ratio of 10-undecenoic acid:HEMA is 1.0:1.2, it explains low molar weights observed from Table 4.4.

The influence of an increase of initiator concentration (2.0 mol%) could be related to an increase of free-radical contribution (self-polymerization), while a reduction of this parameter (0.5 mol%) could be related with low terminate rates, resulting both in an increase of M_w. However,

for these reactions with IMHU, effects as changes in percentage of initiator and increase of temperature could be compensate by the great fraction of non-reacted HEMA, which leads to this compound to act as a chain stopper in thiol-ene polymerization due to have only one double bond in its molecule.

These results justify the application of a purification process in order to reduce the presence of HEMA. All following reactions were performed using a purified monomer (93% of purity) described in section 3.2.4.4.

Table 4.5 represents distribution of molar weight under different thiol-ene polymerization parameters as temperature, mol% of initiator and application (or not) of N_2 purging for 2 min before reaction. Molar ratio of MHU:1,4-BDT was maintained 1:1.

Table 4.5: Distribution of molar weight of pure monomer (MHU) under different thiol-ene polymerization parameters in bulk system.

Reaction	Initiator (mol%*)	Temperature (°C)	M_w (kDa)	M_n (kDa)	Đ	N_2 purging
B10	1.0	60	7.1	3.8	1.8	YES
B11	1.0	70	7.3	3.5	2.0	YES
B12	1.0	80	8.8	4.1	2.2	YES
B13	0.5	80	5.8	3.2	1.8	NO
B14	1.0	80	4.8	2.9	1.7	NO
B15	2.0	80	4.8	2.9	1.6	NO
B16	1.0	85	4.9	2.9	1.7	NO
B17	2.0	85	4.9	2.9	1.7	NO
B18	1.0	80	13.6		2.9	YES
B19	1.0	80	29.1		4.8	YES

*Related to 1,4-BDT.

Comparing reactions B10, B11 and B12, the enhancement of temperature from 60 to 80 °C caused an increase in M_w (from 7.1 to 8.8 kDa), which also affected Đ (increased from 1.8 to 2.2). Machado *et al.* (2017)

have also observed an increase of M_w in 5.82, 14.00 and 21.42 when compared polymerizations performed at 60, 70 and 80 °C, respectively. Temperature affects half-life of AIBN and kinetic constants (propagation, chain-transfer and termination) which could explain this behavior.

Relative to effect of N_2 purging, comparing reactions B12 with B14, with the same mol% of initiator (1 mol%) and same temperature (80 °C), the use of N_2 purging for 2 min increased M_w from 4.8 (B14) to 8.8 kDa (B12). Despite of these reactions present little inhibition by oxygen presence, this reduction of M_w can be explained by the formation of an alkyl peroxy radical when a methacrylate react with oxygen, which could instantly conduct to termination step due to low reactivity of this radical (HOYLE *et al.*, 2003). In this case, comparing B13 with B14 and B15, a lower amount of initiator leads to a lower termination rate, increasing M_w from 4.8 (B14 and B15) to 5.8 kDa (B13).

In terms of reactivity and conversion of double bonds after thiol-ene reactions, Figure 4.2 shows a comparison in terms of ^{1}H NMR spectrum between MHU and the polymer resulted from thiol-ene polymerization (B12). Despite of lower reactivity of methacrylate double bond compared to the other from 10-undecenoic acid (HOYLE; LEE; ROPER, 2004), ^{1}H NMR spectrum shows chemical shifts in 5.52 and 4.67 ppm (10-undecenoic contribution) and 5.84 and 5.30 ppm (HEMA contribution) completely disappeared after polymerization, which indicates both double bond types were able to react with thiol in reaction conditions applied in this study.

Free-radical polymerization reactions were perform to test the potential of this material in order to act as a cross-linker. Table 4.6 presents gel content of this reactions.

Table 4.6: Gel content of free-radical polymerizations.

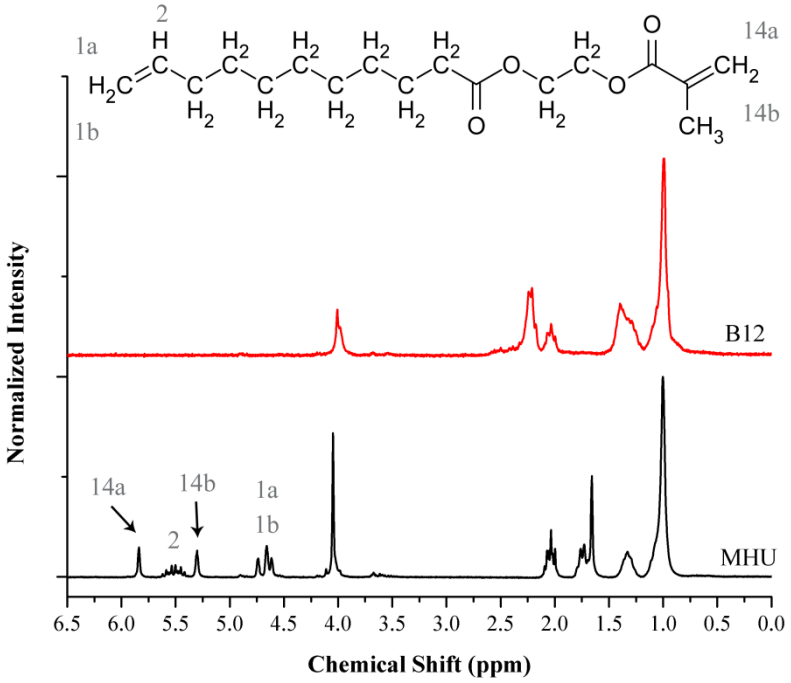
Reaction	Initiator (mol%*)	Temperature (°C)	Gel content (%)	N_2 purging (2 min)
B20	1.0	80	70±5	YES
B21	2.0	80	71±5	YES

*Related to MHU.

Polymer from a diene free-radical polymerization is almost insoluble in solvents, since after cross-linking there is a formation of a network of carbon chains, making solvent solvation process difficult, then a common analysis of molar weight as GPC is impaired. The solution is to

measure the gel content of polymers. Using MHU as cross-linker resulted in gel content of about 70%.

Figure 4.2: ^{1}H NMR spectrum from the polymer (PHU) obtained after thiol-ene polymerization (reaction B12) comparing with spectrum of MHU.



4.3.1.2 Thermal properties

Three different molar ratio of MHU:1,4-BDT, 1.0:1.0 (B12), 1.0:0.9 (B18) and 1.0:0.75 (B19) were submitted to thiol-ene polymerization at 80 °C, for 4 h and after 2 min of N_2 purging, in order to investigate its influence in thermal behavior through DSC analysis. Figure 4.3 and Table 4.7 show, respectively, DSC curves obtained from the second heating run and melting temperatures related to these polymers, comparing with B20 (after free-radical polymerization).

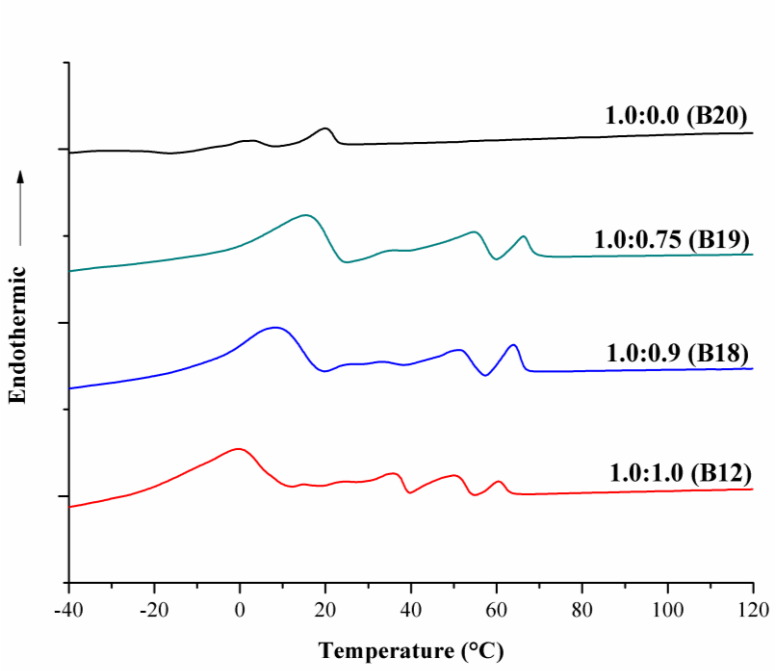
A decrease of melting temperature (T_m) was observed as the amount of 1,4-BDT increased. Higher molar weights resulted in an increase of T_m, which suggests that the reduction of 1,4-BDT

concentration could make the free-radical mechanism more important than the stoichiometric reaction between the double bond and the dithiol.

Table 4.7: Thermal behavior from bulk polymers from MHU through DSC analysis.

Sample	MHU:1,4-BDT	T_m (°C)	M_w (kDa)	Mn (kDa)	Đ
B20	1.0:0.0	20	-	-	-
B19	1.0:0.75	15	29.1	6.0	4.8
B18	1.0:0.9	8	13.6	4.8	2.9
B12	1.0:1.0	0	8.8	4.1	2.2

Figure 4.3: DSC curves of different molar ratio of MHU:1,4-BDT, 1.0:1.0 (B12), 1.0:0.9 (B18), 1.0:0.75 (B19) and 1.0:0 (Free-radical, B20).



Sánchez-Soto *et al.* (2002) have reported an increase of T_m with the enhance of M_w until 4 kDa, higher values of molar weights presented a stabilization in melting temperatures of poly(ethylene oxide) samples.

Wu *et al.* (2015), working with synthesis of poly(β-thioether ester) and poly(β-thioether ester-*co*-lactone) from methyl 3-((2-hydroxiethyl)thio) propanoate (MHETP) and ε-caprolactone have observed an increase in T_m from 5 to 47 °C with the enhancement of molar ratio between ε-caprolactone and MHETP from 0.5:0.5 to 0.8:0.2, which caused an increase in M_n from 44.4 to 51.4 kDa.

Multiple melting peaks are common in semicrystalline polymers, being reported, for example, in poly(ethylene terephthalate) (HIRAMATSU; HIRAKAWA, 1980), poly(ether ether ketone) (BLUNDELL, 1987), propylene-ethylene copolymers (FENG; JIN; HAY, 1998), poly(butylene-2,6-naphthalate) (JU; CHANG, 2001) and polymer from methyl 10-undecenoate (TÜRÜNÇ; MEIER, 2010). Recently, studies with poly(thioether esters) have observed double melting points (MACHADO *et al.*, 2017), and multiple melting temperatures during the first heating (ZHANG; DUMONT, 2018). In these cases, lower peak is related to partial melting of crystals and during intervals there is a continuous recrystallization and melting, while the higher peak represents the melting of organized crystallite (BLUNDELL, 1987).

4.3.1.3 Thiol-ene and free-radical miniemulsion polymerization of MHU

Nanoparticles were obtained by thiol-ene and free-radical miniemulsion polymerizations at 80 °C during 4 h from MHU. Table 4.8 shows intensity average size of droplets and particles though DLS analysis using two different surfactants (Lut50 and SDS). These surfactants were applied in concentrations of 9.0 $\mu mol \cdot cm^{-3}$ for SDS (a mass fraction of 2 %) and Lut50 (20 %) and 13.5 $\mu mol \cdot cm^{-3}$ for Lut50 (30 %). Figure 4.4 shows intensity percent distribution of particles applying Lut50 (2460 $g \cdot mol^{-1}$) and SDS (288.68 $g \cdot mol^{-1}$).

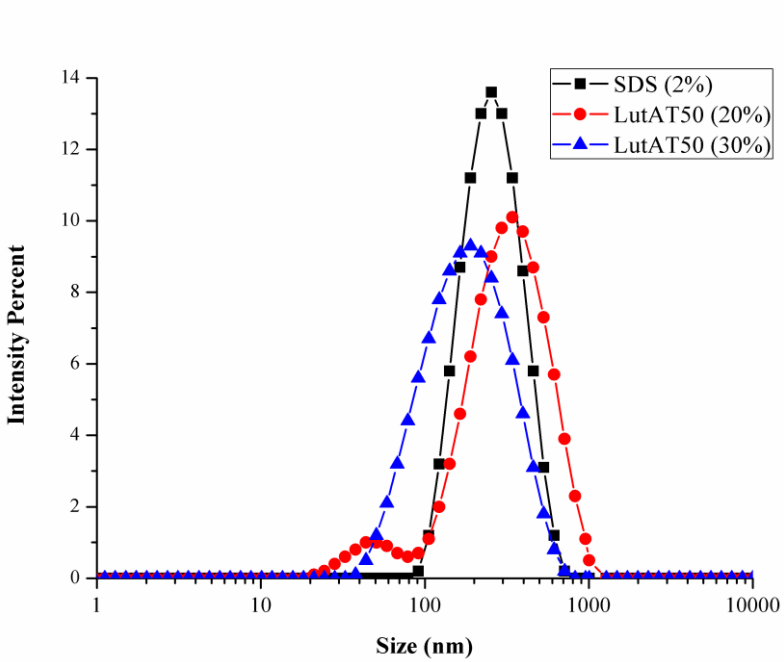
Comparing SDS (2%) and Lut50 (20%), with the same molar concentration, SDS was more efficient in order to protect droplets, giving better PDI, smaller droplet and particle diameters and higher stability, which could be seen in Figure 4.4. The mechanism of colloidal stability from SDS is different compared to Lut50, while the first one presents an eletrostatic coverage, the second one stabilize by steric protection. An increase in Lut50 concentration was necessary in order to promote a better stability. Machado *et al.* (2017) have compared particle size with SDS and Lut50, as surfactants, in same molar concentration, observing lower particle diameters with eletrostatic surfactant. Cardoso *et al.* (2018) have observed similar behavior when SDS and Lutensol AT80 were applied.

Table 4.8: Average droplet and particle size, respectively, and dispersion from DLS for miniemulsion polymerizations. Reactions were performed at 80 °C for 4h using 2 mol% of AIBN.

Reac-tion	Surf.	wt%*	Dp_0 (nm)	PDI_0	Dp (nm)	PDI
M01	Lut50	20	261±2	0.24±0.02	236±1	0.37±0.01
M05	Lut50	30	163±1	0.12±0.01	154±1	0.22±0.02
M13	SDS	2	226±2	0.15±0.01	230±2	0.15±0.01

*Related to the monomer.

Figure 4.4: Particle size distribution for miniemulsion polymerizations with used surfactants. Reactions were performed at 80 °C for 4h using 2 mol% of AIBN.



Due to its hydrophobicity, this monomer enabled stable dispersions in miniemulsion, with particle diameters of approximately 200 nm, without the presence of a co-stabilizer, which is quite interesting since more compounds generally make a system more expensive and complex.

TEM images are presented in Figure 4.5. Thiol-ene polymer nanoparticles are not completely solid due to low molar weights, which causes difficulties in this technique because there is melting during analysis. Machado *et al.* (2017) and Cardoso *et al.* (2018) have reported a melting of poly(thioether esters) due to electron beam of TEM equipment.

On the contrary, cross-linked nanoparticles, from free-radical polymerization (M15), showed a well-defined spherical morphology, while similar diameters were observed for particle population.

Table 4.9 presents molar weight distribution after thiol-ene and free-radical polymerizations in miniemulsion. In terms of molar weight, an enhance of amount of initiator had a slight increase in M_n, for both tested temperatures (70 and 80 °C), while an enhance of temperature from 70 to 80 °C allowed the growth from 5.9 to 9.2 kDa in M_w. Cardoso *et al.*(2018) have observed that an increase from 1.0 to 1.5 mol% of initiator allowed the reduction in M_n from 15.3 to 8.1 kDa. Machado *et al.* (2017) have presented an increase of M_w from 34.5 to 38.3 kDa at 70 and 80 °C, respectively, attributing this effect to reduction of viscosity, since 40% of polymerization degree was reached in beginning of reaction (5 min).

Despite of these results presenting lower M_w and M_n comparing with those obtained starting from dienes based on 10-undecenoic and 1,3-propanediol (408.7 $g \cdot mol^{-1}$) or isosorbide (478.7 $g \cdot mol^{-1}$), they seem compatible when compared with monomers with similar molar weight. Cardoso (2016) have shown M_w from 10.6 to 13.7 kDa when 1,3-propylene dipent-4-enoate (240 $g \cdot mol^{-1}$) was applied as monomer in thiol-ene polymerization.

Figure 4.5: TEM images of samples from miniemulsion after thiol-ene polymerization with Lut50 (M05) (a) and (b), and SDS (M13) (c) and (d). TEM images (e) and (f) are related to miniemulsion free-radical polymerization with Lut50 (M15).

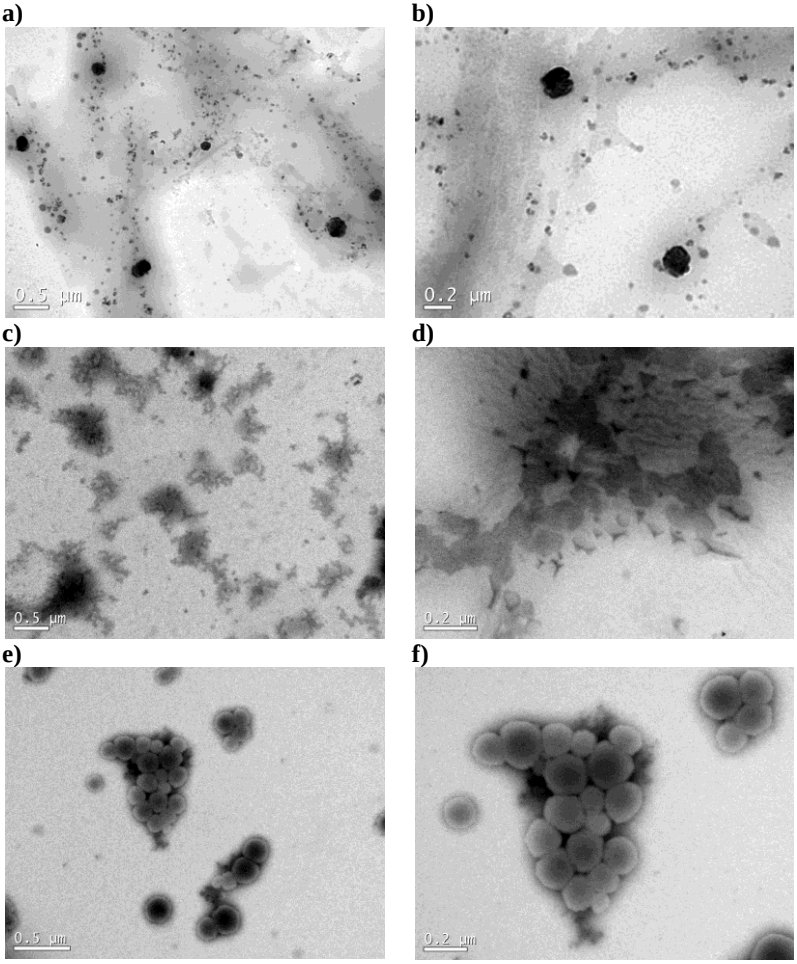


Table 4.9: Distribution of molar weight of MHU under different thiol-ene and free-radical polymerization parameters in miniemulsion system.

Reaction	MHU:1,4-BDT	I (mol%)	T (°C)	Dp	PDI	M_w (kDa)	Đ
M02[1]	1.0:1.0	1.0	70	161±1	0.17	5.9	1.8
M03[1]	1.0:1.0	2.0	70	158±1	0.20	5.9	1.8
M04[1]	1.0:1.0	1.0	80	153±1	0.21	9.1	2.4
M05[1]	1.0:1.0	2.0	80	154±1	0.22	9.2	2.3
M06[1]	1.0:0.9	1.0	70	240±4	0.24	15.4	3.3
M07[1]	1.0:0.9	2.0	70	201±1	0.25	15.5	3.4
M08[1]	1.0:0.9	1.0	80	181±1	0.25	14.7	3.4
M09[1]	1.0:0.9	2.0	80	237±3	0.25	16.2	3.7
M10[2]	1.0:0.9	1.0	70	201±1	0.25	16.6	4.0
M11[2]	1.0:0.9	2.0	70	196±1	0.25	18.0	3.8
M12[2]	1.0:0.9	1.0	80	243±4	0.15	17.0	3.5
M13[2]	1.0:0.9	2.0	80	230±2	0.15	15.3	3.5
M14[1]*	-	1.0	80	139±1	0.19	-	-
M15[1]*	-	2.0	80	140±1	0.19	-	-

[1] Lut50 surfactant; [2] SDS surfactant; * Free-radical polymerization.

Tables 4.5 and 4.9 shows molar weights are similar considering the comparison between M_w values of bulk (B12) and miniemulsion (M04) polymerizations being 8.8 and 9.1 kDa, respectively. Cardoso (2016) have worked with thiol-ene polymerization of symmetric terminal diene esters (1,3-propylene dipent-4-enoate and 1,3-propylene diundec-10-enoate), observing similar molar weights in bulk and miniemulsion.

However, the discrepancy between M_w is more pronounced when the ratio MHU:1,4-BDT is 1:0:0.9, since bulk presented M_w of 13.6 kDa while miniemulsion reached 14.7 (Lut50) and 17 kDa (SDS) under the same polymerization conditions. Machado *et al.* (2017) have observed an increase in molar weight in miniemulsion polymerization, attributing this

behavior to radical compartmentalization effect, which causes suppression of termination in free-radical mechanism (LANDFESTER, 2003). In fact, since the stoichiometry between MHU and 1,4-BDT is modified, reducing 1,4-BDT amount, the importance of free-radical mechanism tend to increase for these reactions..

According to Cramer *et al.* (2003), double bond type and stoichiometry between monomer and thiol are important factors in thiol-ene reaction. The average number of radicals per particle do not seem trivial to analyze comparing with free-radical polymerization, involving different tendencies for different monomers.

4.3.2 Modification of MHU through Michael Addition reaction

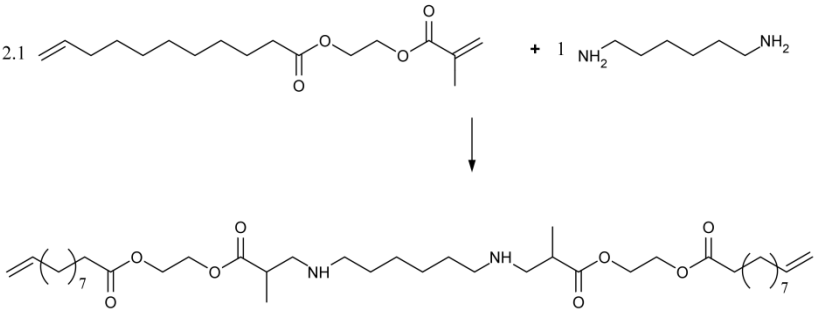
4.3.2.1 Synthesis of functionalized MHU using a diamine

There are differences in reactivity when an asymmetrical monomer is applied in polymerization reactions, since depending on technique, double bonds could behave differently. However, it is not always a problem, such feature could be used in cases wherein a double bond is reactive with a compound while another one is inert in the presence of this same compound.

Taking advantage of selectivity of Michael addition, MHU was reacted with a diamine (HMD) in order to build a new functionalized monomer, this time a symmetrical molecule containing olefin double bond in both sides of carbon chain.

Figure 4.6 presents the scheme of Michael addition reaction and the expected product.

Figure 4.6: Scheme of Michael addition between MHU and HMD.



Source: Author.

Navarro *et al.* (2017) have performed reaction between 2-(acryloyloxy)ethyl methacrylate and dibutylamine, in chloroform at 40 °C for 3 h, resulted in conversion of 85% even without catalyst. When they compared solvents, chloroform and acetonitrile were capable to conduct rapid conversions. On the other hand, THF and acetone performed the lowest conversions, which is related to weakly basic oxygen functions of these solvents, which interferes in protonation equilibrium and conducts little or no conversion (WABNITZ; SPENCER, 2003).

These reactions were then performed using chloroform as solvent for up to 72 h at 50 °C.

Figure 4.7 shows a comparison between ^{1}H NMR spectra from MHU with from modified MHU with HMD after 72 h. Peaks related to olefin double bonds was not affected by Michael addition as observed comparing intensities of chemical shifts 5.52 and 4.67 ppm in MHU and MHU-HMD, then a strategy to calculate conversion is the integration of peaks from methacrylate double bond, in other words, chemical shifts in 5.84 and 5.30 ppm, comparing with one of those belonged to olefin.

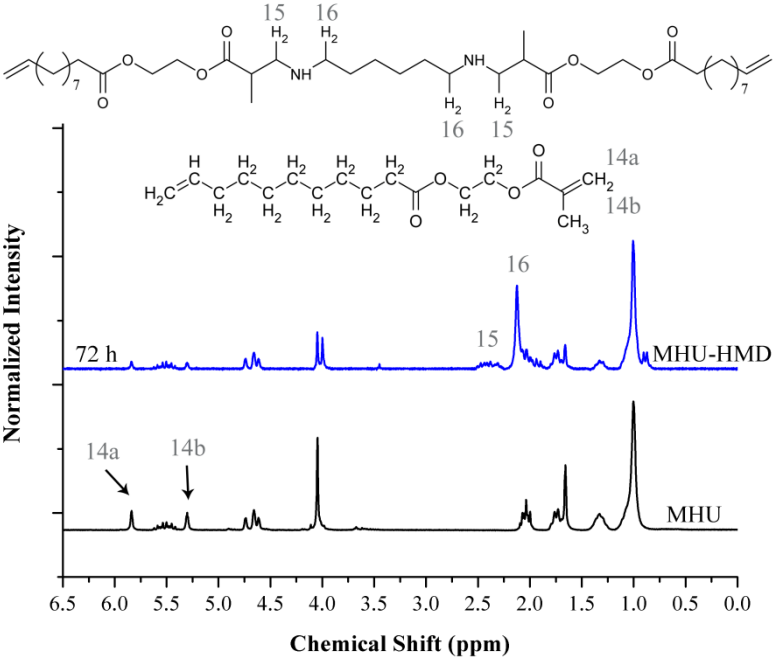
An excess of MHU was used to ensure that its ester would be attached in both sides of diamine, being considering in calculation of conversion. Reaction was performed using 0.0017 mol of MHU and 0.0008 mol of HMD, which indicates an excess of 0.125.

After integration of peaks in Figure 4.7 (MHU-HMD after 72 h), the relation of $\delta C{=}C_{HEMA}/\delta C{=}C_{olefin}$ was calculated and gave 0.32. In order to calculate conversion, contributions of excess of MHU (0.125) was discounted from $C{=}C_{HEMA}$ (0.320). Then a conversion of 80.5% was obtained. Using the same procedure, conversion of reaction after 24 h was reached as 45.8%.

Wu *et al.* (2004) have observed that reactivity of amines changed with the running of polymerization during Michael addition of diacrylates with trifunctional amines. An acceptable reactivity sequence is defined as 2° amine > 1° amine, however, after reaction, formed 2° amines had a reactivity smaller than 1° amines. Researchers have attributed this behavior to steric hindrance of carbon-chain, which causes difficulty in interacting nucleophile with double bond.

González *et al.* (2015) have observed that a substitution of an acrylate by a methacrylate group conducted to a reduction in conversion from 87 to 9% in Michael addition with poly(ethyleneimine) and bisphenol A glycerolate diacrylate. They attributed this fact to the reduction of electrophilic behavior because of the steric hindrance caused by methyl group.

Figure 4.7: ^{1}H NMR spectra from MHU modified with HMD (MHU-HMD) after 72 h, in comparison with spectrum of MHU.



These studies indicated that primary amine is more reactive than a secondary amine already reacted in Michael Addition due to steric hindrance, in addition, methacrylate also confers steric hindrance because of its methyl group, which suggests that reactions with MHU conduct to a partial addition in amine functional group, preventing two methacrylates could react with the same amine group from HMD, leading to the obtainment of the symmetrical monomer proposed in Figure 4.6.

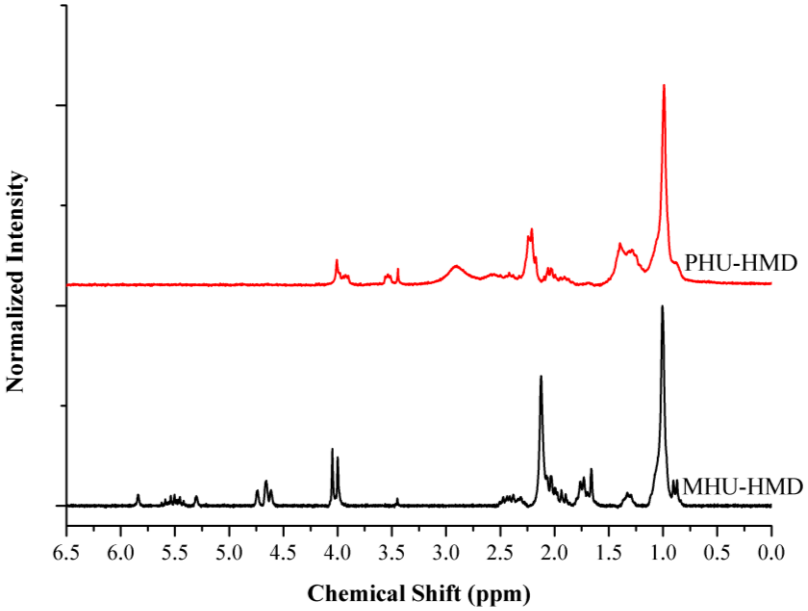
4.3.2.2 Polymerization of MHU-HMD

In order to test reactivity of this new monomer, a thiol-ene polymerization was performed using 1 mol% of AIBN as initiator, at 80 °C and a molar ratio between monomer and 1,4-BDT was 1:1. Figure 4.8 presents spectrum of polymer (PHU-HMD), which can be seen a disappearance of peaks related to H of double bonds (5.84,5.52, 5.30 and 4.67 ppm).

This polymer, on the contrary of previous reactions, was not soluble in THF, hindering GPC analysis. A strategy to estimate its molar weight was using DLS analysis to obtain Debye plot. An adjusted R^2 of 0.84 was obtained after linear regression, resulting in an intercept of $8.87 \cdot 10^{-5}$ Da^{-1}, which gives a M_w of 11.3±0.9 kDa. Comparing this result with M_w obtained through thiol-ene polymerization of MHU in the same conditions B12 (8.8 kDa), this data shows a higher M_w value which could be related to the chain length of MHU-HMD that is higher than MHU. Figure 4.9 shows graph treatment for DLS measures considering refractive index increment (dn/dc) of 0.095 $mL \cdot g^{-1}$.

Kroeger *et al.* (2013) have measured a dn/dc of 0.0948 $mL \cdot g^{-1}$ for a dendronized linear polymer, containing ester and amine groups in its molecule, in chloroform at the same wavelength of 633 nm.

Figure 4.8: ^{1}H NMR spectrum from PHU-HMD after thiol-ene polymerization.



In terms of thermal properties, Table 4.10 and Figure 4.10 show, respectively, DSC melting temperatures and endothermic curves obtained

from the second heating run of polymers from bulk thiol-ene polymerizations at 80 °C using 1 mol% of AIBN as initiator and a molar ratio between monomer and 1,4-BDT of 1:1. This result shows PHU-HMD also presents a semicrystalline characteristcs, considering multiple melting peaks. However, its T_m was higher than T_m obtained by B12, which could be related to different functionality conferred on PHU-HMD polymer, since M_w is quite similar to obtain a DSC curve and T_m completely different.

Figure 4.9: Debye plot in order to obtain molar weight of PHU-HMD.

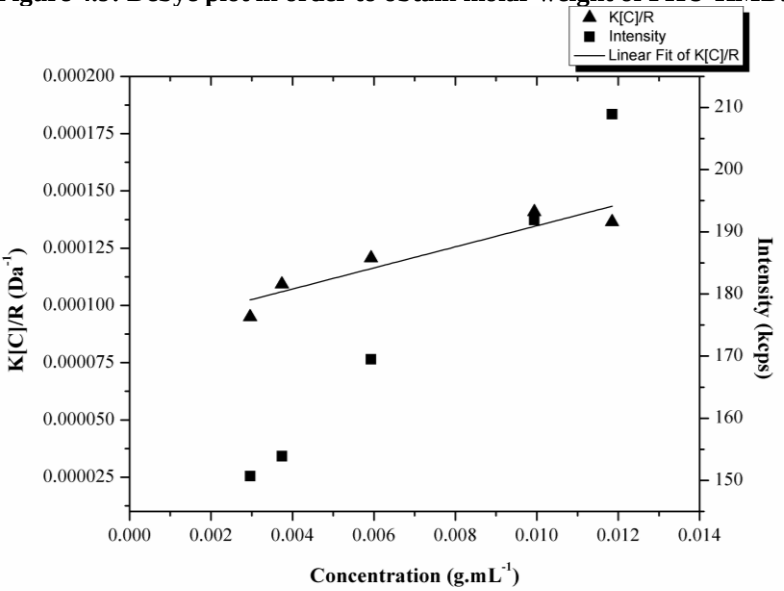
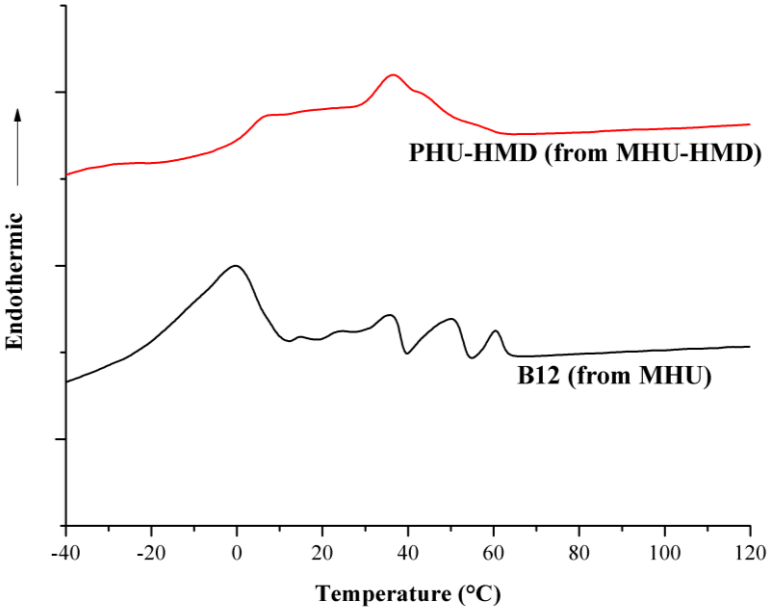


Table 4.10: Thermal behavior of bulk polymers from monomers MHU-HMD and MHU through DSC analysis.

Sample	Initiator (mol%)	T (°C)	t (h)	Monomer	Ene:thiol	T_m (ºC)
PHU-HMD	1.0	80	4	MHU-HMD	1:1	36
B12	1.0	80	4	MHU	1:1	0

Figure 4.10: DSC analysis of polymers PHU-HMD (from MHU-HMD monomer) and B12 (from MHU monomer) obtained by thiol-ene polymerization at 80 °C using 1 mol% of AIBN as initiator and a molar ratio between monomer and 1,4-BDT of 1.0:1.0.



4.4 FINAL CONSIDERATIONS

The diene ester monomer MHU, obtained from enzymatic esterification and partially derived from a plant oil, was properly polymerized by thiol-ene polymerization. This technique was an useful method for the synthesis of renewable poly(thioether ester) and it could be an interesting substitute for petroleum-derived polymers with specific functionalities. The stoichiometric imbalance was able to compensate the lower molar weights.

In addition, the use of the miniemulsion technique for the polymerization processes is important to minimize the application of organic solvents, which makes the process even more environmentally friendly.

Through Michael addition reaction, MHU was capable to be chemically tranformed into a symmetric compound, which was also polymerized by thiol-ene polymerization in order to obtain a poli (amino-thioether ester), showing different chemical-thermal properties when compared to the polymer obtained from the thiol-ene polymerization of MHU diene ester monomer.

CHAPTER V

5 FINAL CONSIDERATION

5.1 CONCLUSION

The application of natural and renewable raw materials is no longer enough in modern industries, it is also important that the processes are more sustainable, so there has been a growth of studies using enzymes as biocatalysts for chemical transformations in recent years.

In this context, this work was successful in studying an enzymatic esterification process for the production of a partially derived natural diene ester monomer, obtaining high conversions in mild temperature conditions even under solvent-free systems. In addition, the results demonstrated that enzymes were able to maintain high conversions even after 10 cycles of reuse, which makes the process less costly.

The diene ester monomer was also capable to be converted to polymers by thiol-ene and free-radical polymerizations in both bulk and miniemulsion systems, avoiding organic solvents as reaction media, despite of its asymmetry. M_w values up to 29 kDa and gel content of 70 %, respectively, was reached. Besides, the miniemulsions were stable with no co-stabilizer, presenting particle diameters of approximately 200 nm and providing higher molar weights in comparison to bulk reactions.

An asymmetric compound such as MHU has challenges since the terminal double bonds contained in its molecule have different reactivities, however, it can be an advantage when it is desired to preserve one double bound over another. By Michael addition, the ester was converted to a symmetric compound and it was able to undergo thiol-ene polymerization, resulting in a final polymer that exhibited functionality of both the diamine (used in synthesis) and the dithiol (of the polymerization step), modifying its solubility in THF, since it presented a more hydrophilic behavior.

Therefore, future studies will be carried out in order to characterize these new polymers in terms of physicochemical properties as well as properties such as biodegradability and biocompatibility.

5.2 FURTHER WORK

- To apply enzymatic synthesis described in this work using other unsaturated carboxylic acids or alcohols;
- To test other thiol molecules in polymerization reaction with MHU through bulk and miniemulsion systems;
- To evaluate the degradation of these polymers in different media in order to test the biodegradability of these materials, since they are derived from an ester capable to undergo hydrolysis.

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