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POLY(GLOBALIDE) NANOPARTICLES PREPARATION AND MODIFICATION FOR POTENTIAL DRUG RELEASE APPLICATIONS

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Felipe Oliveira Lima

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O presente trabalho em nível de mestrado foi avaliado e aprovado, em 12 de abril de 2023, pela banca examinadora composta pelos seguintes membros:

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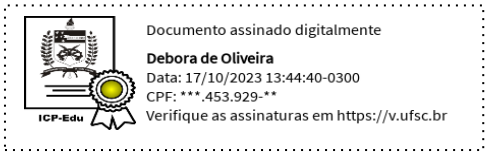
Certificamos que esta é a versão original e final do trabalho de conclusão que foi julgado adequado para obtenção do título de mestre em Engenharia Química



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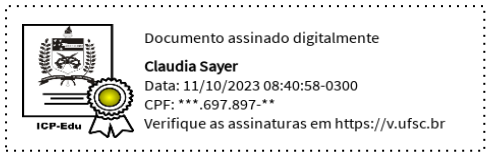

Profª. Drª. Claudia Sayer
Orientadora

Florianópolis, 2023.

Aos meus pais

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“Now I'm a scientific expert; that means I know nothing about absolutely *everything*.”
(Arthur C. Carke, 1968)

RESUMO EXPANDIDO

Introdução

O desenvolvimento de polímeros biocompatíveis e biodegradáveis os quais apresentam propriedades de interesse para aplicações biomédicas, por exemplo, vem sendo estudado extensivamente nas últimas décadas. Nesse contexto, poliésteres emergem como uma classe de polímeros promissora como alternativas para materiais tradicionais devido a características como biocompatibilidade, biorresorbabilidade e biodegradabilidade. Além disso, a possibilidade desses materiais serem obtidos por meio da polimerização por abertura de anel enzimática apresenta diversas vantagens em relação a catalisadores convencionais como condições de reação mais brandas e menor geração de resíduos tóxicos. Entre os poliésteres recentemente estudados, o poli(globalide) (PGl) apresenta um grande potencial de aplicações devido à presença da insaturação em sua unidade de repetição que pode ser modificada através de reações tiol-eno, mudando propriedades como grau de reticulação, cristalinidade e degradabilidade que podem influenciar na liberação de fármacos. A liberação sustentada de fármacos pouco solúveis é um problema significativo para diversos tratamentos atualmente. Doenças Tropicais Negligenciadas, como a doença de Chagas, afetam milhões de pessoas em todo mundo anualmente e seu tratamento ainda apresenta obstáculos como eficácia limitada e fortes efeitos colaterais causados pelo fármaco hidrofóbico utilizado, o benznidazol (BNZ). Nesse contexto, a utilização de nanopartículas (NPs) poliméricas vem sendo investigada como forma de melhorar formulações de agentes terapêuticos com o BNZ como forma de melhorar seu desempenho aumentando sua dispersibilidade e biodisponibilidade em condições fisiológicas, modulando sua liberação sustentada contribuindo para a adesão de pacientes ao tratamento e eficácia do medicamento. O presente trabalho relata a síntese de PGl por polimerização enzimática por abertura de anel e sua subsequente reticulação com 2,2-(etilenodioxi)dietanotiol (EDDT) via reação tiol-eno em presença do iniciador térmico azobisisobutironitrila (AIBN). O polímero obtido foi usado na preparação e reticulação de nanopartículas pelo método de miniemulsificação-evaporação de solvente e, em seguida, foi realizada a encapsulação de BNZ adaptando as formulações utilizadas. Por fim os materiais obtidos foram caraterizados e um ensaio de liberação sustentada foi realizado.

Objetivo

Objetivo Geral

Este trabalho teve como objetivo geral preparar avaliar a utilização de nanopartículas de poliéster insaturado modificado (poly)globalide como potenciais nanocarreadores do fármaco benznidazol.

Objetivos específicos

a) Preparar poliglobalide através de polimeirização por abertura de anel (e-ROP) caracterizá-lo;

b) Modificar PGl através da reticulação em solução com 2,2-(etilenodioxi)dietanotiol (EDDT) via reação tiol-eno em presença do iniciador térmico azobisisobutironitrila (AIBN) em diferentes formulações e caracterizar os materiais obtidos;

c) Preparar NPs de PGl através da técnica de miniemulsificação/evaporação de solvente e caracterizá-las em relação a seu tamanho, morfologia, propriedades térmicas e estabilidade;

d) Realizar testes de encapsulação do fármaco hidrofóbico BNZ também através da técnica de miniemulsificação/evaporação de solvente e modificá-las, em processo análogo à reticulação em solução mencionada anteriormente e, em seguida, caracterizá-las em relação a seu tamanho, morfologia, estabilidade, propriedades térmicas e eficiência de encapsulação;

e) Conduzir ensaios de liberação sustentada de NPs de PGl e PGl modificado para avaliar e entender os efeitos da reticulação na taxa de liberação.

Metodologia

A síntese do polímero foi realizada pelo método de polimerização enzimática por abertura de anel de globalida (Gl). A reticulação foi realizada em solução via reação tiol-eno com EDDT e em presença do iniciador térmico AIBN com 4 formulações diferentes variando a relação tiol-eno e a concentração de iniciador. Os polímeros obtidos foram caracterizados em termos de massa molar, estrutura química através das técnicas convencionais de cromatografia de permeação em gel (GPC), espectroscopias por ressonância magnética nuclear (NMR) e de infra-vermelho por transformada de Fourier (FT-IR). Propriedades térmicas como grau de cristalinidade, entalpia e temperatura de fusão dos polímeros foram determinadas através de calorimetria diferencial de varredura (DSC). Visando a nanoencapsulação de BNZ, nanopartículas (NPs) de PGl e PGl reticulado foram produzidas pelo método de miniemulsificação-evaporação de solvente e, em seguida, NPs carregadas de fármaco foram produzidas pelo mesmo método. As NPs foram então caracterizadas em relação a propriedades como distribuição de tamanho de partícula e potencial zeta utilizando as técnicas de espalhamento dinâmico de luz (DLS) e a morfologia por meio de microscopia eletrônica de transmissão (TEM). As propriedades térmicas também foram verificadas por DSC. Por fim, a eficiência de encapsulação foi determinada pela quantificação por espectrofotometria de luz ultravioleta e visível (UV-vis), e um ensaio de liberação em condições controladas análogas ao meio fisiológico (pH=7.3 e T=37ºC) foi conduzido com quantificação também por UV-Vis.

Resultados e Discussão

A reticulação do PGl reduziu significantemente a cristalinidade do polímero formando polímeros com características amorfas que podem aumentar a biodegradabilidade e, por conseguinte, viabilizar sua aplicação várias aplicações biomédicas incluindo a liberação sustentada de fármacos. Nanopartículas estáveis com tamanho em torno de 100 nm e baixo PDI (<0,2) foram produzidas e sua caracterização indicou também uma diminuição de cristalinidade com a reticulação com EDDT e completa amorfização dos materiais com a incorporação do fármaco na matriz polimérica. As eficiências de encapsulação nas NPs de PGl e PGl reticulado foram de 92 e 85%, respectivamente, corroborando a aplicabilidade do poliéster para encapsulação de BNZ e outros fármacos hidrofóbicos em potencial. Os ensaios de liberação mostraram que a reticulação aumentou a taxa de liberação de BNZ em condições fisiológicas, mostrando que é possível modular as propriedades de liberação pela metodologia utilizada.

Considerações Finais

A reticulação do PGl tanto em solução e como em nanopartículas via reação tiol-eno foi realizada reduzindo significantemente a cristalinidade dos materiais obtidos demonstrando potencial para aplicações biomédicas como o carreamento de fármacos e sua liberação sustentada. Além disso, altas eficiências de encapsulação e a capacidade de modulação da taxa de liberação através da modificação das NPs de PGl foram verificadas demonstrando o a possibilidade de preparo de nanocarreadores através de um método direto de encapsulação que pode ser útil como forma de melhorias para tratamentos terapêuticos variados

Palavras-chave: poli(globalide), benznidazol, nanopartículas, liberação sustentada de fármacos, reação tiol-eno.

RESUMO

Poliésteres despontaram como uma categoria em potencial de polímeros biodegradáveis que podem servir como alternativa a materiais convencionais no campo biomédico devido às suas propriedades, incluindo biocompatibilidade, bioabsorção e biodegradabilidade. A polimerização enzimática por abertura do anel (e-ROP) de lactonas é uma alternativa "verde" para preparar poliésteres devido à ausência de subprodutos tóxicos e à possibilidade de reaizar reações em condições brandas. As nanopartículas poliméricas são um sistema promissor para o transporte e liberação sustentada de fármacos pouco solúveis em aplicações biomédicas devido às suas propriedades únicas, tais como alta estabilidade, biocompatibilidade, e a capacidade de incorporar uma grande variedade de substâncias. O benznidazol (BNZ) é um medicamento antiparasitário cuja eficácia é diminuída pela sua elevada toxicidade e baixa solubilidade. A utilização de nanocarreadores poliméricos é uma abordagem proeminente para melhorar a absorção de ativos hidrofóbicos, aumentando a eficiência e rapidez de efeitos farmacológicos. Esta abordagem pode melhorar, por exemplo, o tratamento e sistemas de liberação sustentada com BNZ. Neste estudo, síntese de PGl foi conduzida seguida de sua modificação por reação tiol-eno para reticulação usando 2,2-(etilenodioxi)dietanotiol (EDDT) como agente reticulante na presença do iniciador térmico azobisisobutironitrila (AIBN). PGl apresentou Mw e Mn de 44926 $g \cdot mol^{-1}$ e 16700 $g \cdot mol^{-1}$, respectivamente e dispersão Đ de 2,69. A cristalinidade dos polímeros reticulados foi significativamente reduzida, sugerindo que as propriedades do polímero PGl podem ser moldadas para atender a aplicações específicas incorporando agentes de reticulação em sua estrutura. Além disso, este trabalho teve como foco o desenvolvimento de uma plataforma para liberação de BNZ por meio da preparação de nanopartículas (NPs) de PGl pelo método de miniemulsificação/evaporação de solvente (MSE). As NPs foram então modificadas usando a reação química tiol-eno em uma abordagem análoga ao procedimento mencionado anteriormente. As NPs obtidas apresentaram formas esféricas, tamanho submicrométrico e distribuição estreita (*ca*. 100 nm e PDI <0,2)). A reticulação de NPs e a incorporação de BNZ em NPs também diminuíram a cristalinidade dos materiais. As eficiências de encapsulação em NPs de PGl e PGl reticulados foram de 92% e 85%, respectivamente, confirmando a aplicabilidade do poliéster para a encapsulação de BNZ. O estudo de liberação sob condições controladas revelou que as NPs reticuladas apresentaram taxas de liberação do fármaco mais rápidas devido a sua menor cristalinidade. Em resumo, o emprego de PGl e um processo simples de preparação de nanocarreadores tem grande potencial para várias aplicações de liberação de fármacos. Essa abordagem pode beneficiar formulações de BNZ e de outros compostos hidrofóbicos que requerem melhorias em seus respectivos tratamentos e administração de dosagem.

Palavras-chave: poli(globalide), benznidazol, nanopartículas poliméricas, liberação sustentada de fármacos, reação tiol-eno.

ABSTRACT

Polyesters have emerged as a potential category of biodegradable polymers that can serve as an alternative to conventional materials in the biomedical field due to their properties, including biocompatibility, bioresorbability, and biodegradability. Enzymatic ring-opening polymerization (e-ROP) of lactones is a green alternative to prepare polyesters due to the absence of toxic byproducts and the possibility of carrying reactions in mild conditions. Polymeric nanoparticles are a promising tool for the delivery of poorly soluble actives and sustained release in biomedical applications because of their unique properties, such as high stability, biocompatibility, and the capacity to enclose various substances. Benznidazole (BNZ) is an antiparasitic medication whose efficacy is diminished by its high toxicity and low solubility. Utilizing polymeric nanocarriers is a promising approach for accelerating the absorption of highly hydrophobic actives, leading to a more rapid and efficient onset of pharmacological effects. This approach could improve, for instance, BNZ therapeutics and controlled release systems. In this study, PGl synthesis by e-ROP was conducted, followed by its modification by thiol-ene crosslinking reactions using 2,2′-(ethylenedioxy)diethanethiol (EDDT) as a crosslinking agent in the presence of thermal initiator azobisisobutyronitrile (AIBN). PGl presented Mw and Mn of 44,926 $g \cdot mol^{-1}$ and 16,700 $g \cdot mol^{-1}$, respectively, and polydispersity Đ of 2.69. The crystallinity of crosslinked polymers was significantly reduced, suggesting that the polymer PGl can be tailored to meet specific applications by incorporating crosslinking agents into its structure. In addition, this work focused on developing a platform for BNZ delivery by preparing polymacrolactone nanoparticles (NPs) via the miniemulsification-solvent evaporation (MSE) method from PGl. The NPs were then modified using thiol-ene chemistry in an analogous approach to the procedure mentioned previously. The obtained NPs presented spherical shapes, small size, and narrow distribution (*ca*. 100 nm and PDI <0.2)). Crosslinking of NPs and BNZ incorporation in NPs also diminished the crystallinity of the materials. The encapsulation efficiencies in PGl and crosslinked PGl NPs were 92% and 85%, respectively, supporting the applicability of the polyester for the encapsulation of BNZ. The release study under controlled conditions revealed that crosslinked samples presented faster drug release rates for reducing crystalline properties. In summary, the employment of PGl and a simple process for preparing nanocarriers has great potential for various drug delivery applications. This approach can benefit BNZ formulations and other hydrophobic compounds that require improvements in their respective therapeutics and dosage administration.

Keywords: poly(globalide), benznidazole, polymeric nanoparticles, sustained drug release, thiol-ene reaction.

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LIST OF ABBREVIATIONS AND SYMBOLS

ζ	Zeta potential
AFM	Atomic Force Microscopy
AIBN	Azobisisobutyronitrile
BNZ	Benznidazole
$CDCl_3$	Deuterated chloroform
CL	Caprolactone
Đ	Molecular weight dispersity
DCM	Dichloromethane
DLS	Dynamic Light Scattering
Dp	Particle diameter
DSC	Differential Scanning Calorimetry
EDDT	2,2′-(Ethylenedioxy)diethanethiol
EE%	Encapsulation Efficiency
e-ROP	Enzymatic Ring-Opening Polymerization
EtOH	Ethanol
FT-IR/ATR	Fourier Transform Infrared/Attenuated Total Reflectance
Gl	Globalide
GPC	Gel Permeation Chromatography
MetOH	Methanol
MIX	Physical mixture
Mn	Number average molecular weight
MSE	Miniemulsification-Solvent Evaporation
Mw	Weight average molecular weight
NFX	Nifurtimox
NMR	Nuclear Magnetic Resonance
NPs	Nanoparticles
NTDs	Neglected Tropical Diseases
Nz435	Novozym 435
PBS	Phosphate-Buffered Saline
PCL	Poly(ε-caprolactone)
PDI	Particle size dispersity
PDL	ω-Pentadecalactone

PE	Polyethylene
PGl	Poly(globalide)
PPDL	Poly(ω-pentadecalactone)
ROP	Ring-Opening Polymerization
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
THF	Tetrahydrofuran
Tm	Melting temperature
TMS	Tetramethyl silane
UV	Ultra-violet
Xc	Degree of crystallinity
ΔH_m	Melting enthalpy

SUMMARY

CONCEPTUAL DIAGRAM

"POLY(GLOBALIDE) NANOPARTICLES PREPARATION AND MODIFICATION FOR POTENTIAL DRUG RELEASE APPLICATIONS"

What?
Enzymatic polymerization of poly(globalide) (PGl) followed by its crosslinking reaction with EDDT and preparation of nanoparticles encapsulating BNZ for drug release applications under controlled conditions.

Why?
• Biocompatible and bioresorbable polyesters present interesting properties as alternative materials for biomedical applications; • Unsaturated chains enable varied modifications that are useful for tuning final materials properties and enhancing synergy and/or conjugation with other components. • The use of nanoscale materials is of great interest in a wide range of medical therapies; • The miniemulsification-solvent evaporation process is a straightforward method of producing and modifying nanoparticles and encapsulating a wide range of compounds; • Drug delivery systems improvement is essential to several medical treatments providing benefits and effectiveness.

State of the art
• Enzymatic ring-opening polymerization (e-ROP) has been utilized to synthesize polyesters using various monomers, including Globalide (Gl), with favorable outcomes; • Modification and crosslinking of PGl double bonds are powerful routes to produce functional NPs;

- There are a few works that studied PGl as a polymer matrix for nanoparticles used in drug delivery systems;
- PGl has not yet been applied in the encapsulation of BNZ.

Hypotheses

- PGl can be modified by thiol-ene reaction forming stable crosslinked nanoparticles;
- BNZ can be incorporated in both PGl and PGl-EDDT nanoparticles;
- The crosslinking process modulates drug release and nanoparticles properties.

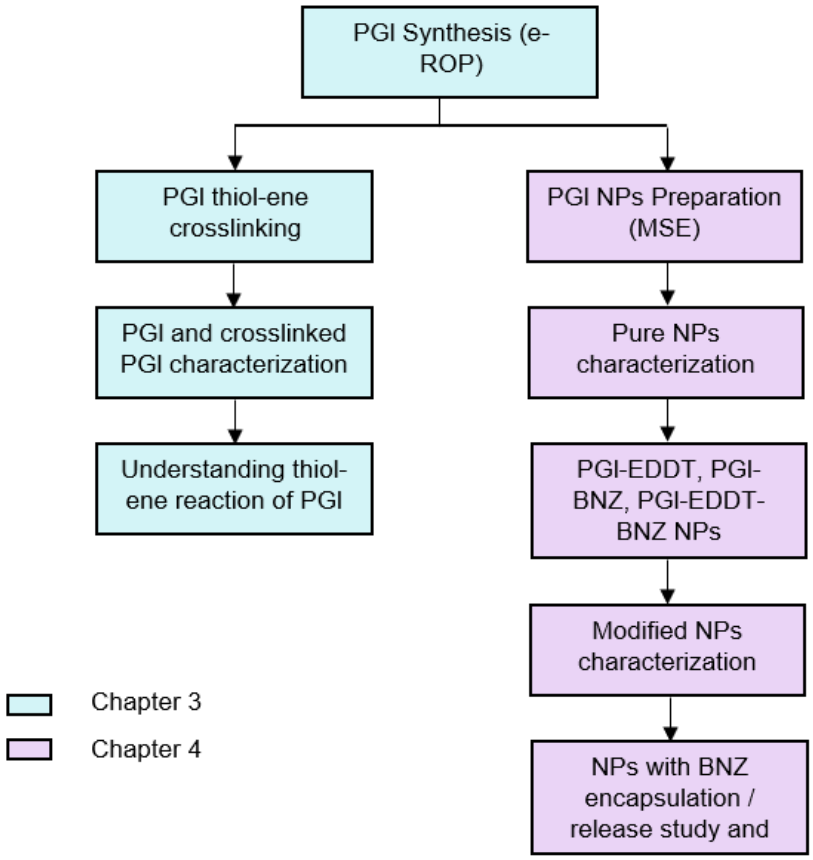
Which steps?

- Synthesis of PGl via e-ROP;
- PGl crosslinking by thiol-ene reaction;
- Characterization of obtained polymers;
- Nanoparticles preparation by miniemulsification-solvent evaporation technique and characterization;
- NPs post crosslinking by thiol-ene reaction;
- BNZ encapsulation by MSE technique;
- PGl-BNZ NPs crosslinking;
- Release study

Expected results

- To understand PGl thiol-ene crosslinking phenomenology and changes that this reaction provides in the material;
- To obtain of stable and narrow-size distributed NPs;
- To achieve high drug loading in BNZ encapsulation with the obtained polymers;
- To comprehend the effect of crosslinking on BNZ release behavior from PGl NPs.

WORK METHODOLOGICAL SEQUENCE FLOWCHART



1 INTRODUCTION

In the domain of polymer development, challenges in the future are dependent on the discovery of novel synthetic components and techniques that can improve the features of polymers, customize their properties, reduce costs, and minimize their impact on the environment and decrease their toxicity (ATANASE *et al.*, 2022). Features such as biodegradability, bioresorbability and biocompatibility are particularly attractive for potential applications in the biomedical field (GUINDANI *et al.*, 2017). From drug delivery systems to tissue engineering, these innovative materials are paving the way for a greener, more sustainable future.

Among the category of biodegradable polymers, aliphatic polyesters represent a broadly used class of materials, especially in biomedical applications, such as sutures, bone screws, tissue engineering scaffolds, and drug delivery systems (SEYEDNEJAD *et al.*, 2011). Long-chain aliphatic polyesters present comparable qualities to conventional polymers, including high hydrophobicity and semicrystalline structure, along with degradability via hydrolysis, biocompatibility, and bioresorbability (KOBAYASHI, 2010; POLLONI *et al.*, 2017a; WILSON *et al.*, 2019). As a result, they are attractive alternatives for conventional polymers used in biomedical applications.

The ring-opening polymerization (ROP) of lactones is a commonly used method to produce various types of aliphatic polyesters in bulk or solution, with multiple potential applications. There are numerous initiators and catalysts that have been described in the literature for the lactone polymerization process (ALBERTSSON, 2002; LECOMTE; JÉRÔME, 2012). The use of enzymes as catalysts in polymerization reactions has been investigated as a greener alternative to traditional chemical catalysts. Enzymatic ring-opening polymerization (e-ROP) offers various advantages over chemical catalysis, including specificity, high efficiency, reusability, low toxicity, and the requirement of mild reaction conditions (CHIARADIA *et al.*, 2018; DUBOIS; COULEMBIER; RAQUEZ, 2009; POLLONI *et al.*, 2017b).

Recently, there has been research on globalide (Gl) which is a macrolactone composed of a mixture of two isomers with 15 carbons containing a double bond in positions 11 or 12 (VAN DER MEULEN *et al.*, 2008). Polyester poly(globalide) (PGl) synthesis by enzymatic route has been explored in several studies and its unsaturation is susceptible to crosslinking or functionalization through thiol-ene reaction

(CHIARADIA *et al.*, 2019; DE OLIVEIRA *et al.*, 2017; GUINDANI *et al.*, 2019; VAN DER MEULEN *et al.*, 2008). Thiol-ene reaction-based modification of polymers is a simple and straightforward process that does not involve multiple steps that may help to tailor the properties and characteristics of a polymer, such as reducing its crystallinity and increasing its hydrophilicity (BELTRAME *et al.*, 2021; GUINDANI *et al.*, 2020).

Regarding biomedical applications, polymeric nanoparticles have become a highly promising approach for drug delivery due to their distinctive features, including high stability, biocompatibility, and ability to encapsulate a diverse range of substances (MISHRA *et al.*, 2018; TANG *et al.*, 2016). For instance, incorporating drugs into nanoformulated systems can improve their dispersibility in water, leading to faster absorption and a quicker onset of pharmacological effects (JOSEPH *et al.*, 2022; ROCHA *et al.*, 2014).

Controlled release of poorly soluble drugs is a significant problem for many treatments today, and especially challenging when it comes to treating endemic diseases. Neglected Tropical Diseases, such as Chagas disease, affect millions of people worldwide each year, and its treatment still faces obstacles such as limited effectiveness and strong side effects caused by the hydrophobic drug used, namely, benznidazole (BNZ) (DOS SANTOS SILVA *et al.*, 2019).

In this scenario, the use of varied nanostructures such as colloidal dispersions, miniemulsions, liposomes, microparticles and nanoparticles based on synthetic or natural polymers has been investigated as a way to improve formulations of therapeutic agents with BNZ (MÜLLER; MÄDER; GOHLA, 2000). The use of such advents can enhance drug performance by increasing its dispersibility and bioavailability under physiological conditions, modulating its release, and contributing to patient compliance to treatment and medication efficacy (MISHRA *et al.*, 2018; RIAL *et al.*, 2017).

The objective of this work was to synthesize and modify polymer PGl using 2,2′-(ethylenedioxy)diethanethiol (EDDT) via thiol-ene reaction in solution and azobisisobutyronitrile (AIBN) as thermal initiator aiming to tune properties of new materials (PGl-EDDT) intended for biomedical applications. Besides, a platform composed of (PGl) and PGl-EDDT-based NPs was prepared by MSE and evaluated in terms of suitability for BNZ encapsulation and sustained release applications.

1.1 OBJECTIVES

1.1.1 General objective

To prepare and evaluate the use of modified aliphatic unsaturated polyester poly(globalide) (PGl) NPs as potential nanocarriers for the Chagas' disease drug benznidazole (BNZ).

1.1.2 Specific objectives

a) To obtain poly(globalide) via enzymatic ring opening polymerization (e-ROP) mechanism with immobilized lipase Novozym 435 and determine its molecular weight (M_n), and, dispersity (Đ).

b) To modify PGl in the presence of 2,2′-(ethylenedioxy)diethanethiol (EDDT) via thiol-ene reaction in solution using azobisisobutyronitrile (AIBN) as thermal initiator in different molar ratios and characterize the obtained materials in respect to their chemical structure and thermal properties.

c) To synthesize PGl NPs through miniemulsification-solvent evaporation (MSE) technique and to characterize their thermal properties, stability, and morphology.

d) To perform encapsulation tests with hydrophobic drug BNZ producing PGl-BNZ NPs through MSE technique and PGl-EDDT-BNZ NPs with the same process followed by thermal crosslinking in the presence of EDDT via thiol-ene reaction with AIBN and characterize their size and morphology, stability, thermal properties, and encapsulation efficiency.

e) To carry out sustained release tests of PGl-BNZ and PGl-EDDT-BNZ NPs under controlled conditions to evaluate and understand the effects of NPs crosslinking.

2 LITERATURE REVIEW

2.1 BIODEGRADABLE POLYMERS

The field of polymer science has made significant progress lately, leading to a change in the way biodegradable polymers are used. These eco-friendly alternatives to conventional plastics are now extensively utilized in biomedical fields such as tissue engineering and drug delivery (MCBRIDE; GILLIES, 2013). Biodegradable polymer generally refers to materials whose chemical and physical characteristics undergo deterioration and completely degrade when exposed to certain conditions (ABHILASH; THOMAS, 2017). Moreover, these polymers must be biologically inactive and not produce any harmful substances during the process of degradation.

Biodegradable polymers are usually composed of polymer structures that are hydrolytically or enzymatically cleaved being degraded into soluble byproducts (ATANASE *et al.*, 2022; TIAN *et al.*, 2012; TSCHAN *et al.*, 2012). These types of polymers can be extracted from natural sources such as polysaccharides, proteins, and bacterial polyesters, or they can be created synthetically such as polyamides, polyureas, polyurethanes, polyesters, polyethers, polyanhydrides, and polypeptides (TSCHAN *et al.*, 2012). The degradation characteristics of these types of polymers, like their physical properties, are affected by various factors, including the chemical composition of the polymer backbone, molecular weight, polydispersity, and crystallinity (TSCHAN *et al.*, 2012). Therefore, in order to effectively tailor and apply biodegradable polymers, it is crucial to have a comprehensive understanding of the properties of the polymer and how they impact the degradation process.

Among the various biodegradable polymers available, aliphatic polyesters have garnered the most interest for biomedical applications because they are relatively easy to synthesize, have tunable properties of interest, and usually exhibit high biocompatibility (POLLONI *et al.*, 2020; TIAN *et al.*, 2012). These characteristics make these polymers sustainable alternatives and comparable to conventional materials such as polyethylene (PE) (GONÇALVES *et al.*, 2017; KOBAYASHI, 2010; WILSON *et al.*, 2019). Commonly used synthetic polyesters such as poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), and poly(ε-caprolactone) (PCL) are extensively used in biomedical applications including tissue engineering scaffolds, and drug delivery systems (ATANASE *et al.*, 2022; URBÁNEK *et al.*, 2019).

Furthermore, polyester chains can be modified by the addition of various functional groups to modulate their biodegradability and physical properties (POLLONI *et al.*, 2020). As a result, aspects of the polymer, such as molecular weight, stereochemistry, flexibility, and crystallinity, can also be impacted. With that in mind, functionalization procedures can be utilized to adjust the polymer properties to meet specific application requirements (ALBERTSSON, 2002; DUBOIS; COULEMBIER; RAQUEZ, 2009). Nonetheless, this approach requires the development of innovative materials that meet a set of overall criteria specified by the targeted application.

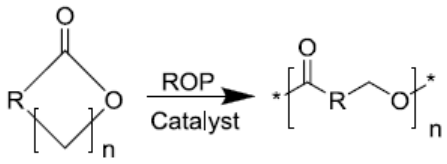
2.2 POLYMERS FROM MACROLACTONES

2.2.1 ROP of lactones

Originally, aliphatic polyesters were synthesized by condensation polymerization of hydroxyl acids or diacids and diols (FLORY, 1946; WILSON *et al.*, 2019). However, condensation polymerization has some drawbacks such as the need for thorough purification of monomers, precise stoichiometry, and high reaction temperatures that can lead to unwanted side reactions (WILSON *et al.*, 2019). There are three distinct methods of polymerization that can be employed to produce aliphatic polyesters: (1) ring-opening polymerization (ROP) of cyclic ketene acetals, (2) step-growth polymerization of lactones, and (3) ROP of lactones (LECOMTE; JÉRÔME, 2012). The first two mechanisms present limitations compared to ROP of lactones, such as low selectivity and relatively broad molecular weight distributions (LECOMTE; JÉRÔME, 2012). Thus, numerous instances of living or controlled polymerization have been reported using this mechanism and various types of catalysts and initiators, which enables the synthesis of high molecular weight aliphatic polyesters with low molecular weight dispersities.

There is a wide variety of initiators and catalysts that have been documented in the literature for the ROP of lactones. The specific type of catalyst utilized will dictate the polymerization mechanism, which typically falls under one of several categories, including cationic, anionic, coordination-insertion, organocatalytic, and enzymatic polymerization (DOVE, 2008). A representation of an ROP reaction for the preparation of polyesters is presented in Figure 1.

Figure 1: ROP for preparation of polyesters



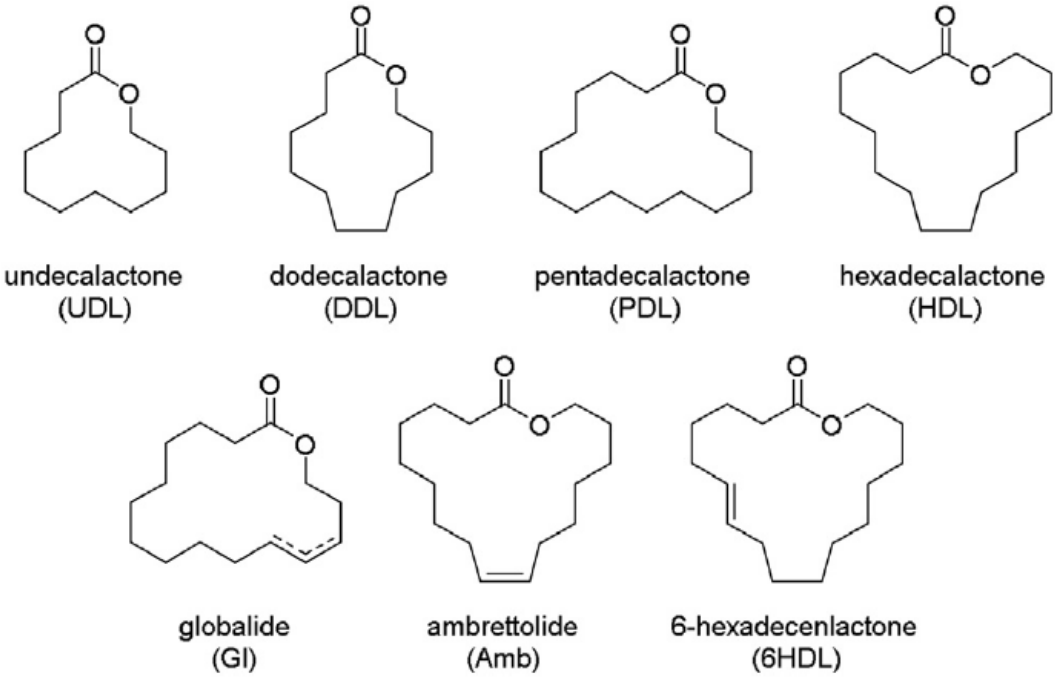
Source: (ATES, 2014)

Aliphatic polyesters can be categorized as either short-chain or long-chain polyesters, depending on the size of their respective monomer (GONÇALVES *et al.*, 2017). The ROP of lactones of small and medium sizes (up to 11 atoms) is driven by the release of angular and transannular strains, which leads to fast rates of polymerization at relatively low reaction temperatures (DUBOIS; COULEMBIER; RAQUEZ, 2009). On the other hand, macrolactones (consisting of 12 or more atoms) have negligible ring strain, and thus, the primary driving force behind the ROP of macrolactones is the entropic gain that results from ring-opening. This enables less hindered chain rotation, making the enthalpic gain minimal (WILSON *et al.*, 2019).

Organometallic catalysts tend to easily polymerize small cyclic lactones with high ring tension (DUDA *et al.*, 2002). However, when the same type of catalyst is employed to polymerize macrolactones, slower kinetics are observed, and polymers with low molecular weights are often produced (DUDA *et al.*, 2002). Thus, in such scenarios, enzymatic ring-opening polymerization is a more appropriate choice.

Over the past few years, research has focused on studying the ROP of macrolactones for numerous applications. The typical structures of some commercially available macrolactones employed as monomers for long-chain polyesters are shown in Figure 2.

Figure 2 – Commercially available macrolactones



Source: (WILSON et al, 2014)

Macrolactones are frequently employed in the pharmaceutical and chemical industries to enhance the scent or fragrance of various products. Macrolactones containing 14-16 carbons are particularly notable for their musk-like odor and are often found in natural sources such as plants and animal hormones (CHIARADIA, 2019). Furthermore, macrocyclic polyesters have been extensively studied for their potential use in synthesizing biodegradable polymers (POLLONI et al., 2017a).

2.2.2 Enzymatic ring-opening polymerization (e-ROP) of macrolactones

Enzymes play a crucial role in catalyzing metabolic reactions through biosynthetic pathways within living cells. They can facilitate the synthesis of various natural macromolecules, including proteins, polysaccharides, and polyesters. Nowadays, both natural and non-natural polymers created through enzymatic catalysis are being explored as a green approach to developing useful materials for technical and biomedical applications.

Enzymatic reactions used in polymer synthesis provide excellent chemoselectivity, regioselectivity, and enantioselectivity, which avoids the necessity for protection chemistry in certain case (FINNVEDEN *et al.*, 2016; KOBAYASHI, 2012).

Additionally, these reactions occur under mild conditions without the use of toxic reagents, contributing to sustainable production practices and facile catalyst recyclability (POLLONI *et al.*, 2018).

Several studies have been conducted on ring-opening polymerization catalyzed by enzymes, primarily lipases. These studies have highlighted numerous advantages, including the production of high molecular weight polyesters. The e-ROP of lactones with various ring sizes, lactides, and cyclic carbonates, resulting in the production of polyesters and polycarbonates, has been the subject of intense research.

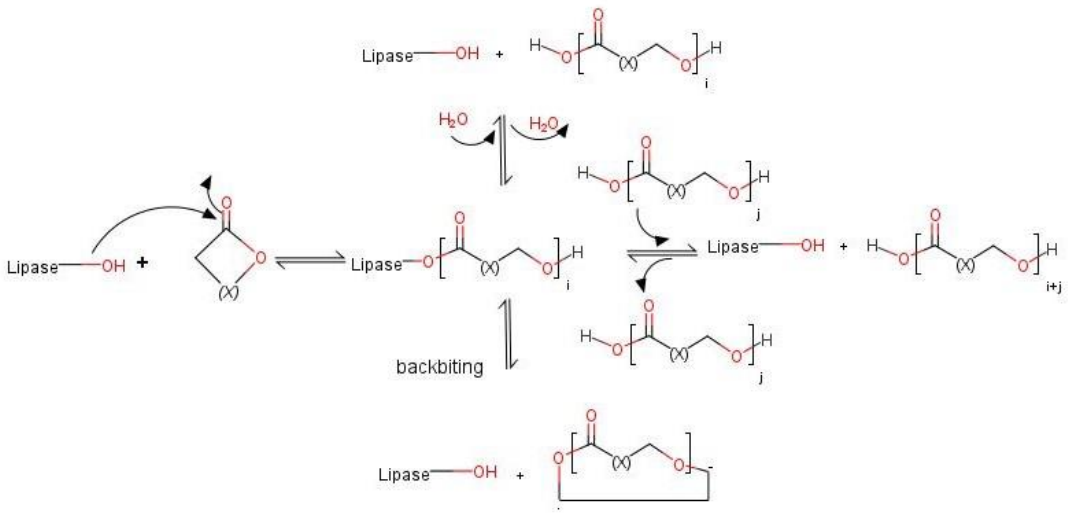
Early e-ROP experiments were explored by two groups in 1993 using *Pseudomonas fluorescens* and commercially-available crude porcine pancreatic lipase to polymerize the lactones ε-caprolactone and δ-valerolactone and produce the corresponding polyesters and copolymers (KNANI; GUTMAN; KOHN, 1993; UYAMA; KOBAYASHI, 1993). Uyama group studied the copolymerization of ε-caprolactone (CL) and ω-pentadecalactone (PDL) with the *Pseudomonas fluorescens* and furtherly, other larger ring lactones such as ω-undecalactone, ω-dodecalactone. Since then, a selection of lactones with varying ring sizes, both substituted and unsubstituted, as well as other cyclic monomers, have undergone polymerization, copolymerization, and catalysis using various lipases (HEVILLA *et al.*, 2021). Notwithstanding, while there have been some efforts to use e-ROP for lactones with larger ring sizes beyond the most available macrolactone, ω-pentadecalactone, the polymerization of smaller cyclic lactones has been more extensively researched and comprehended (HEVILLA *et al.*, 2021; WILSON *et al.*, 2019). Therefore, there is a growing interest in expanding the research on the use of other macrolactones with larger ring sizes for producing highly hydrophobic polyesters, especially those derived from fatty acids (VAN DER MEULEN *et al.*, 2008).

Novozym 435 (Nz435), an immobilized lipase B from Candida Antarctica, is currently one of the most used lipases in enzymatic polymerization for the synthesis of polyesters including macrolactones (POLLONI *et al.*, 2017a; VAN DER MEULEN *et al.*, 2008, 2011a; ZHANG *et al.*, 2014a). There is a wide range of works available in the literature on its activity and catalyst efficiency and, the use of these biological catalysts have gained attention in the biomedical field, for example, as an alternative to reduce toxicity problems (GUINDANI *et al.*, 2017). Additionally, Nz435 exhibits thermosetting immobilization and displays significant activity in a variety of organic solvents (ZHANG *et al.*, 2014a).

The e-ROP mechanism occurs through the role of a nucleophile initiator and the lipase active site regeneration. Water content plays a crucial role in e-ROP. Bisht and co-workers (1997) observed that higher water amounts in reactions increase polymerization rates while it decreases the molecular weight of PPDL. That behavior is justified by the fact the same time that water may favor enzyme activity it also generates more propagating chains (BISHT *et al.*, 1997).

A proposed kinetic model based on the work of Johnson *et al.* (2011) is well accepted as description of mechanism of e-ROP of cyclic structures such as lactones and macrolactones. Figure 3 shows a representation of Johnson and co-workers' adaptation for an enzymatic reaction for a generic lactone e-ROP (JOHNSON; KUNDU; BEERS, 2011; MEI; KUMAR; GROSS, 2003).

Figure 3: Mechanism for the e-ROP of lactones



Source: Adapted from (JOHNSON et al., 2011)

The model steps consist in the following main reactions: 1) macrolactone (in Johnson's work, the small ring size ε-caprolactone) e-ROP; 2) chain propagation; and two parallel reactions: 3) chain propagation/degradation enzyme and polymer chain interactions in the presence of water, and 4) cyclic structure formation by backbiting. It can be noticed that there is the production of three fundamental chain types: the first one is the 'enzyme-activated polymer chain' wherein the macrolactone chain is connected to the active site of the lipase by an ester bond; the second one is a macrolactone chain with both hydroxyl and carboxylic acid end groups; the third category is cyclic chains generated from enzyme-activated polymer chains.

2.3 POLYESTERS FOR BIOMEDICAL APPLICATIONS

Aliphatic polyesters are highly versatile and exhibit excellent mechanical properties, hydrolyzability, and biocompatibility, making them top contenders in the biomedical and pharmaceutical industries. They can be used as resorbable implant materials as well as components of controlled drug delivery systems and other medical applications (ALBERTSSON; VARMA, 2003).

Biodegradable polyesters have been regularly studied as nanocarriers for the sustained release of drugs. The addition of macrolactones to these systems increases their hydrophobicity, which has an impact on drug loading and degradation behavior as well as contributes to nanoparticles stability (TINAJERO-DÍAZ; MARTÍNEZ DE ILARDUYA; MUÑOZ-GUERRA, 2020; WILSON *et al.*, 2019). Over the last decade, researchers have extensively investigated the potential of using polyesters made from macrolactones as biodegradable carriers for drug delivery applications.

Despite their inherent potential, the homopolymers of macrolactones are generally poorly biodegradable/bioresorbable due to their semi-crystalline structure and high hydrophobicity (VAN DER MEULEN *et al.*, 2011a). Among the macrolactones that have recently been explored, poly(globalide) (PGl) has gained attention for its use in several applications. PGl is a biocompatible and non-toxic unsaturated poly(macrolactone) derived from the monomer globalide, which is a macrolactone with 16 members and a double bond at the 11 or 12 positions (VAN DER MEULEN *et al.*, 2008). Globalide is easily polymerizable via e-ROP due to its macrolactone nature, and it has been studied for its modification/functionalization potential, versatility, and biomedical material synthesis applications. Recent studies have explored in-situ post-modification/crosslinking of PGl, and this feature has been investigated in works by Polloni *et al.*, (2020), Chiaradia (2019), Guindani *et al.*, (2019), De Oliveira *et al.* (2017) and Van der Meulen *et al.* (2008).

2.3.1 Polymeric nanoparticles for drug delivery

The growing attention towards NPs stems from the fact that their mechanical, chemical, optical, electrical, and magnetic properties differ from those of their bulk counterparts, and these properties can be altered by varying the size of NPs (PULINGAM *et al.*, 2022). Nanoparticles present ideal features for delivering

therapeutic drugs to specific locations within the body. Unlike larger particles, which are rapidly eliminated by the immune system, NPs have demonstrated superior efficacy as drug carriers. Due to their increased surface area, NPs can effectively penetrate cells and traverse the blood-brain barrier while remaining easily degradable (PULINGAM *et al.*, 2022).

One of the most challenging tasks for pharmaceutical formulation researchers is to create effective safe and efficient biodegradable NPs for protein, DNA, drug, and peptide delivery and incorporation (ASSEM *et al.*, 2016). Typically, these systems involve the use of either polymers or lipids as carriers for the drug, and their diameter is generally defined in a range from 5 to 1000 nm (MORA-HUERTAS; FESSI; ELAISSARI, 2010; PATHAK; THASSU, 2016). The use of NPs as drug carriers offers multiple benefits, such as shielding the active substance from degradation *in vivo* and *in vitro*, prolonging its biological activity, enhancing therapeutic effectiveness, and managing drug release rate (ASSEM *et al.*, 2016; BEGINES *et al.*, 2020). Their potential use is justified by their ability to be used as nanostructures that can help enhance the dispersibility of a drug in water, leading to quicker absorption and a faster onset of pharmacological effects. Thus, polymeric NPs have been studied as important components of drug delivery systems in medicine and related biomedical fields (FAROKHZAD; LANGER, 2009; JOSEPH et al., 2022; ROCHA et al., 2014).

In recent years, nanotechnology has significantly increased focus on the development of less toxic/damaging drug delivery systems by utilizing biodegradable polymers as the primary carrier material (MISHRA *et al.*, 2018). Advanced therapeutic systems based on nanomaterials have several benefits compared to traditional therapies (MISHRA *et al.*, 2018). In this context, polyester NPs such as PCL, poly(lactic acid), poly(propylene fumarate), poly(lactic-co-glycolic acid), polyhydroxyalkanoates, and poly(butylene succinate) have been widely used in drug delivery, biosensing, bioimaging, and tissue engineering applications (GUPTA *et al.*, 2021).

One key advantage of NPs is that the rate at which drugs are removed by scavenger cells can be reduced by adjusting their size. Additionally, these systems can encapsulate and transport drugs that are poorly soluble in water, which is advantageous for pharmaceutical applications and increase bioavailability (MISHRA *et al.*, 2018; MORA-HUERTAS; FESSI; ELAISSARI, 2010). Controlled drug release also allows for better management of drug pharmacokinetics and can reduce toxic side effects. Lastly, these systems can enhance the distribution of drugs throughout the

body and improve their ability to penetrate cells, thereby increasing their effectiveness (MISHRA *et al.*, 2018; TANG *et al.*, 2016).

Polymeric nanoparticles can be produced through various methods, such as direct polymerization of monomers using techniques like emulsion polymerization, surfactant-free emulsion polymerization, mini-emulsion polymerization, micro-emulsion polymerization, and microbial polymerization. Emulsion methods are frequently employed for generating nanoparticles that aim to achieve excellent encapsulation efficacy, strong durability, and minimal toxicity (JENJOB *et al.*, 2019; PULINGAM *et al.*, 2022). Alternatively, preformed polymers can be dispersed to create NPs, which can be achieved through methods such as nanoprecipitation, emulsification-solvent evaporation, emulsification solvent diffusion, and salting-out (PULINGAM *et al.*, 2022). The choice of preparation technique for NPs relies on the characteristics of interest and the intended applications for the obtained products and significantly influences the materials' morphologies, size distributions, and particle properties (JENJOB *et al.*, 2019).

2.3.2 The emulsification-solvent evaporation method

Emulsions refer to a combination of two or more liquids that do not dissolve in each other, where one or more liquids are scattered within another liquid. Different types of emulsions, such as water-in-oil (W/O), oil-in-water (O/W), oil-in-water-in-oil (O/W/O), or water-in-oil-in-water (W/O/W), are frequently utilized for pharmaceutical purposes (JENJOB *et al.*, 2019). Emulsions, miniemulsions (or nanoemulsions), and microemulsions are all types of colloidal dispersions, where one substance (the dispersed phase) is dispersed in another substance (the continuous phase). However, they differ in terms of the size of the dispersed phase droplets and the methods used to prepare them.

Emulsions: An emulsion is a colloidal dispersion in which small droplets of one liquid are dispersed in another immiscible liquid. Emulsions are typically prepared by mixing two liquids together and applying mechanical energy to break up the dispersed phase into small droplets. Emulsions are typically macroscopic in size, with a wide range of droplet diameters and require surfactants for stability and a co-stabilizer to avoid diffusional degradation (GUPTA *et al.*, 2016; MCCLEMENTS, 2012).

Miniemulsions (Nanoemulsions): A miniemulsion is a type of emulsion where the dispersed phase droplets are much smaller, typically in the range of 50 to 500 nm. Miniemulsions are prepared using a surfactant to stabilize the droplets and prevent them from coalescing. They are typically prepared using high-energy methods, such as ultrasonication or high-pressure homogenization (KALE; DEORE, 2017). The range of miniemulsion applications spans diverse fields including drug delivery, the food industry, and cosmetic industry.

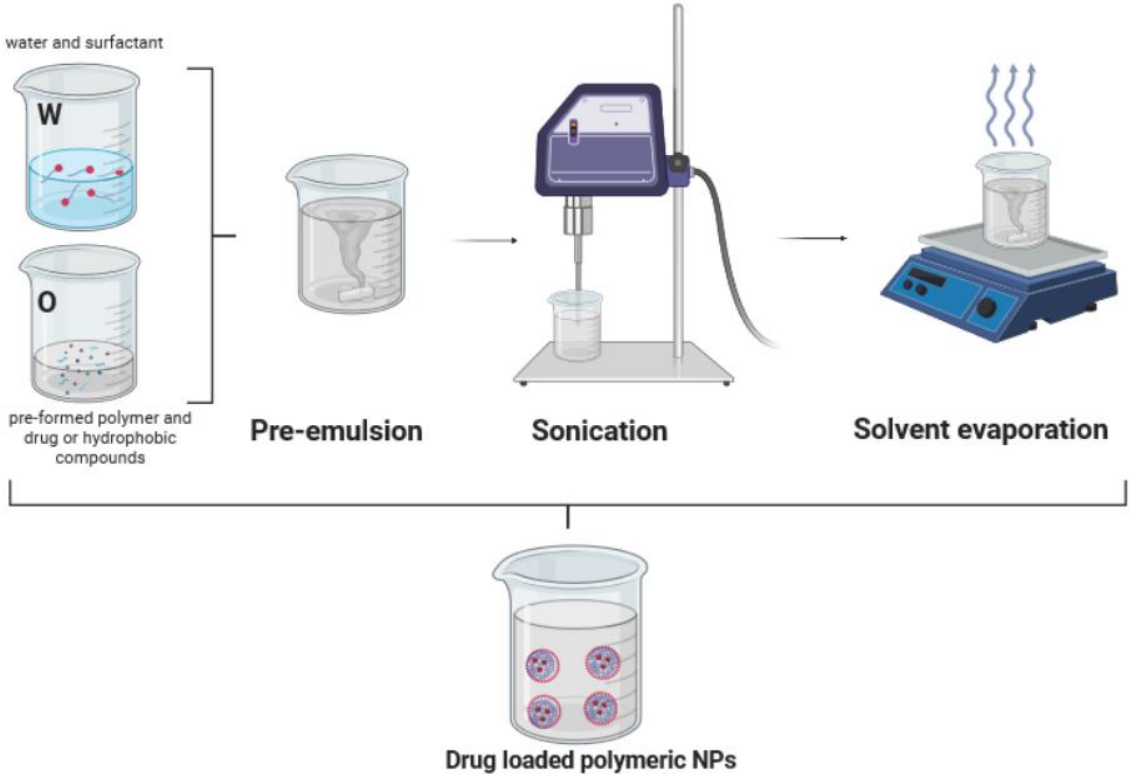
Microemulsions: A microemulsion is a type of emulsion where the dispersed phase droplets are even smaller, typically in the range of 10 to 50 nanometers. Microemulsions are stabilized by a combination of surfactants and co-surfactants, and they are typically prepared using low-energy methods, such as phase inversion or spontaneous emulsification. Microemulsions are often transparent or translucent and are thermodynamically stable (KALE; DEORE, 2017).

In 1979, Vanderhoff *et al.* first introduced the emulsification-solvent evaporation method as an alternative to the emulsification-polymerization process for producing polymer latexes. This process involves mixing a dispersed phase containing polymers, solvents, and/or drugs with a continuous phase, and then evaporating the solvents present in droplets to form an emulsion (JENJOB *et al.*, 2019) . This approach offers a significant advantage in relation to the emulsification-polymerization that is the absence of toxic residual monomers, unreacted agents, or catalysts that may be present in the emulsification-polymerization method. Since then, this method has been readily modified to produce polymer NPs specifically designed for nanoencapsulation processes and drug delivery systems (MENDOZA-MUÑOZ; ALCALÁ-ALCALÁ; QUINTANAR-GUERRERO, 2016).

Various factors influence the emulsification process, including the amount of energy provided, duration of shear energy exposure, the concentration of surfactant, ratio of oil to water, viscosity of the continuous phase, hydrostatic pressure, gas content, and pre-emulsification characteristics (MENDOZA-MUÑOZ; ALCALÁ-ALCALÁ; QUINTANAR-GUERRERO, 2016). If the emulsification of organic and aqueous phases happens with the aid of high-efficiency dispersion equipment like an ultrasonic probe, and results in the formation of submicron-sized particles, then this technique is referred to as miniemulsification-solvent evaporation (MSE). This process is distinct from emulsification-solvent evaporation in terms of the initial size of precursor droplets, which are generally in the micron range for emulsification (PERES, 2016).

The emulsification-solvent evaporation technique for direct emulsion (O/W) preparation generally involves the preparation of (1) an oil phase (O) dissolving the drug and biodegradable polymer in a volatile organic solvent and (2) an aqueous phase (W) containing surfactant. The aqueous phase solution is then added to the oil phase while being continuously sonicated to produce an emulsion. The resulting emulsion is continuously stirred to facilitate the evaporation of the volatile organic solvent, leaving behind polymeric nanoparticles loaded with the drug that remain suspended in the aqueous surfactant solution (PASWAN; SAINI, 2017). A schematic representation drug loaded polymeric nanoparticles preparation process by emulsification-solvent evaporation is shown in Figure 4.

Figure 4: Schematic representation of polymeric nanoparticles preparation by emulsification-solvent evaporation method



Source: Author (2023)

Under suitable circumstances, when the organic solvent evaporates, polymer particles in the shape of nanospheres, with a diameter of a few hundred nanometers, are formed. These nanoparticles are able to integrate drugs with lipophilic properties into the organic phase (DOS SANTOS *et al.*, 2020). Purification processes can be

performed using ultracentrifugation to retrieve the polymer nanoparticles, followed by washing them with distilled water to eliminate the stabilizer and allow the drug to be released (PERES, 2016). Purification of NPs is an essential step when dealing with certain applications such as biomedical ones to remove excess surfactant and potentially harmful impurities for the intended application (PATHAK; THASSU, 2016).

Surfactants play a crucial role in the, stabilization of NPs latexes due to their capacity to decrease the interfacial tension between the aqueous and organic phases upon initial emulsion formation (MORA-HUERTAS; FESSI; ELAISSARI, 2010). Their ability to prevent the coalescence of newly formed nanoparticles is attributed to the steric and/or electrostatic repulsion forces generated by the stabilizer that adheres to the surface of the particles. These forces become active when another nanoparticle approaches sufficiently close (MENDOZA-MUÑOZ; ALCALÁ-ALCALÁ; QUINTANAR-GUERRERO, 2016).

MSE technique has been applied as a promising approach for the entrapment of several compounds in nanoparticles of different substrates such as polymers and lipids (DOS SANTOS *et al.*, 2020; FREIBERGER *et al.*, 2015). This technique also allows the production of uniform stable emulsions that enables the preparation of nanoparticles or nanocapsules with sustained release properties, which can improve the efficacy and safety of the encapsulated active ingredients (GUPTA *et al.*, 2016; JENJOB *et al.*, 2019; PULINGAM *et al.*, 2022).

Zhao *et al.* group tested nanoencapsulation of hexadecane as a model component in a series of commercially available polymers such as poly(L-lactide), poly(methyl methacrylate), poly(vinyl formal), poly(vinyl acetate), poly(2,6-dimethyl-1,4-phenylene oxide), and poly(vinyl cinnamate) using sodium dodecyl sulfate (SDS) as a surfactant, and chloroform and dichloromethane (DCM) as solvent.. The combination of miniemulsification and solvent evaporation was used to produce nanocapsules with sizes from 232 to 362 nm showing the versatility of the technique for encapsulating hydrophobic compounds (ZHAO *et al.*, 2012).

Also using MSE method, Mattos dos Santos and coworkers successfully incorporated magnetic nanoparticles into stable biobased poly(thioether-ester) NPs with an average size of 150 nm as an alternative to the targeted delivery of antitumor drugs and cancer treatment for hyperthermia (MATTOS DOS SANTOS *et al.*, 2018). Guindani *et al.* used the same technique to produce poly(globalide-co-ε-caprolactone) NPs to furtherly form functionalized conjugates with bovine serum albumin by thiol-ene

reaction. The NPs obtained were stable and had mean particle diameter of around 146 nm and PDI lower than 0.1 showing the feasibility of the NPs preparation process (GUINDANI *et al.*, 2019).

While much is known about certain technological aspects of the emulsion-solvent evaporation method, further research is needed to address several other issues. These include exploring the use of new technologies such as dispersion and homogenization equipment, improving large-scale industrial production using solvent displacement, finding less toxic ingredients, incorporating green processes into operating conditions, developing multifunctional formulations with better targeting properties, and discovering new methods to eliminate residual solvent and stabilizers (MENDOZA-MUÑOZ; ALCALÁ-ALCALÁ; QUINTANAR-GUERRERO, 2016). In conclusion, MSE is a useful tool with various possibilities and plenty of room for new research to assess and develop nanoparticles formulations for drugs and pharmaceutical purposes.

2.3.3 Controlled drug release systems

Particulate systems for drug entrapment, such as O/W emulsions, liposomes, microparticles and nanoparticles based on synthetic polymers or natural macromolecules have been extensively studied (MÜLLER; MÄDER; GOHLA, 2000). The effective clinical application of earlier drug delivery systems at the macro- and micro-scale has resulted in the development of controlled release nanodrug delivery platforms. These platforms have the ability to overcome limitations of pharmacology and offer significant benefits over traditional forms of medication (KAMALY *et al.*, 2016).

As previously mentioned, one of the reasons of using this type of configuration is to enable controlled drug release reducing side effects (MÜLLER; MÄDER; GOHLA, 2000). Also, therapeutic actives can have short half-lives implying in a brief duration of their action in the organism, making the development of specific nanocarriers to overcome these drawbacks (KAMALY *et al.*, 2016; TANG *et al.*, 2016).

The use of polymeric NPs offers a range of significant benefits due to their highly adaptable nature and the ability to regulate the release of therapeutics. The ability to produce and tailor properties of polymeric nanoparticle drug delivery systems that can provide sustained drug delivery, resulting in more efficient therapeutic

outcomes, has been made possible by advancements in synthetic methods, fabrication techniques, and mathematical models used to study controlled drug release mechanisms (KAMALY *et al.*, 2016).

2.4 POST-MODIFICATION OF POLYMERS

The modification of polymeric materials refers to the chemical modification of pre-synthesized polymers, allowing for the introduction of new functionalities and properties (GÜNAY; THEATO; KLOK, 2012). This technique involves the reaction of functional groups within the polymer structure or at the end of the polymer chains with other molecules or reagents (GÜNAY; THEATO; KLOK, 2012; ZOU *et al.*, 2018). Post-modification can alter the physical, chemical, and biological properties of polymers, making them suitable for a wide range of applications such as drug delivery, tissue engineering, and sensoring technologies (RESETCO *et al.*, 2017). After undergoing the process of modification, the characteristics of the polymers, including viscosity, solubility, adhesion, crystallinity, hydrophobicity, and others, are typically altered (CHIARADIA, 2019). The versatility and controllability of post-modification reactions make them an attractive method for tailoring polymer properties to specific applications (RESETCO *et al.*, 2017; ZOU *et al.*, 2018).

2.4.1 Thiol-ene reaction

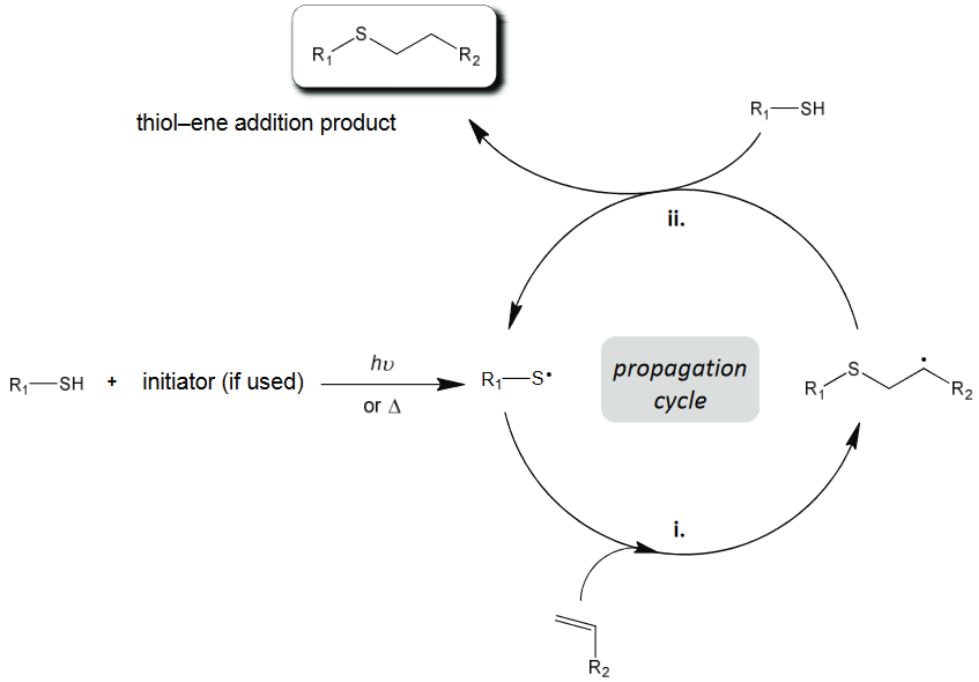
Thiol-ene *click-chemistry* is a highly beneficial method for functionalizing unsaturated polymers, as it offers fast reaction rates, a diverse range of accessible thiols with different functional groups, and the ability to initiate reactions using light. Additionally, this technique can tolerate exposure to air/oxygen and water (ATES; HEISE, 2014). In general, thiol-ene reactions occur by adding a thiol to an alkene bond, *i.e.*, a C=C bond. This addition can occur via a radical or nucleophilic pathway. (SINGHA; SCHLAAD, 2012). The radical thiol-ene addition can be initiated either through thermal or photochemical means, which create radicals that mediate the reaction (GÜNAY; THEATO; KLOK, 2012).

The scheme shown in Figure 5 illustrates the typical process of hydrothiolation via radical mechanism for a single unsaturated bond, which involves two distinct steps: 1) initiation: The radical initiator (such as temperature or UV light) generates free radicals RS• from thiol groups R-SH and 2) propagation: The free radical reacts with

the alkene double bond, generating a new radical on the carbon atom. This new radical can then react with the thiol, forming a new C-S bond and generating another radical on the thiol (CLAUDINO, 2011).

The initiator keeps producing thiyl radicals, which enables the reaction to continue in a cyclical manner until the thiol-ene species are consumed. The reaction is terminated by the combination of radicals (either thiyl or β-carbon), but this type of termination is not as common as propagation and chain transfer reactions. Thiol-ene modification/polymerization processes are first order kinetics reactions limited by the propagation and chain transfer constants (CHIARADIA, 2019).

Figure 5: Mechanism of thiol-ene addition via free radicals



Source: (CLAUDINO, 2011)

2.4.2 Crosslinked polymers for controlled drug release

Crosslinking reactions, such as thiol-ene chemistry, can modulate the drug release from nanoparticles by controlling the degree of crosslinking within the nanoparticle network. A higher degree of crosslinking leads to smaller pores within the nanoparticle structure, which in turn results in a slower rate of drug release. Furthermore, the degree of crosslinking may modify polymer swelling degree enabling modulation of drug diffusion rates (ASADI *et al.*, 2011; DEIRRAM *et al.*, 2019).

Additionally, thiol-ene chemistry can be utilized to increase the biodegradability of polymers under certain conditions, for example, ether groups added to polymer backchains can be degraded under physiological conditions causing faster release (DEIRRAM *et al.*, 2019; ZHANG *et al.*, 2014b, 2015). Functionalization of copolyesters with aminoethanethiol via thiol-ene reaction was studied by Tinajero-díaz *et al.* (2020) produced doxorubicin pH-responsive nanocarriers with controlled drug release rate. The degradability of polymers can be also increased incorporating functional groups into the polymer structure, which can produce enzymatically degradable polymers through crystallinity and hydrophobicity reduction by double bonds functionalization and/or crosslinking by thiol-ene reaction (ATES *et al.*, 2014; ATES; THORNTON; HEISE, 2011; VAN DER MEULEN *et al.*, 2011b).

Therefore, by adjusting the degree of crosslinking in thiol-ene crosslinked nanoparticles, it may be possible to modulate the rate and duration of drug release. This control over drug release can be further enhanced by incorporating stimuli-responsive crosslinking moieties that allow drug release to be triggered by specific environmental cues such as changes in pH or temperature (TINAJERO-DÍAZ; DE ILARDUYA; MUÑOZ-GUERRA, 2020; TINAJERO-DÍAZ; MARTÍNEZ DE ILARDUYA; MUÑOZ-GUERRA, 2019). Overall, thiol-ene crosslinking provides a versatile approach to tailor nanomaterials used in drug delivery systems with controlled drug release kinetics.

2.5 NEGLECTED TROPICAL DISEASES

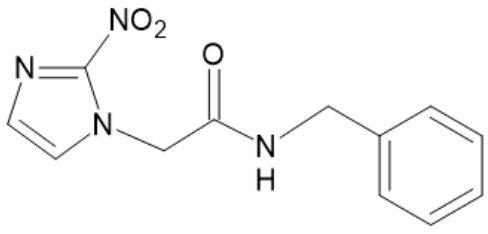
Neglected tropical diseases (NTDs) are a class of seventeen infectious diseases that are primarily prevalent in tropical areas of the globe, in communities where there is a lack of adequate healthcare infrastructure (WORLD HEALTH ORGANIZATION, 2023). According to the World Health Organization's 2023 forecast, 1.65 billion individuals globally were said to need mass or individualized care for NTDs. Chagas disease, one of remarkable examples of NTDs, is still a serious public health issue, primarily in America, especially in the most isolated parts of Brazil (VOLPEDO *et al.*, 2019; WORLD HEALTH ORGANIZATION, 2023).

2.5.1 Chagas disease treatment

Benznidazole (BNZ) and nifurtimox (NFX) are the only currently available therapy alternatives for Chagas disease, despite the fact that it was first recognized more than 100 years ago. One of the elements that affect how well a treatment works is the disease's stage. (ARRÚA *et al.*, 2019). Despite being commonly used, the treatment with both drugs presents severe negative effects and cross resistance (QUIJIA QUEZADA *et al.*, 2019). NFX, for example, can lead to various adverse effects such as psychiatric and neurological disorders, as well as gastrointestinal complications. Similarly, patients receiving BNZ treatment may experience cutaneous eruption, fever, generalized edema, as well as complications in the bone marrow, polyneuritis, and polyneuropathy (ARRÚA *et al.*, 2019; MOLINA *et al.*, 2014).

BNZ (Figure 6) is a popular antiparasitic medicine used to treat Chagas disease, which is caused by the parasite Trypanosoma cruzi. Benznidazole is well stablished as a treatment, but it also provokes serious adverse effects (DOS SANTOS SILVA *et al.*, 2019; VINUESA *et al.*, 2017). In addition, due to the drug's limited capacity to overcome biological barriers—primarily brought about by its high toxicity and low solubility in water (0.4 g/mL)—its efficacy is decreased in the chronic stage of the disease (DOS SANTOS-SILVA *et al.*, 2017; DOS SANTOS SILVA *et al.*, 2019).

Figure 6: BNZ chemical structure



Source: Author (2023)

The use of biomaterials and nanoparticles for drug delivery provides the ability to surpass biological barriers, enable drug integrity in physiological conditions and control its release making them a promising approach to enhance the efficacy of BNZ and reduce its toxicity (QUIJIA QUEZADA *et al.*, 2019). The nanoparticulate systems for antichagasic agents that have been developed in recent years are varied such as nanocrystals, liposomes, micelles, nanoemulsions, polymeric and non-polymeric

nanoparticles (ARRÚA *et al.*, 2019; QUIJIA QUEZADA *et al.*, 2019). Polymeric nanoencapsulation of the hydrophobic drug is an interesting tool that enables surface modification that may help therapeutic enhancement (ARRÚA *et al.*, 2019; ESTEVA *et al.*, 2016).

The combination of biomaterials and nanoparticles for drug delivery in Chagas disease treatment is a relatively new field, and much more research is needed to fully understand the potential benefits and limitations of these technologies (ARRÚA *et al.*, 2019). Nonetheless, the development of new types of nanostructures offers a promising approach to enhance the efficacy of benznidazole and reduce its toxicity in the treatment of Chagas disease.

2.5.2 Nanocarriers for BNZ delivery systems

The latest approach for improving the effectiveness of chemotherapy and reducing the adverse side effects of medications is the development of nanostructures for drug delivery systems. Nanotechnology offers a promising alternative for improving treatment of Chagas disease, as nanostructures have unique properties that make them suitable for various oral dosage forms. However, these delivery systems still face challenges with physical and chemical stability, low encapsulation capacity, and particle aggregation or precipitation, which can impact the release and absorption of drugs and result in products with varying quality attributes, such as low bioavailability or inconsistent biological activity.

Over the last decades, polymeric nanoparticles from synthetic or natural resources have emerged as therapeutic agents constituents and used to entrap, encapsulate or adsorb drugs such as BNZ and NFX (QUIJIA QUEZADA *et al.*, 2019). Seremeta *et al.* (2019) successfully encapsulated BNZ in Eudragit® micro- and nanoparticles by nanoprecipitation and freeze drying technique (SEREMETA *et al.*, 2019). Encapsulation efficiency reached up to 95% and nanoparticle size varied from 200 to 300 nm. In vitro release studies were performed showing an increase in dissolution rate compared to the raw drug and possible reduction in BNZ crystallinity. In another study, Dos Santos Silva and co-workers (2017) prepared BNZ-loaded cationic stable nanoparticles with less than 150 nm and narrow PDI by emulsification-solvent evaporation method using a copolymer matrix of copolymer of poly(methylmethacrylate) and Eudragit® E PO (DOS SANTOS-SILVA *et al.*, 2017).

In conclusion, the combination of benznidazole with nanocarriers for drug delivery represents a promising strategy to improve the treatment of Chagas disease. By enhancing drug delivery and targeting, prolonging drug release, and improving drug stability, these technologies offer the potential to enhance the efficacy and reduce the toxicity of benznidazole, and ultimately improve the outcomes for patients with Chagas disease.

2.6 STATE OF THE ART

Polymeric NPs have become popular drug delivery systems due to their potential for targeted delivery, long-term stability, low toxicity, and relatively low production cost (BEGINES *et al.*, 2020). The core-shell structure of polymeric NPs facilitates encapsulation of hydrophobic drugs and sustained drug release, extending the circulation time (RAI; ALWANI; BADEA, 2019). Controlled drug release is important in drug delivery to ensure therapeutic efficacy and minimize side effects. Various strategies have been developed to modify polymeric NPs to achieve controlled drug release.

One approach is to modify the surface of the NPs with stimuli-responsive groups that respond to changes in the microenvironment, such as pH, temperature, and enzymes (TINAJERO-DÍAZ; MARTÍNEZ DE ILARDUYA; MUÑOZ-GUERRA, 2019). In addition, modifying the polymeric NPs with biodegradable polymers that degrade in response to specific stimuli or over time, might contribute to controlled drug release (TINAJERO-DÍAZ; DE ILARDUYA; MUÑOZ-GUERRA, 2020). Thus, modifying the surface of polymeric NPs, the polymer itself can be modulated to achieve specific sustained drug release rates.

Overall, modified polymeric NPs have shown great potential for controlled and sustained drug release applications. The various modification strategies can be combined to achieve synergistic effects and improve drug delivery efficacy. However, challenges remain in scaling up production and ensuring reproducibility and safety for clinical use (TANG *et al.*, 2016). Further research is needed to optimize the design and properties of modified polymeric NPs for drug delivery applications.

Among the class of biodegradable polymers, PGI is a compelling candidate for biomedical applications for it is a non-toxic and biocompatible material with unique

mechanical properties (VAN DER MEULEN *et al.*, 2008). The presence of unsaturation in the PGI chain enables it to be easily functionalized and crosslinked using thiol-ene reactions (ATES; THORNTON; HEISE, 2011; VAN DER MEULEN *et al.*, 2011b). By functionalizing the polymer, it is possible to decrease its crystallinity and hydrophobicity and increase the polymer's affinity for various human cells and tissues as well as modulate controlled drug release properties (ATES *et al.*, 2014; ATES; THORNTON; HEISE, 2011; VAN DER MEULEN *et al.*, 2011b).

Modified polymeric NPs development offers a promising alternative for improving treatment of Chagas disease, as nanostructures have unique properties that make them suitable for various oral dosage forms. However, these delivery systems still face challenges with physical and chemical stability, low encapsulation capacity, and particle aggregation or precipitation, which can impact the release and absorption of drugs and result in products with varying quality attributes, such as low bioavailability or inconsistent biological activity.

Chagas disease is a neglected tropical disease that affects millions of people in Latin America. The current treatment for Chagas disease involves the use of Benznidazole, which is an effective medication if given soon after infection. However, the drug has some limitations such as low solubility, poor bioavailability, and significant side effects, including skin rashes and gastrointestinal issues. Therefore, there is a need to develop alternative drug delivery systems that can improve the efficacy and safety of Benznidazole in Chagas disease treatment.

Nanocarriers with BNZ have been proposed as a promising strategy for overcoming the limitations of the conventional drug delivery system (ARRÚA *et al.*, 2019; QUIJIA QUEZADA *et al.*, 2019). Nanostructures can improve drug solubility and bioavailability, enhance drug stability and reduce side effects (DOS SANTOS-SILVA *et al.*, 2017). Additionally, these systems can target the parasite more effectively, increasing the drug concentration in the infected cells while reducing the drug concentration in healthy cells (ARRÚA *et al.*, 2019; QUIJIA QUEZADA *et al.*, 2019; SEREMETA *et al.*, 2019). Thus, the development of nanotechnological approaches for BNZ treatment can significantly improve the therapeutic outcomes for patients with Chagas disease.

The release rate of a drug in the human organism can be significantly altered by its incorporation in a polymeric matrix (DEIRRAM *et al.*, 2019; KAMALY *et al.*, 2016). Moreover, the degradability of polymers can be enhanced by adding functional groups to the polymer structure and or by crosslinking its chain network (DEIRRAM *et al.*, 2019; ZHANG *et al.*, 2014b, 2015). These modifications can lead to enzymatically degradable polymers by reducing crystallinity and hydrophobicity through double bond functionalization and/or crosslinking through the thiol-ene reaction, for example (ATES *et al.*, 2014; ATES; THORNTON; HEISE, 2011; VAN DER MEULEN *et al.*, 2011b).

In the presented literature review, important results, which are directly related to the theme of this project, were revised. However, there are still a significant number of challenges to be overcome, especially regarding the functionalization/modification of nanocarriers used in BNZ incorporation and its characterization. Considering the topics covered, the combination these with the application of an alternative biodegradable/biocompatible polymer such as PGl and its subsequent modification aiming to explore and tune its properties represents the present work objective and innovation.

CHAPTER 3

3 POLY(GLOBALIDE) ENZYMATIC RING-OPENING POLYMERIZATION AND THIOL-ENE POST-MODIFICATION BY THERMAL CROSSLINKING

This chapter shows the enzymatic ring-opening polymerization (e-ROP) of unsaturated macrolactone globalide was performed in solution. Homopolymer poly(globalide) (PGl) was characterized in relation to molecular weight and distribution, chemical structure, and thermal properties and then modified via thiol-ene reaction using 2,2′-(ethylenedioxy)diethanethiol (EDDT) as a crosslinking agent in the presence of thermal initiator azobisisobutyronitrile (AIBN) in solution in different molar ratios of thiol:ene.

3.1 INTRODUCTION

Polymer technology today has gradually shifted to more environmentally friendly methods. Polymers are widely used in natural and synthetic technologies as highly adaptable and varied macromolecular materials with exceptional structural and multifunctional properties (MÜLHAUPT, 2013). In the field of polymer development, future obstacles rely on identifying new synthetic building blocks and methodologies that can enhance functionalities, tailor properties, decrease expenses, and mitigate environmental consequences (RESETCO *et al.*, 2017). Thus, research on alternative functionalized polymers, which are biodegradable/bioresorbable, expanded in several directions, including biomedical applications in drug delivery systems and tissue engineering (GUINDANI *et al.*, 2017).

In this scenario, for instance, the search for viable substitutes for common and massively produced non-biodegradable polyolefins, such as polyethylene (PE), has constantly been discussed (MYERS *et al.*, 2017; ZHU; ROMAIN; WILLIAMS, 2016). The use of biodegradable polymers as biomedical and pharmaceutical materials is an instinctive pathway for many drawbacks in modern medicine being applied as sutures, bone screws, tissue engineering scaffolds, drug delivery systems (DE OLIVEIRA *et al.*, 2017). Long chain aliphatic polyesters derived from lactones represent an interesting choice not only for being generally biodegradable/compatible but also for having good processability and promising tunable properties, which can be compared to traditional materials such as PE (DE OLIVEIRA *et al.*, 2017; STEMPFLE; ORTMANN; MECKING, 2016; ZHU; ROMAIN; WILLIAMS, 2016). Their similar characteristics to PE such as

high hydrophobicity and semicrystalline nature as well as degradability by hydrolysis, biocompatibility, and bioresorbability make them compelling candidates to replace conventional polymers used in the biomedical field (KOBAYASHI, 2010; WILSON *et al.*, 2019).

The ring-opening polymerization of lactones is a well-known route to obtain a variety of aliphatic polyesters in bulk or solution with a wide range of applications. Numerous initiators and catalysts have been documented in literature for the polymerization of lactones. The selection of the initiator and catalyst types determines the polymerization mechanism, which includes cationic, anionic, coordination-insertion, organocatalytic, and enzymatic polymerization (ALBERTSSON, 2002; LECOMTE; JÉRÔME, 2012).

Enzyme-catalyzed polymerization reactions have been extensively studied as synthesis routes of many of these polymers for their greener nature and other advantages compared to the chemically catalyzed ones, such as specificity, efficiency, reusability, low toxicity, and mild reaction conditions required (CHIARADIA *et al.*, 2018; POLLONI *et al.*, 2017a). Particularly, the enzymatic route is not only a standard method but more efficient and suitable for the polymerization of macrolactones (large lactones rings that contain more than 12 atoms) (DUBOIS; COULEMBIER; RAQUEZ, 2009). The demand for these specific lactones has rapidly grown, particularly for manufacturing highly hydrophobic polyesters derived from fatty acids. They have been used in various chemical industries, including fragrances and pharmaceuticals. Recently, they have also been studied as monomers to produce a broad range of functional polymers and naturally numerous approaches have been developed for their synthesis (WILSON *et al.*, 2019)

Functionalizing double bonds is a widespread method in the development of polymers and materials due to its high efficiency, versatility, and ability to occur under mild conditions (RESETCO *et al.*, 2017). Polyglobalide (PGl) is an unsaturated poly(macrolactone) that is both biocompatible and non-toxic. It is synthesized from the monomer globalide, which is a 16-membered macrolactone containing a double bond at the 11 or 12 positions. that enables in-situ post-modification/crosslinking that has been explored in recent works (CHIARADIA *et al.*, 2019; DE OLIVEIRA *et al.*, 2017; GUINDANI *et al.*, 2019; VAN DER MEULEN *et al.*, 2008). As a macrolactone, globalide tends to be easily polymerizable via e-ROP compared to smaller-sized lactones and its interesting features, modification/functionalization proneness, and versatility have

been explored in several studies for biomedical materials synthesis (ATES; HEISE, 2014; CHIARADIA *et al.*, 2018; POLLONI *et al.*, 2017a). However, its semicrystalline nature and hydrophobicity hinder hydrolytic degradation, which can difficult its applicability in biomedical fields (WILSON *et al.*, 2019).

Considering the double bond present in the PGl backbone, the thiol-ene "click reaction" is a promising tool for polymer modification, providing a versatile and cost-effective method (BELTRAME *et al.*, 2021). Thiol-ene polymerization is frequently regarded as a click-chemistry reaction due to its ability to occur under mild conditions and typically produce high yields of polymer with harmless byproducts (MACHADO; SAYER; ARAUJO, 2017). Unlike other synthesis methods, this approach does not require multiple steps. Consequently, it allows facile modification of a polymer's characteristics and properties, including reduced crystallinity and increased hydrophilicity, as well as being non cytotoxic (BELTRAME *et al.*, 2021; GUINDANI *et al.*, 2020). Nonetheless, meeting and understanding the reactions nature of each process is crucial for the intended application. Thus, the range of polymer synthesis, modification and properties presents the possibility of customizing materials to suit various biomedical applications.

In that sense, the present chapter proposes to develop a modification process of promising polyester poly(globalide) using 2,2′-(ethylenedioxy)diethanethiol (EDDT) as a crosslinking agent and AIBN as a thermal initiator. Understanding this process is useful to tailor PGl or other similar materials' properties to achieve specific application requirements and related challenges.

3.2 MATERIALS AND METHODS

3.2.1 Materials

Globalide (Gl) was kindly donated by Symrise. Toluene P.A was purchased from Dinâmica Química Contemporânea LTDA (Brazil). Dichloromethane P.A 99.8% (DCM), ethanol (EtOH) P.A 99,8, methanol (MetOH) P.A 99,5 and tetrahydrofuran P.A. 99,9% (THF) were purchased from Neon Química (Brazil) and used as received. Novozym 435 (N435 – commercial lipase B from Candida antarctica immobilized on polyacrylate beads was a kind gift from Novozymes). Azobisisobutyronitrile 98% (AIBN) and 2,2′-(ethylenedioxy)diethanethiol (EDDT) were acquired from Vetec Química (Brazil).

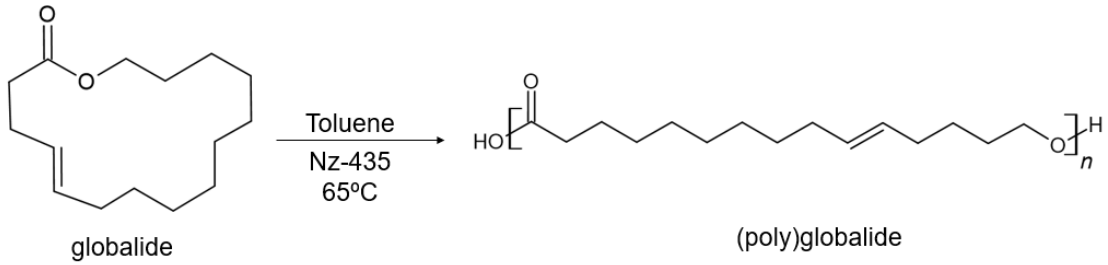
Gl and Nz435 were previously dried for 24h at 60ºC under vacuum and stored desiccator over silica and 4 Å molecular sieves and AIBN was recrystallized before reactions.

3.2.2 Synthesis of poly(globalide) via enzymatic ring-opening polymerization

The synthesis of PGl was carried out in a sealed glass vial in a 2:1 monomer (Gl): solvent (toluene) ratio. Monomer, solvent, enzyme, and molecular sieves were directly weighted in an analytical precision balance (ATX224 Shimadzu) in the reaction vial. Enzyme and molecular sieves concentrations were 5 wt.% and 0.5 wt.%, respectively, in relation to the monomer. Reaction was conducted under magnetic stirring at 65ºC for 2h in a sand bath. These conditions were based on the previous works of the group that involved globalide homo- and copolymerization and other similar polyesters (CHIARADIA *et al.*, 2019; GUINDANI *et al.*, 2017; POLLONI *et al.*, 2018).

As the polymerization process ceased, reaction's viscosity increased, dichloromethane (DCM) was added to the vial to allow the separation of the enzymes and molecular sieves by filtration, followed by precipitation of PGl in cold methanol. In sequence, the polymer was placed in petri dishes and dried overnight at 60ºC. Yields were measured gravimetrically and were about 75%. This polymerization was carried out in triplicate but only one of them, the third triplicate was used for further modifications. A schematic representation of e-ROP of Gl is showed in Figure 7.

Figure 7 – Schematic representation of PGl synthesis via e-ROP



Source: Author (2023)

3.2.3 Thiol-ene crosslinking reaction of poly(globalide) with EDDT

Crosslinking of produced PGl was carried out in solution in a one-pot procedure. In order to understand the reaction behavior, two different ene to thiol ratios and initiator (AIBN) percentages in relation to thiol were tested as F1 (1:1, 1%); F2 (1:1, 5%); F3 (1:2, 1%) and F4 (1:2, 5%). Tetrahydrofuran volume was adjusted to keep constant the total concentration of repeat units. PGl, AIBN and EDDT were weighted in an analytical precision balance (ATX224 Shimadzu) and disposed in a vial. Then, the volume of THF was verted into each respective vial. All samples were purged with nitrogen before reaction and well-sealed, carried out in a sand bath under continuous magnetic stirring at 70ºC for 8h. The formulations are presented in Table 1, and a schematic representation of the crosslinking reaction is shown in Figure 8.

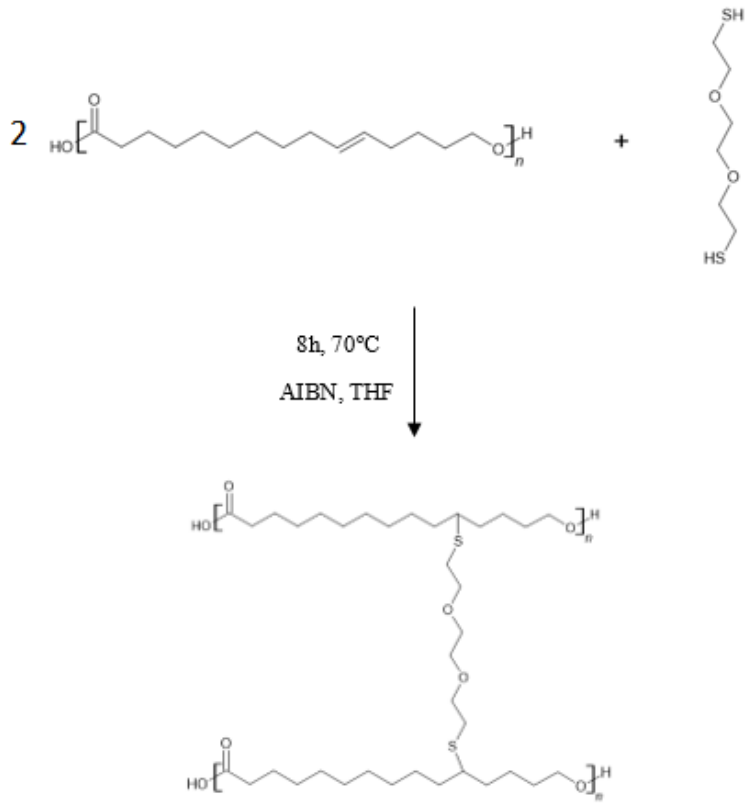
After crosslinking, samples were purified by precipitation in cold ethanol followed by drying at 60ºC for 48h to remove any remaining solvent or unreacted EDDT and then stored.

Table 1 – Formulations of crosslinking of PGl via thiol-ene reactions with EDDT.

Entry	PGl (mg)	EDDT (mg)	AIBN (mg)	THF (mL)
F1 (1:1, 1%)	242	92	1	4.4
F2 (1:1, 5%)	238	93	5	4.4
F3 (1:2, 1%)	239	182	2	5.6
F4 (1:2, 5%)	243	180	10	5.6

Source: Author (2023)

Figure 8 – Crosslinking of PGl with EDDT



Source: Author (2023)

3.2.4 Molecular weight distribution of PGl

Number average molecular weight (M_n), weight average molecular weight (M_w), and dispersity (Đ) were determined by Gel Permeation Chromatography (GPC) with a high performance liquid chromatography equipment (HPLC, model LC 20-A, Shimadzu) with a refraction index detector (RID-10A), and Shim Pack GPC800 Series columns (GPC804 and GPC807, Shimadzu). THF was used as eluent with volumetric flow rate of 1 mL·min^{-1} at 40 °C. Calibration was performed against styrene standards with molecular weight ranging from 580 to 3,000,000 g mol^{-1}. For the analysis, approximately 0.02 g of the PGl was dissolved in 4 mL of THF and filtered through a nylon syringe filter, pore: 0.45 µm, diameter: 33 mm.

3.2.5 Chemical structure

The chemical composition of PGl was determined by ^{1}H NMR spectroscopy using a on a Bruker AC-200F NMR, operating at 200 MHz. Chemical shifts are reported in ppm, relative to tetramethyl silane (TMS) 0.01 % (v/v) (δ=0.00). Samples of 10 mg were solubilized in 0.5 mL of $CDCl_3$ (δ=7.26 for ^{1}H NMR).

Poly(globalide) polymer: Yield: 75%. ^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm) = 5.55-5.30 (m, CH=CH), 4.18-4.00 (m, $CH_2O(C=O)$), 2.39-2.23 (m, $CH_2(C=O)O$), 2.13-1.95, 1.75-1.55, 1.45-1.15 (m, CH2).

Fourier transform infrared (FT-IR/ATR) spectroscopy was performed to identify other chemical structure characteristics PGl and crosslinked polymers. The analysis was determined by Attenuated Total Reflectance (ATR) on thin solid samples Cary 600 model (Model: Agilent Technologies CARY 600) spectrometer with ZnSe window Transmission infrared spectra were recorded in a wavenumber range of 650-4000 cm^{-1}.

3.2.6 Thermal properties

Pure PGl and crosslinked samples thermal properties were determined by differential scanning calorimetry (DSC). Samples of approximately 9 mg of pure PGl and of crosslinked polymers PGl-EDDT were analyzed using a Perkin-Elmer JadeDSC, under inert atmosphere (N_2, 50 $mL \cdot min^{-1}$), from -30 to 120 °C at a heating rate of 10 $°C \cdot min^{-1}$. Thermal history was removed, and the melting temperatures (T_m) were taken from the second heating runs and melting enthalpies (ΔH_m) and areas from first heating curves. Crosslinked samples crystallinity was estimated using their ΔH_m and ΔH_m of a standard of 100% crystalline poly(ω-pentadecalactone) (PPDL) using Eq 1:

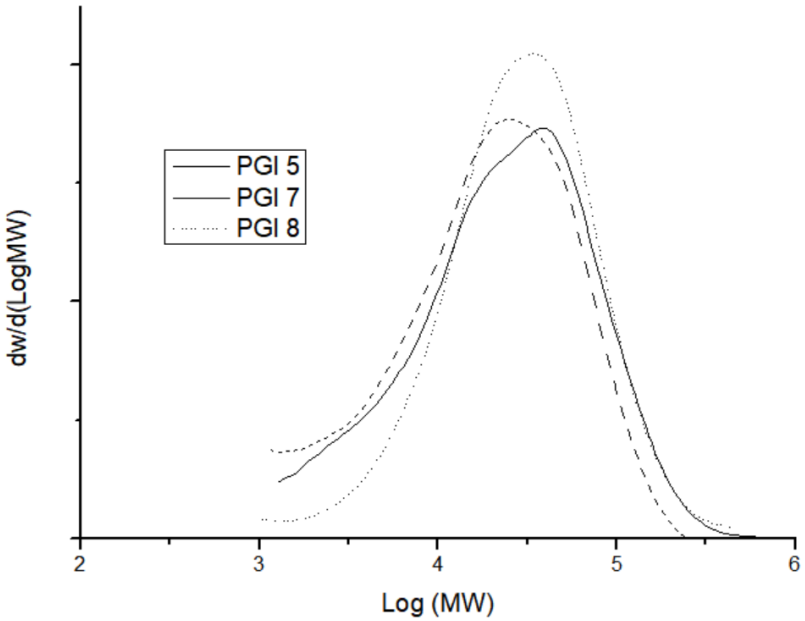
$$T_m^0(X) = \left[\frac{\Delta H(T_m^0, X)}{\Delta H(T_m^0)}\right] T_m^0 \quad (1)$$

where $\Delta H(T_m^0, X)$, $\Delta H(T_m^0)$, $T_m^0(X)$ and T_m^0 are the melting enthalpies and melting temperature of the crosslinked polymer and homopolymer respectively (CRESCENZI *et al.*, 1972; GUINDANI *et al.*, 2017).

3.3 RESULTS AND DISCUSSION

The e-ROP yield was on average 75% (experiments conducted in triplicate). For the last batch (PGl 8) the number (Mn) and weight (Mw) average molecular weights of PGl determined by GPC were 16,700 g·mol^{-1} and 44,926 g·mol^{-1}, respectively, resulting in a polydispersity Đ of 2.69. The previous reactions (PGl 5 and PGl 7) molecular weights, dispersities and GPC traces are presented in Table 2 and Figure 9. The variation of the results obtained can be explained by the arbitrary loss of oligomers during purification step and increase on system viscosity that could have decrease chain mobility in some cases and lead to higher dispersity (POLLONI *et al.*, 2017a). PGl homopolymer is white and opaque with a waxy aspect after purification and drying. Interestingly, samples after thiol-ene reaction with EDDT presented a rubbery yellowish complexion (Figure 10) and an apparently higher tear resistance when manipulated suggesting crosslinking and the presence of a less crystalline structure as well as thiol incorporation in polymer matrices.

Figure 9 – Normalized GPC traces of PGl samples GPC of different synthesized samples of PGl



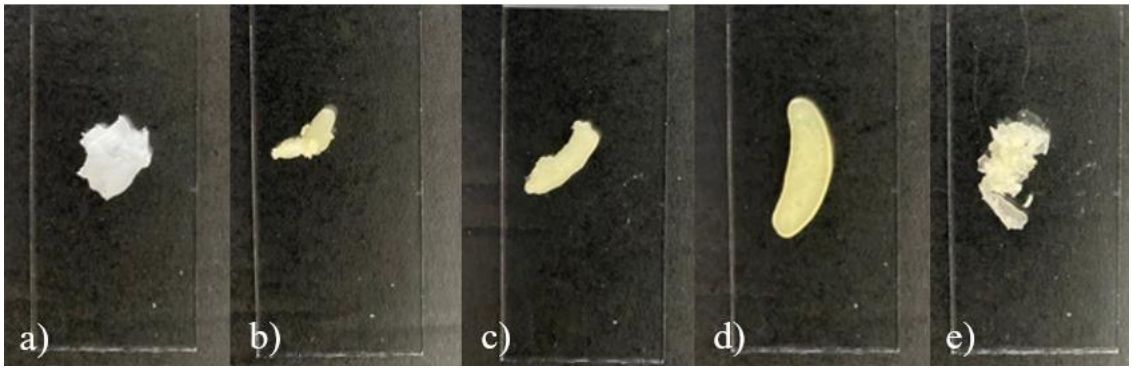
Source: Author (2023)

Table 2 – Weight average (Mw) and number average (Mn) molecular weights and dispersity measured by GPC of different synthesized samples of PGl

Sample	Mw (g/mol)	Mn (g/mol)	Đ
PGl 5	42,510	11,604	3.66
PGl 7	33,720	10,404	3.24
PGl 8	44,926	16,700	2.69

Source: Author (2023)

Figure 10 – Samples of a) PGl; and crosslinked samples using different thiol-ene ratios and AIBN concentrations b) F1 (1:1, 1%); c) F2 (1:1, 5%); d) F3 (1:2, 1%) and d) F4 (1:2, 5%)

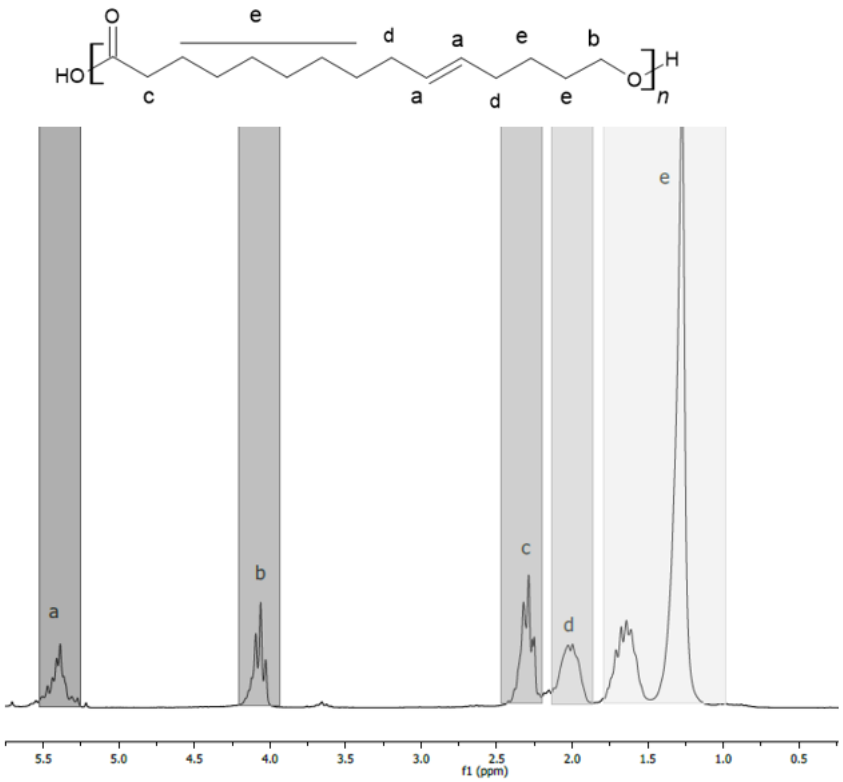


Source: Author (2023)

3.3.1 Chemical Structure

^{1}H NMR spectroscopy confirmed chemical structure of PGl in accordance to literature as Figure 11 shows (ATES; THORNTON; HEISE, 2011; CHIARADIA, 2019; GUINDANI *et al.*, 2017; VAN DER MEULEN *et al.*, 2008). Crosslinked samples were not analyzed due to their insolubility in $CDCl_3$. Notwithstanding, this fact indicates a high gel content in all tested reactions.

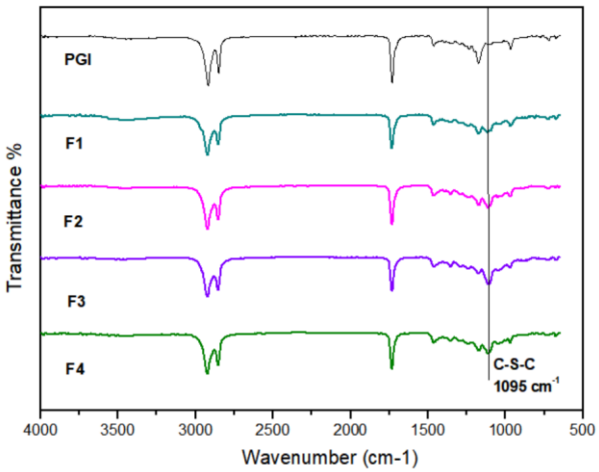
Figure 11 - ^{1}H NMR spectrum of PGl and the respective peak attributions to its chemical structure



Source: Author (2023)

FT-IR/ATR spectra (Figure 12) indicated all bands identified in PGl (SAVIN *et al.*, 2018): 721 cm^{-1} (*cis* RCH = CHR); 966 cm^{-1} (*trans* RCH=CHR); 1177 cm^{-1}(COC; 2918, 2848 and 1463 cm^{-1} (C–H); and 1730 cm-1 (C=O). PGl modification in samples F1 to F4 was observed with the appearance of C-S-C stretch (1095 cm^{-1}) present in 2,2′-(ethylenedioxy)diethanethiol (EDDT) ratifying crosslinking agent incorporation in the modified samples.

Figure 12 – FTIR spectra of PGL and crosslinked samples using different thiol ene ratios and AIBN concentrations b) F1 (1:2, 1%); c) F2 (1:2, 5%); d) F3 (1:1, 1%) and d) F4 (1:1, 5%)



Source: Author (2023)

3.3.2 Thermal properties

DSC thermograms (Figure 13) suggest that PGl tends to become less crystalline upon cross-linking as the melting peaks area decrease as thiol and initiator concentration increase. When analyzing both components separately, a pattern of ΔH_m decrease. These result is in line with previous works that approached crosslinking of PGl (CHIARADIA *et al.*, 2019; VAN DER MEULEN *et al.*, 2008). The degree of crystallinity in the materials were estimated by Eq. 1 using the value of a PPDL sample as reference. PGl homopolymer displayed highest crystallinity (49.54%) confirming its semicrystalline characteristic (Table 3). Also, the theoretically more crosslinked sample (F4, with the highest thiol and initiator concentrations) presented a complete disappearance of its melting peak indicating a completely amorphous feature as Figure 13 shows. Regarding melting temperatures, a less prominent decrease was observed. The modification in thermal properties are expected with the modification of PGl backbone since the crosslinking may lead to a greater distance of polymer chains which can reduce crystallinity behaviour (CHIARADIA, 2019).

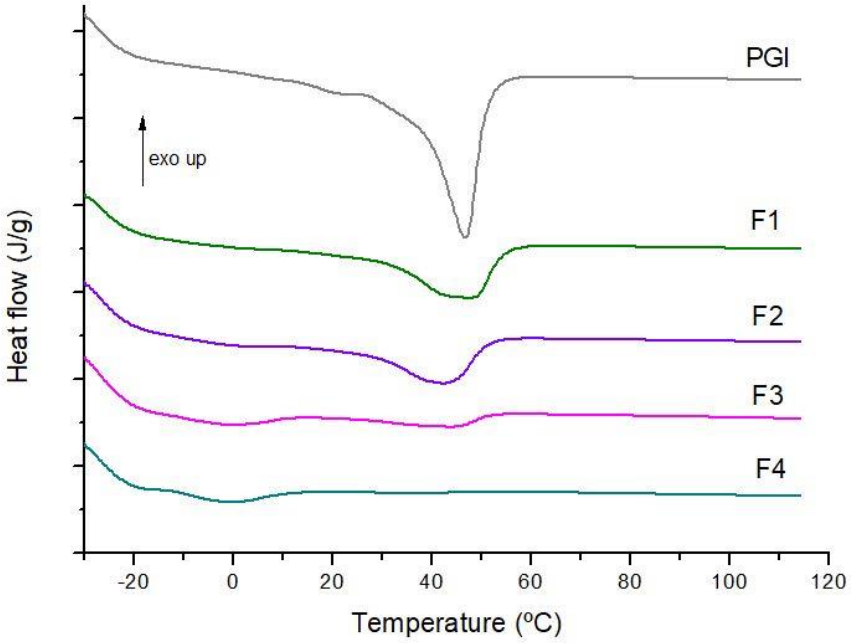
Table 3 – Thermal properties determined by DSC analysis for PGI and crosslinked samples (ΔH_m: melting enthalpy; T_m: melting temperature peak; X_C: degree of crystallinity, calculated from the fusion enthalpy value of a PPDL 100% crystalline sample (CRESCENZI *et al.*, 1972; GUINDANI *et al.*, 2017)).

Sample	ΔH_m(J/g)	Area (mJ)	T_m (C°)	X_C (%)
PGI	91.49	839.00	46.54	49.54
F1	62.00	501.54	47.50	33.56
F2	46.41	418.91	42.50	25.12
F3	9.53	86.80	44.24	5.16
F4	2.78	23.30	-*	1.50

*could not be determined

Source: Author (2023)

Figure 13 – DSC thermograms of PGI and crosslinked samples using different concentrations of EDDT and AIBN



Source: Author (2023)

3.4 CONCLUSIONS

The production of polymers derived from macrolactones through enzymatic polymerization is a promising field due to their unique range of properties, versatility and sustainability. In this chapter, enzymatic ring opening polymerization of macrolatone globalide was carried out obtaining a polymer with Mw and Mn of 44,926 $g \cdot mol^{-1}$ and 16,700 $g \cdot mol^{-1}$, respectively, and polydispersity Đ of 2.69. PGl was chemically characterized by ^1H NMR and FTIR/ATR corroborating its structure. In addition, thiol-ene crosslinking modification of resulting poly(macrolactone) poly(globalide) was studied in solution varying thiol:ene ratios and initiator concentration. Thermal properties of the materials were significantly changed mainly exhibiting a decrease in crystallinity as theoretical crosslinking increased. The results of this study suggest that PGl can be adjusted to meet specific requirements by introducing crosslinking agents into the polymer structure.

CHAPTER 4

4 BENZNIDAZOLE ENCAPSULATION IN CROSSLINKED POLYESTER NANOPARTICLES

In this chapter, polymacrolactone nanoparticles NPs were prepared from poly(globalide) (PGl) by miniemulsification-solvent evaporation (MSE) method and posteriorly modified by thiol-ene crosslinking reaction with 2′-(ethylenedioxy)diethanethiol (EDDT) using azobisisobutyronitrile (AIBN) as thermal initiator. The obtained NPs were characterized in respect to size distribution, zeta potential, morphology and thermal properties. The encapsulation efficiency and release profile of BNZ in both linear PGl and modified PGl (PGl-EDDT) NPs were evaluated by UV-Vis spectrometry.

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4.1 INTRODUCTION

Neglected tropical diseases (NTDs) are a group of contagious diseases that are mostly found in the world's poorest regions, where healthcare infrastructure is insufficient. The World Health Organization's 2023 report reveals 1.65 billion people required mass or individual treatment and care for NTDs worldwide. Among the NTDs, Chagas disease is still a major public health problem mainly in the American continent, including most remote areas of Brazil (WORLD HEALTH ORGANIZATION, 2023).

Benznidazole (BNZ) is an antiparasitic medication commonly used to treat Chagas disease caused by the parasite *Trypanosoma cruzi*. Despite its effectiveness, benznidazole is associated with significant side effects (DOS SANTOS SILVA *et al.*, 2019; VINUESA *et al.*, 2017). Also, drug efficacy is reduced in the chronic stage of the disease due to its limited ability to overcome biological barriers, which is primarily caused by its high toxicity and low solubility in water (0.4 g/mL) (DOS SANTOS-SILVA *et al.*, 2017; DOS SANTOS SILVA *et al.*, 2019). As an illustration, 20% of patients discontinue treatment due to drug adverse hypersensitivity effects (VINUESA *et al.*, 2017). As multiple disease treatments, drug delivery systems have been improved to enhance therapeutic efficacy and patient management. In the particular case of BNZ, several works have developed and tested nanocarriers to improve the drug delivery systems (DOS SANTOS-SILVA *et al.*, 2017; DOS SANTOS SILVA *et al.*, 2019; VINUESA *et al.*, 2017). Aiming the overcome of BNZ treatment drawbacks, colloidal systems such as liposomes, nanoparticles as well as other combinations have been

developed improving bioavailability and reducing toxicity of the drug(DOS SANTOS-SILVA *et al.*, 2017; DOS SANTOS SILVA *et al.*, 2019; ESTEVA *et al.*, 2016; MORILLA; PRIETO; ROMERO, 2005).

Particularly, polymeric nanoparticles have emerged as a promising drug delivery system due to their unique properties, such as high stability, biocompatibility, and versatility in encapsulating a wide range of drugs thus having an exceptional potential in medicine and related biomedical fields (FAROKHZAD; LANGER, 2009). Also, the use of nanostructures helps enhancing the dispersibility of a drug in water can lead to faster absorption, resulting in a quicker onset of pharmacological effects (JOSEPH *et al.*, 2022; ROCHA *et al.*, 2014).

Mainly, the versatility of these polymers enables the optimization of properties of their respective nanoparticles by tuning and improving their interaction with the biological environment. For instance, surface modification of polymeric nanoparticles with targeting ligands or functional groups can enhance their specificity and selectivity towards targeted cells or tissues as well as influence their pharmacokinetics and biodistribution (JOSEPH *et al.*, 2022; LI; HUANG, 2008; NAIR; LAURENCIN, 2007). As such, the development of nanoparticles with tailored properties holds great promise for revolutionizing medicine and related fields.

Nanoparticles can be prepared and modified through numerous methods. Typically, there are six traditional techniques to create nanoparticles, namely nanoprecipitation, emulsion-diffusion, double emulsification, emulsion-coacervation, polymer-coating, and layer-by-layer (MORA-HUERTAS; FESSI; ELAISSARI, 2010). However, alternative methods, such as emulsion-solvent/evaporation and the procedures for producing polymer liposomes, have also been employed (MORA-HUERTAS; FESSI; ELAISSARI, 2010). MSE is a widely used method particularly for the encapsulation of hydrophobic drugs or bioactive compounds being possible to produce NPs with small size and narrow PDI (ALEXANDRINO *et al.*, 2014; ROCHA *et al.*, 2014). This technique involves the formation of a nanometric scale emulsion, where the oil phase containing the hydrophobic compound is dispersed in an aqueous phase containing surfactants. The subsequent removal of the solvent through evaporation results in the formation of nanoparticles with a high drug-loading and bioavailability capacity (LEMOS-SENNA *et al.*, 1998; SZCZĘCH; SZCZEPANOWICZ, 2020).

In this context, the choice of polymers and methods used in nanoparticles for drug delivery applications is important for ensuring effective drug delivery. The

selected material should be biocompatible and non-toxic, biodegradable, have a high drug loading capacity and specific release profile. Among the most common polymers, polyesters-based nanoparticles show great promise as effective and versatile platforms for drug delivery and tissue engineering in the biomedical field (BEGINES *et al.*, 2020; CRAPARO *et al.*, 2020). These materials are an alternative to conventional polymers to due to their properties as biodegradability, biocompatibility, and non-toxicity being successfully applied for encapsulation of several compounds and as nanocarriers for sustained drug release (ALBERTSSON; VARMA; SRIVASTAVA, 2009; KOBAYASHI, 2015). Furthermore, the possibility of post-modifying a polymer can be useful in drug delivery systems, by improving the properties of the polymer in a targeted and specific manner (ZHONG *et al.*, 2018).

PGl has gained significant interest in the field of biomedical applications due to its unsaturated backbone, which facilitates its functionalization and/or crosslinking through click chemistry-based reactions like diels-alder and thiol-ene addition (CHIARADIA *et al.*, 2019; VAN DER MEULEN *et al.*, 2008). One of the primary challenges in utilizing PGl for biomedical applications is its high crystallinity, which restricts its application. In that sense, several researchers observed reduction in its crystallinity by different approaches such as copolymerization, crosslinking and modification at the PGl chain double bond via well-established thiol-ene reaction (ATES; THORNTON; HEISE, 2011; CHIARADIA, 2019; GUINDANI *et al.*, 2017; VAN DER MEULEN *et al.*, 2011a). Thiol-ene functionalization of PGl and other copolymers has been explored in several works to produce potential drug loading materials such as cross-linked polymers, grafted polymers and bioconjugates (ATES *et al.*, 2014; ATES; HEISE, 2014; DE OLIVEIRA *et al.*, 2017).

Within this framework, the development of drug delivery systems alternatives for poorly water-soluble drugs and their improvement is of great interest for many therapeutic compounds. This work aims to prepare and modify poly(globalide) nanoparticles crosslinking via thiol-ene reaction. In addition, encapsulation of BNZ in PGl NPs was carried out and analyzed as a potential drug delivery system to be used in Chagas's disease treatment.

4.2 MATERIALS AND METHODS

4.2.1 Materials

Globalide (Gl) was kindly donated by Symrise. Poly(globalide) (PGl) was prepared as described in Chapter 3. Dichloromethane P.A 99.8% (DCM) was purchased from Neon Química (Brazil). Azobisisobutyronitrile 98% (AIBN), Methanol P.A. 99.5% and 2,2′-(Ethylenedioxy)diethanethiol (EDDT) were acquired from Vetec Química (Brazil) and the former was recrystallized before use. Surfactant sodium dodecyl sulfate (SDS) and Benznidazole (BNZ) were purchased from Sigma-Aldrich (Germany). Water was deionized by a Milli-Q water purification system.

Phosphate-buffered saline (PBS; pH 7.4) - 0.01M PBS was prepared by dissolving 18 g NaCl, 0.18 g KCl, 0.8 g KH_2PO_4, and 5.8 g $Na_2HPO_4 \cdot 12H_2O$ in 200 mL of distilled water.

4.2.2 Poly(globalide) enzymatic polymerization

Poly(globalide) was synthesized as described in Chapter 3.

4.2.3 Poly(globalide) nanoparticles preparation

Nanoparticles were prepared by the MSE method based on the work of Guindani et al, 2019 (GUINDANI *et al.*, 2019) with a few adjustments depending on the formulation. For the preparation of PGl nanoparticles, both aqueous and organic phase were weighted individually in a precision balance (ATX224 Shimadzu). The aqueous phase was prepared in a beaker 7g of distilled water and SDS (0.2% w/w) and the organic phase was prepared in a sealed vial with 50 mg of PGl and 1.75 g of DCM. Both phases were homogenized by magnetic agitation first separately (5 min, 500 rpm) and then aqueous phase was added to the organic phase and then stirred again (20 min, 1000 rpm) forming a coarse emulsion. Sonication was then carried out using an ultrasound probe (Sonics Vibra Cell, VC505) with a 1/8” microtip for 3 min (pulse 10s on, 10s off) with an amplitude of 40%. Solvent was evaporated at 50ºC for

2h and the resulting nanoparticles colloidal dispersion stored at 4ºC for further analyses.

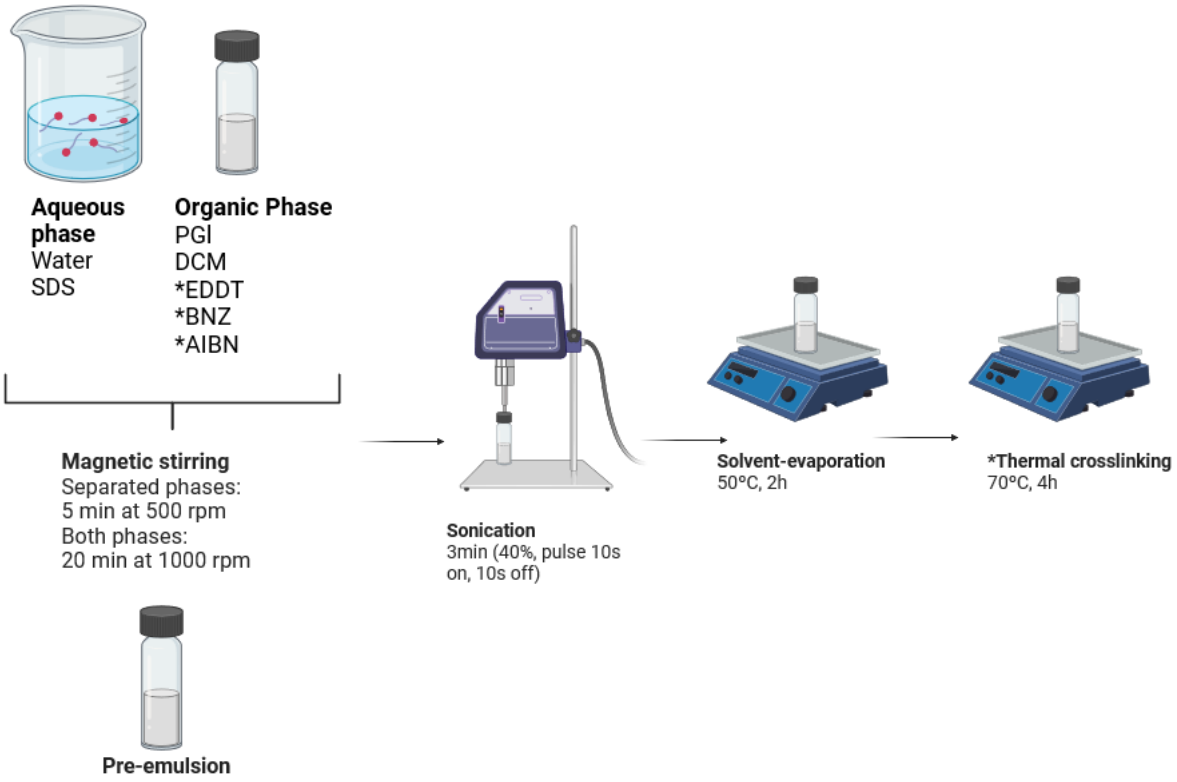
4.2.4 Nanoparticles crosslinking via thiol-ene reaction

For NPs crosslinking (PGl-EDDT), first NPs were prepared as described in the previous section with an extra crosslinking step after solvent evaporation. Therefore, the crosslinking agent EDDT and thermal initiator AIBN were added to the organic phase prior to the emulsification. Thiol-ene molar ratio was set as 1:1 (38.3 mg of EDDT) and the mass of initiator was 0.5 mg (1% w/w in relation to the polymer). Solvent mass (3.09 g) was adjusted in relation to the total solids amount to maintain the viscosity conditions during agitation/sonication and for very low masses of components, a stock solution in DCM was prepared before weighting. Thermal crosslinking was carried out in the same vial at 70 ºC for 4h under continuous magnetic stirring. After reaction completion, colloidal dispersions of the crosslinked NPs were refrigerated at 4ºC.

4.2.5 Nanoencapsulation of benznidazole

Benznidazole was encapsulated in either PGl or PGl-EDDT NPs (PGl-BNZ and PGl-EDDT-BNZ) by the same procedure from section 4.2.3, with the addition of the hydrophobic drug in the organic phase (Figure 14). BNZ concentration was established as 10% w/w (5 mg) in relation to PGl. Samples containing the drug were carefully protected by light throughout the experiment with aluminum foil due to its photosensitivity. DCM amount was also adjusted (1.925g to PGl-BNZ and 3.27g to PGl-EDDT-BNZ). A schematic diagram of MSE/nanoencapsulation process is presented in Figure 14.

Figure 14 – Schematic representation of experimental steps of the PGI NPs preparation by the MSE process and NPs crosslinking (Compounds and step preceded by * were only added when specifically mentioned).



Source: Author (2023)

4.2.6 NPs characterization

Intensity average diameter (Dp), and polydispersity indexes (PDI) of NPs by dynamic light scattering (DLS) and zeta potential (ζ) was measured by electrophoretic light scattering were determined on a Zetasizer Nano S equipment (Malvern Instruments). Samples were diluted in distilled water at approximately 1:100 before each measurement. All measurements were carried out in triplicate but ζ which was measured in duplicate.

One-way analysis of variance, ANOVA, and Tukey's Honestly Significant Difference (HSD) test were employed for statistical analysis of Dp and PDI values. The one-way ANOVA assessed whether there were significant differences in means between the groups, while the post-hoc Tukey's HSD test identified specific pairs of groups with significant mean differences. These statistical analyses allowed us to investigate relationships between the groups while controlling for potential Type I

errors due to multiple comparisons. All calculations were performed using the Python programming language.

NPs morphology was evaluated by transmission electron microscopy (TEM) using a JEOL microscope (JEM-1011). One drop of the diluted latex (solids content of 0.7%) was placed on a carbon coated grid and dried overnight at room temperature.

4.2.6.1 Thermal properties

NPs thermal properties were determined by differential scanning calorimetry (DSC) after purification by Amicon filters to remove excess surfactant. Since NPs could not be separated by simple centrifugation, Amicon Ultra 0.5 filter (Millipore®, 100 KDa - 100,000 NMWL) were used for this purpose. About 500 µL of NPs colloidal dispersion was verted in an Eppendorf coupled with a filter and ultracentrifuged for 5 min at 13,300 rpm. Supernatant was stored and the Amicon filter was inverted and coupled with another Eppendorf to collect the solid material adhered in the filter by centrifugation for another 5 min at 13,300 rpm. This process was repeated 5 times or until the filter was completely clogged. To remove the remaining polymer more efficiently a small amount of DCM was added in each inversion. The collected solids were dried for 24 h at 60 ºC and stored for further analysis.

Samples of approximately 9 mg pure PGl and purified samples of all formulations were analyzed using a Perkin-Elmer JadeDSC, under inert atmosphere (N_2, 50 mL·min-1), from -30 to 200 °C at a heating rate of 10 °C/min. Thermal history was removed and the melting temperatures (T_m) were taken from the second heating runs and melting enthalpies (ΔH_m) and areas from first heating curves. Crosslinked samples crystallinity was estimated using their ΔH_m and ΔH_m of a standard of 100% crystalline poly(ω-pentadecalactone) (PPDL) using eq. 3.1. (CRESCENZI *et al.*, 1972; GUINDANI *et al.*, 2017). In addition, DSC analysis of pure BNZ and a physical mixture of BNZ and PGl with the same drug:polymer ratio of NPs was performed from 25 to 250 °C and -30 to 200ºC.

4.2.6.2 Encapsulation efficiency

For drug loading quantification and release assay a calibration curve was obtained using different dilutions of BNZ in methanol due to drug greater solubility in this solvent. First, a stock solution of 200 µg mL^{-1} (200 ppm) was prepared and then

diluted in lower concentration solutions which values ranged from 1 to 40 μg mL^{-1}. Each solution was analyzed at 315 nm in a UV-Vis spectrophotometer (HITACHI U-1900).

Previous tests were performed with PBS and distilled water and values did not show significant variation for encapsulation efficiency values and UV-Vis analysis using methanol as a solvent in the cuvettes. Thus, the curve could be used without further alteration in the release experiment.

For encapsulation efficiency determination, an aliquot 400 μL of each sample was centrifuged in an Amicon Ultra 0.5 filter (Millipore®, 100 KDa - 100,000 NMWL) for 30 min at 13,300 rpm. An aliquot of 100 μL of the supernatant was then diluted in 2 mL of distilled water in a quartz cuvette and subsequently analyzed at 315 nm (peak of BNZ) in UV-Vis equipment. The encapsulation efficiency was determined by Eq. 2.

$$EE\% = \frac{(m_{total} - m_{free})}{m_{total}} \times 100 \quad (2)$$

where m_{total} and m_{free} stand for the starting amount of BNZ used and the amount of free BNZ determined by UV–Vis spectrophotometry, respectively. The experiments of this section were performed in triplicate.

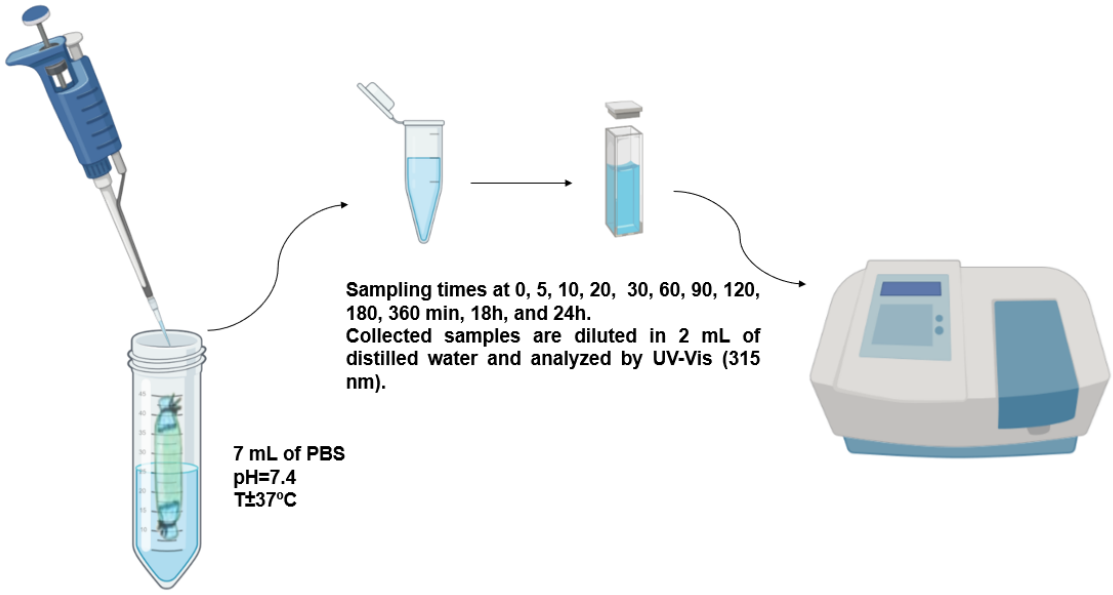
Statistical analysis was performed by a two-way analysis of variance (ANOVA) was conducted at a 95% confidence level, along with Tukey's multiple comparisons test, to determine statistical differences. A significance level of $p < 0.05$ was used to identify statistically significant differences between the groups or conditions being compared. Repeated observations were analyzed using these statistical methods to assess the presence of significant differences.

4.2.6.3 Release experiment

Release study was performed in a PBS (pH 7.4) solution at 37ºC. The release apparatus consituted of a dyalisys bag with 1 mL of latex inside a falcon tube filled with 7 mL of buffer solution under continuous agitation and controlled temperature. Aliquots of 100 μL of the buffer solution containing the permeate were collected at pre-determined time intervals (0, 5, 10, 20, 30, 60, 90 min, and 2, 3, 6, 18 and 24h) and diluted in a quartz cuvette with 2mL of water and measured in the UV-Vis equipment. Experimental apparatus and sequence are presented in Figure 15. In order to maintain sink conditions, medium was replaced each measurement and the concentration change was recalculated. All measurements were performed in duplicate and values

were determined by interpolation on BNZ calibration curve. The statistical analysis was pefromed as mentioned in the previous section.

Figure 15 – Experimental procedure of BNZ release study



Source: Author (2023)

4.3 RESULTS AND DISCUSSION

4.3.1 Nanoparticles characterization

DLS measurements revealed that MSE method provided nanoparticles from 99 to 107 nm with all tested formulations with a narrow PDI (<0.2) indicating a uniform size distribution and their suitability for the intended applications. Zeta potential values were ranged from -21.83 to -28.32 mV. The negative charge is related with the presence of anionic surfactant SDS as it is showed in Table 4.

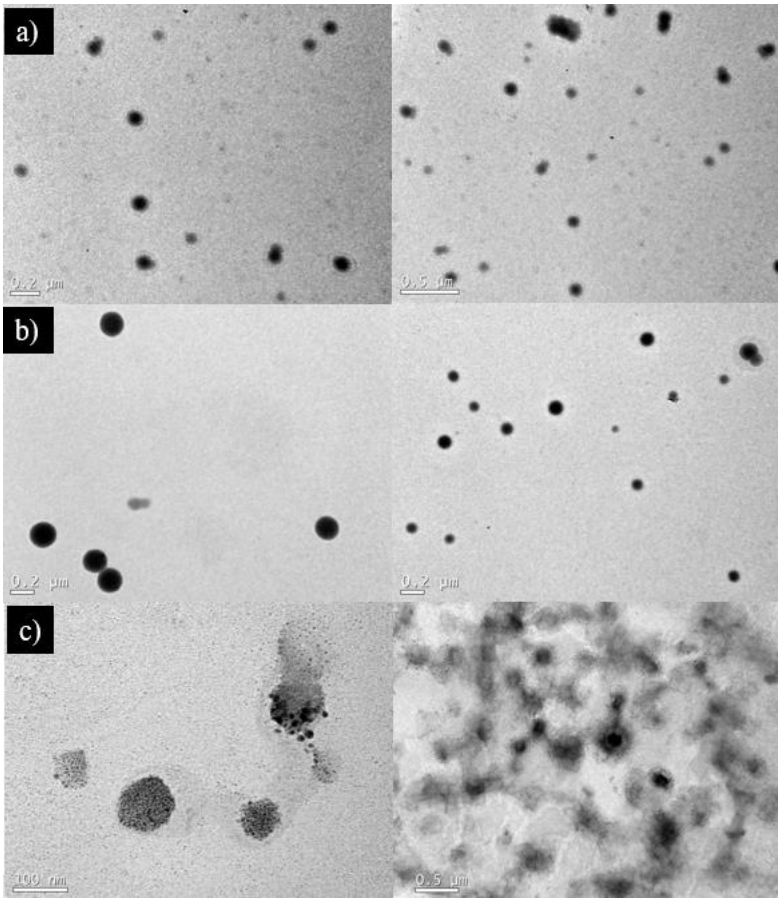
Table 4 – Intensity average particle size (Dp), dispersity (PDI) and zeta potential (ζ) of PGI nanoparticles and encapsulation efficiency (EE) of benznidazol

Sample	Dp ± SD	PDI±SD	ζ (mV)	EE%
PGI	99.9 ± 1.4	0.12 ± 0.01	-25.9	-
PGI-EDDT	107.4 ± 2.2	0.12 ± 0.02	-24.3	-
PGI-BNZ	99.2 ± 1.4	0.13 ± 0.01	-21.8	92.18 ± 3.33
PGI-EDDT-BNZ	105.2 ± 1.7	0.13 ± 0.02	-28.3	85.07 ± 8.36

Source: Author (2023)

TEM images after purification confirmed nanometric scale (around 100 to 200 nm) of non-BNZ loaded NPs with spherical shapes (Figure 16). Statistical analysis showed significant difference ($p<0.05$) in between the following nanoparticles formulations: PGI and PGI-EDDT ($p=0.0052$); PGI and PGI-EDDT-BNZ ($p=0.0335$); PGI-EDDT and PGI-BNZ-EDDT ($p=0.003$); and PGI-BNZ and PGI-EDDT-BNZ ($p=0.0179$). No significance differences were observed in the pairs of PGI and PGI-BNZ ($p=0.9664$) and PGI-EDDT and PGI-EDDT-BNZ ($p=0.522$). These results indicate that the addition of BNZ did not alter significantly particle diameter as the crosslinking reaction. Crosslinked samples without BNZ presented sharper contours than pure PGI NPs. The samples with the drug (PGI-EDDT-BNZ) in Figure 16 (c) had relatively more noise in imaging probably due to NPs and drug degradation due to its photosensitivity during sample analysis (potential beam damage) and manipulation (environment light). This fact suggest that another qualitative magnification method could be useful for these NPs such as scanning electron microscopy (SEM) or atomic force microscopy (AFM).

Figure 16 – TEM images of a) PGl, b) PGl-EDDT and c) PGl-EDDT-BNZ NPs



Source: Author (2023)

4.3.2 Thermal properties

In order to identify thermal events all relevant components and their respective changes during nanoparticles processing thermographs of pure PGl and a physical mixture were obtained. Figure 17 shows the disappearance of BNZ endothermic peak (186.4^{o}C in the pure sample) in the second heat curves of the simple physical mixture suggesting that an amorphization occurred in the polymeric matrix during analysis. NPs DSC graphs are represented in Figure 18. Clearly, it can be observed crosslinked purified nanoparticles (PGl-EDDT and PGl-EDDT-BNZ) constituted of completely amorphous materials, i.e., they suffered a great reduction in their crystallinity. A less prominent crystallinity reduction was observed in the samples without crosslinking agent EDDT as Table 5 shows. This decrease in crystallinity is probably due to remaining surfactant that could have retained water in the polymer matrix. BNZ endothermic peak (186.4^{o}C in the pure sample) was not observed in the second heat

curves of neither NPs contained the drug nor the physical mixture suggesting an amorphization in polymeric matrix during the encapsulation process which was observed in literature with the BNZ polymeric NPs (SEREMETA *et al.*, 2019). Another interesting change was the reduction of melting temperatures (T_m) of PGl and PGl-BNZ NPs (33.11 and 35.19ºC) in relation to their respective bulk material samples (46.54 and 43.64ºC). The thermal properties of polymer nanoparticles can be different from bulk polymers due to the unique physical and chemical properties that arise from their small size and changes in the crystalline structure after processing or thermal history. Bunjes et al (2000) observed a reduction in melting temperatures up to 5 ºC in solid lipid nanoparticles produced via emulsification in relation to their bulk material (BUNJES; KOCH; WESTESEN, 2000). Another similar study with polycaprolactone (PCL) homopolymer and PCL/PCL-b-poly-(ethylene oxide) (PEO) nanoparticles was conducted revealing a reduction of 3 to 4 ºC in their T_m (CHO *et al.*, 2006). It is important to highlight that these changes in endothermic peaks do not necessarily indicate potential incompatibility of the drug in polymer matrix but a solubilization complete of BNZ in its polymeric excipient during encapsulation could be inferred (BARRERA *et al.*, 2020; VERMA; GARG, 2005). Thus, with different nanoparticles materials and processing methods a more significant change could be observed in the present, suggesting a greater modification in the crystalline structure of the polymer.

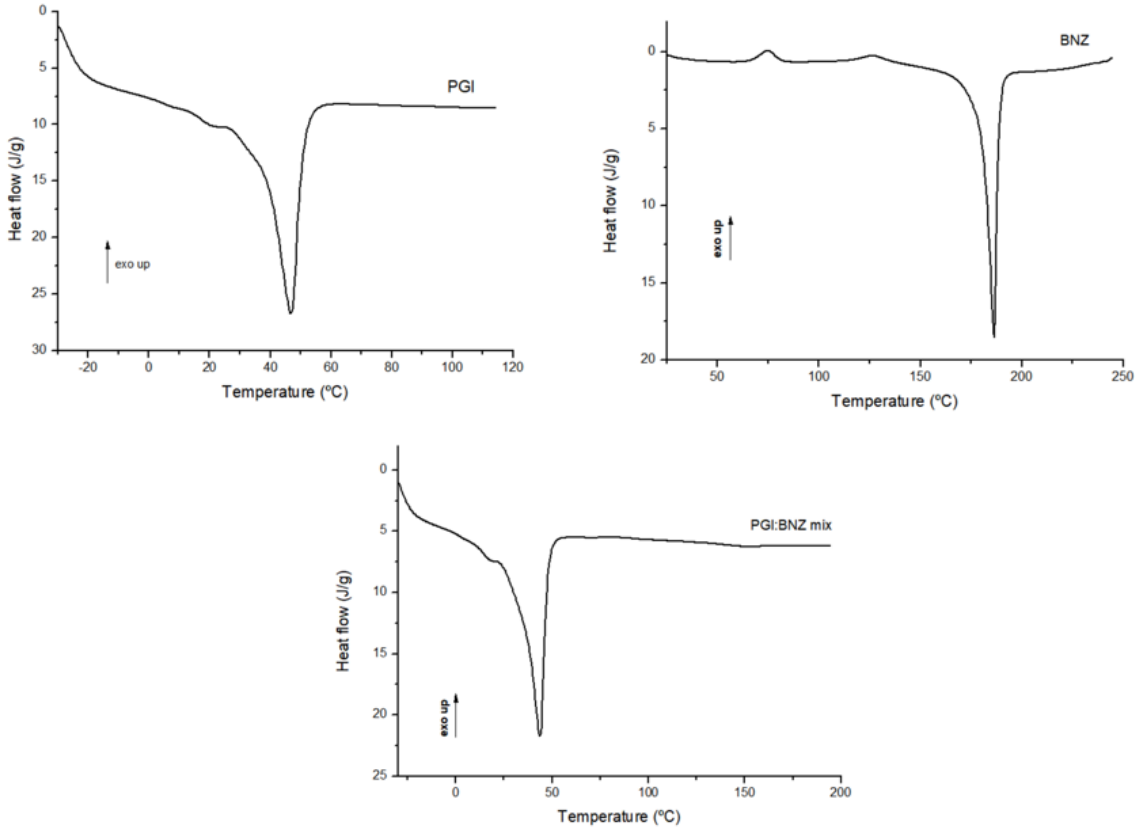
Table 5 – Thermal properties of linear PGl, purified polymeric nanoparticles and BNZ-PGl mixture (ΔH_m: melting enthalpy; T_m: melting temperature peak; X_C: degree of crystallinity, calculated from the fusion enthalpy value of a PPDL 100% crystalline sample (CRESCENZI *et al.*, 1972; GUINDANI *et al.*, 2017)).

Sample	**ΔH_m(J/g)**	**Area (mJ)**	**Tm (Cº)**	**Xc (%)**
PGl	91.49	839.00	46.54	49.54
PGl NPs	56.55	542.02	33.11	30.62
PGl-BNZ NPs	49.45	477.82	35.19	26.77
PGl-EDDT NPs*	-	-	-	-
PGl-EDDT-BNZ NPs*	-	-	-	-
PGl-BNZ MIX	70.92	648.68	43.64	38.39

*could not be determined

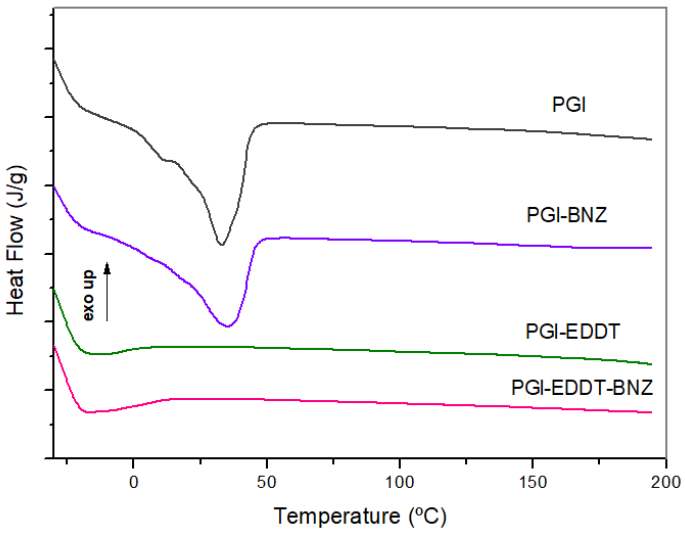
Source: Author (2023)

Figure 17: DSC thermograms of pure PGI, BNZ and PGI-BNZ physical mixture



Source: Author (2023)

Figure 18 – DSC thermograms of obtained NPs obtained by miniemulsification-solvent evaporation method.



Source: Author (2023)

4.3.3 Encapsulation efficiency

Before BNZ encapsulation efficiency and release experiment, UV-Vis previous measurements revealed that the solvent did not interfere significantly in the drug quantification as the group of SOUSA *et al.* (2021) also observed for BNZ incorporation in polyethylene glycol (PEG 4000) modified microparticles.

The EE% of BNZ in nanoparticles is variable depending on nanocarrier material formulation and encapsulation method. The present methodology showed average values of EE% of 92% and 85% for PGl and PGI-EDDT NPs, respectively. The statistical analysis showed that there was no significant difference between the two types of nanoparticles ($p=0.1511$). Usually when applying solvent evaporation method, the encapsulation of hydrophobic drugs are generally around 60% (SOUSA *et al.*, 2021). Remarkably, Vinuesa *et al.* prepared in solid lipids NPs (SLNs) and nanostructured lipid NPs (NLCs) emulsion solvent evaporation and melt homogenization with BNZ encapsulation efficiency from 83 to 95% for BNZ encapsulated in polymeric nanoparticles. Therefore, the present result indicates an interesting approach of increasing bioavailability of BNZ in PGI NPs for future Chagas disease treatment improvements.

4.3.4 Release experiment

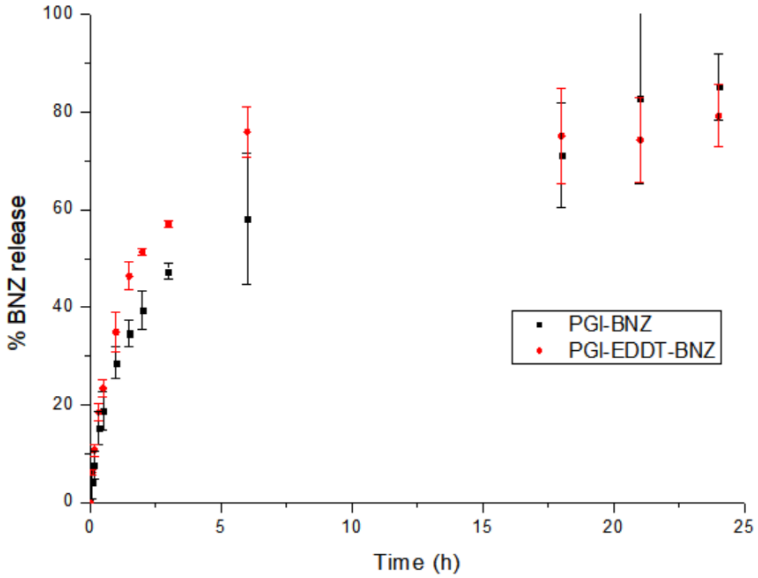
As showed in section 4.3.3, NPs crosslinking with EDDT decreased completely polymers crystallinity. Theoretically, the drug release rate can be influenced by various factors. Crystallinity is related to polymer matrix behavior including drug release properties, since it can alter, for instance, properties such as biodegradability (CHIARADIA *et al.*, 2018).

The release experiment showed a slightly higher release for crosslinked nanoparticles with an initial burst in the first 3 h, reaching 50% of released BNZ followed by a slower release rate up to 6 h and then a plateau (Figure 19). Comparing the two formulations, the significance level represented by p-value was 0.2799 ($p>0.05$) which means that, based on these results, it was not possible to conclude that there is a significant difference between the means of PGI-BNZ and PGI-EDDT-BNZ nanoparticles release. However, when analyzing the data up to 3 hours – where the standard deviation was lower – the slope of crosslinked nanoparticles was

significantly different (y=9.2199x and y=7.2898x) with p-value lower than 0.05. This result suggests higher release rate in crosslinked nanoparticles in the first hours of the experiment.

Besides crystallinity reduction, release behavior might be modified because crosslinked nanoparticles can swell when exposed to certain stimuli, such as changes in pH or temperature. This swelling may increase the volume of the NP matrix, creating extra space for drug diffusion, thus increasing release (ASADI *et al.*, 2011; DEIRRAM *et al.*, 2019). In addition, the ether groups present in EDDT chains can be degraded under physiological conditions which can also contribute to a more rapid release as it was observed in previous works that studied thiol-crosslinked nanostructures (DEIRRAM *et al.*, 2019; ZHANG *et al.*, 2014b, 2015). In this work, the crystallinity featured in the linear NPs hinders drug diffusion and retards the release, whereas the amorphous crosslinked counterparts allow a faster drug release and still are able to attain high encapsulation efficiency. The crosslinking process of PGI NPs illustrates how chemical modifications and alterations in molecular architecture can be used to tailor drug release depending on the demand, *i.e.,* faster or slower release kinetics. Furthermore, variations in reactions conditions could be implemented so this approach can still be useful to modulate BNZ release kinetics even further.

Figure 19 – Release assay of BNZ from PGI-BNZ and PGI-EDDT-BNZ NPs



Source: Author (2023)

4.4 CONCLUSIONS

PGI nanoparticles were successfully prepared via MSE technique with varied formulation resulting in nanometric scale (around 100 nm) with low polydispersity (PDI < 0.2) materials. PGI main-chain unsaturations enable nanoparticles crosslinking through thiol-ene reaction which was carried out in the presence of thermal initiator AIBN. Thermal characteristics of the obtained materials were significantly altered suggesting a significant loss of crystallinity. Furthermore, NPs applicability as nanocarriers was tested by encapsulating of BNZ also by MSE reaching over 85% of encapsulation efficiency in both linear and the crosslinked PGI. A released study showed that crosslinking renders faster drug release kinetics by eliminating crystalline features of PGI, which affects drug diffusion under physiological conditions. In conclusion, the use of a versatile polymeric biomaterial as PGI combined with a facile straightforward process to prepare nanocarriers is undoubtedly attractive for a range of potential biomedical applications, not only for BNZ but other hydrophobic compounds that may need therapeutic and administration improvements.

CHAPTER 5
5 CONCLUDING REMARKS

Micro- and nanoencapsulation of drugs may present several advantages in relation to directly delivered compounds. The selection of a polymer as a nanocarrier depends on its characteristics as well as the encapsulated drug properties of the drug as well the other components of the formulation. This work aimed to synthesize and characterize poly(globalide) (PGl) and PGl-based nanoparticles loaded with benznidazole (BNZ) and evaluate their release behavior.

Enzymatic polymerization of macrolactones to create polymers is a promising area, as these polymers offer a diverse range of unique properties and are both versatile and sustainable. In this work, the enzymatic polymerization of macrolactone globalide resulted in the production of a polymer with Mw and Mn values of 44,926 $g \cdot mol^{-1}$ and 16,700 $g \cdot mol^{-1}$, respectively, and a polydispersity index of 2.69. The structure of the resulting PGl was confirmed through chemical characterization using ^1H NMR and FTIR/ATR. Furthermore, the thiol-ene crosslinking modification of the resulting poly(macrolactone) poly(globalide) was studied by varying the thiol:ene ratios and initiator concentration in solution. The thermal properties of the resulting materials (PGl-EDDT) were significantly altered compared to PGl, with a prominent decrease in crystallinity observed as the theoretical crosslinking degree increased. These findings suggest that introducing crosslinking agents into the polymer structure can be used to tailor PGl to meet specific requirements.

Poly(globalide) NPs were successfully prepared via MSE technique with varied formulations resulting in sizes around 100 nm and low polydispersity indexes (PDI < 0.2). The main-chain unsaturation in PGl allowed for crosslinking of nanoparticles through a thiol-ene reaction, which was carried out in the presence of thermal initiator AIBN. The thermal properties of the resulting materials were markedly changed, indicating a significant decrease in crystallinity. Additionally, the potential of the nanoparticles to be used as nanocarriers were evaluated by encapsulating BNZ through the mini emulsification-solvent evaporation method. The results showed that crosslinked PGl achieved over 85% encapsulation efficiency of BNZ, while linear PGl NPs presented 92%.

The release study under controlled conditions showed that crosslinking enhances the rate of drug release by eliminating the crystalline properties of PGl, which

can impede drug diffusion under physiological conditions. This suggests that PGl, as a versatile polymeric biomaterial, in combination with a simple and straightforward method for preparing nanocarriers, holds significant potential for a wide range of biomedical applications, not only for BNZ but also for other hydrophobic compounds that require improvements in their therapeutic and administration properties.

Thus, this work demonstrated the suitability of the proposed nanocarrier system as a contender for BNZ delivery applications. The use of PGl has proven to be a highly effective matrix by its crosslinking. It is susceptible to other types of functionalization to overcome the drawbacks of low-solubility drug therapies. Also, it was possible to verify the influence of crosslinking in drug release rates of the prepared NPs that can be furtherly developed to more specific finalities.

To future works, it is suggested to study deeply BNZ release kinetics under varied controlled conditions and different crosslinking degrees as well as incorporate other functional groups in the formulation since MSE technique and thermal crosslinking offer relatively simple possibilities of adjustments. Finally, improved systems' cytotoxicity can be evaluated *in vitro* and *in vivo*.

With the results achieved herein, posterior changes in the applied methodology offer a wide range of tunable properties with PGl or copolymers that might be useful to produce feasible nanocarriers in the future.

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