

Bioderived Cyrene For Sustainably 3D Printing Organo/Hydrogels

by

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Author's Declaration

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Abstract

Hydrogels possess unique properties that make them suitable for a wide range of applications. Recent advances in 3D printing techniques, such as stereolithography (SLA), have enabled the precise customization of hydrogel structures with complex geometries and controlled mechanical properties. Further development of advanced hydrogel properties remains a key research focus, with particular attention to improving properties such as mechanical integrity, electrical conductivity, and responsiveness to environmental stimuli. Strategies such as incorporating fillers and nanofillers into hydrogel matrices have been explored to enhance these properties. Additionally, the use of organic solvents instead of water, leading to the formation of organogels, has begun to expand the range of printable materials, addressing some limitations associated with hydrogel-based SLA printing, such as structural disintegration.

This thesis investigates the use of Cyrene, a bioderived and environmentally friendly solvent, as an alternative to traditional organic solvents in 3D-printed hydrogel systems. Organogels can be 3D printed, and afterwards a simple solvent exchange with water can convert organogels into the desired hydrogels materials, maintaining the advantage of biocompatibility along with the complex structures produced by SLA 3D printing. This research work started by exploring Cyrene's role in hydrogel formulation, printability, and mechanical performance, comparing its effectiveness with the synthesis route using Cyrene and conventional solvents such as dimethyl sulfoxide (DMSO). Specifically, this study focuses on Cyrene's application in mask stereolithography (mSLA) 3D printing of organogels. It demonstrates Cyrene's excellent performance in enhancing hydrogel stretchability and swelling behavior after solvent exchange. Additionally, structural stability during organogel printing is improved due to the application of a previously developed acrylate salt. The findings reported in this work suggest that Cyrene-based hydrogels have promising applications in biomedical fields and soft robotics.

Furthermore, we extend this investigation by examining Cyrene as a dispersion medium for graphene, and its use in hydrogel nanocomposites. The study highlights Cyrene's ability to stabilize graphene dispersion without the need for chemical surfactants, aiming to produce hydrogels with enhanced mechanical strength, electrical conductivity, and multifunctional properties. These advancements open new investigations for applications in flexible electronics, biosensing, and tissue engineering.

The research findings provide a comprehensive understanding of Cyrene's potential as a sustainable solvent in the synthesis of organogels that can be converted into hydrogels after solvent exchange and used for a wide range of applications. The insights gained from this research contribute to the advancement of high-performance, eco-friendly hydrogel materials by demonstrating how Cyrene can serve as a sustainable alternative to conventional organic solvents. By utilizing a bioderived solvent to create organogels, followed by solvent exchange with water, the mechanical properties, structural stability, and biocompatibility of 3D-printed hydrogels can be enhanced. Additionally, the ability to stabilize graphene dispersion in hydrogels without chemical surfactants opens new opportunities for developing conductive and multifunctional hydrogel-based devices. These findings not only support the ongoing shift toward greener material synthesis but also lay the foundation for future innovations in sustainable material science and additive manufacturing.

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Dedication

I dedicate this thesis to my family, whose unwavering love, encouragement, and sacrifices have been the foundation of my journey. Your belief in me has given me the strength to persevere through every challenge. To my parents and my aunt, for their endless support and for instilling in me the values of hard work and resilience. Your guidance has shaped me into the person I am today. To my sisters, for their constant encouragement, for always celebrating my achievements, and for lifting me up during difficult times.

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List of Abbreviations

SLA: Stereolithography

mSLA: Mask stereolithography

DMSO: Dimethyl sulfoxide

CNTs: Carbon nanotubes

PEGDA: Poly(ethylene glycol) diacrylate

DMF: N,N-dimethylformamide

NMP: N-Methyl-2-pyrrolidone

scCO₂: Super critical carbon dioxide

DESs: Deep eutectic solvents

GVL: γ -Valerolactone

DMI: Dimethyl isosorbide

MOFs: Metal-organic frameworks

PAI: Poly(amide-imide)

LCD: Liquid crystal display

UPW: Ultrapure water

DI: Deionized Water

SPA: 3-sulfopropyl acrylate

Qch: Quaternized chitosan hydroxide

QCh:SPA: Neutralized Quaternized Chitosan Hydroxide Salt

HEA: 2-Hydroxyethyl acrylate

EGDA: Ethylene glycol diacrylate

Dp: Depth of penetration

Ec: Critical energy

Cd: Thickness of cured resin

E0: Exposure dose

PDMS: Polydimethylsiloxane

SEM: Scanning electron microscopy

ESEM: Environmental scanning electron microscopy

RH - Relative humidity

Chapter 1 Introduction

Hydrogels, three-dimensional networks of hydrophilic polymers, have garnered significant attention in recent years due to their unique properties and diverse applications.¹ Composed of either natural or synthetic polymers, hydrogels can be tailored to exhibit specific characteristics such as high water content, biocompatibility, and tunable mechanical strength.² These features make them ideal for various biomedical applications, including tissue engineering, drug delivery systems, and wound dressings.³⁻⁵ Additionally, among hydrogel fabrication techniques, 3D printing has emerged in recent years, enabling the precise customization of structures with complex geometries and controlled mechanical properties.⁶

In particular, stereolithography (SLA) has revolutionized the fabrication of intricate hydrogel structures with high precision and resolution.⁷ However, challenges remain in enhancing the mechanical stability, environmental sustainability, and compatibility of hydrogels with photopolymerization-based printing techniques.^{8,9} The development of advanced hydrogel properties remains a key research focus, with efforts directed toward improving mechanical integrity, electrical conductivity, and responsiveness to environmental stimuli. Strategies such as incorporating fillers and nanofillers into hydrogel matrices have been explored to enhance these properties.¹⁰⁻¹² Additionally, the use of organic solvents instead of water, leading to the formation of organogels, has begun to be exploited to expand the range of printable materials, which may address some limitations associated with hydrogel-based SLA printing.¹³

As the field evolves, there is increasing interest in utilizing sustainable and green solvents like Cyrene for organogel synthesis. Derived from cellulose waste, Cyrene presents a promising alternative to traditional solvents, aligning with green chemistry principles and potentially reducing environmental impact.¹⁴ Despite its advantages, its role in hydrogel synthesis and 3D printing remains largely unexplored. This thesis investigates the potential of Cyrene as a sustainable solvent for advancing 3D-printed hydrogel systems.

In chapter 3, we will focus on the use of Cyrene in mask stereolithography (mSLA) 3D printing of organogels, examining its impact on printability, mechanical performance, and structural stability. mSLA printing of hydrogels often leads to structure deformation or collapse due to the hydrogel low

crosslinking density and mechanical stress generation during the 3D printing process, alternatives are needed.¹³ The use of organogels during 3D printing can increase intermolecular forces within the material, decreasing the likelihood of structural disintegration.¹³ Organogels can be 3D printed, and afterwards a simple solvent exchange with water can convert organogels into the desired hydrogels materials, maintaining the advantage of biocompatibility along with the complex structures produced by SLA 3D printing.¹⁵ The selection of a solvent becomes a critical factor, as it greatly influences the properties of the resulting hydrogel. Particularly, solvent toxicity and safety are critical considerations for applications involving contact with living systems or the environment.¹⁶⁻¹⁹ Cyrene can be a potential alternative for avoiding the use of hazardous solvents, such as dimethyl sulfoxide (DMSO), which can deeply penetrate the skin and generate hazardous by-products.

Our recent study has begun exploring Cyrene as a solvent for organogel synthesis and 3D printing.²⁰ We previously demonstrated the chemical process for creating conductive supramolecular hydrogels using a developed crosslinking strategy with poly(ionic) salts and Cyrene as a medium.²⁰ In light of these considerations, this study expands on the Cyrene-based organogels by making a comparison with those produced using the typical solvent DMSO, analyzing differences in viscosity, polymer-solvent interactions, and mechanical behavior. The findings provide insights into how Cyrene influences the final properties of hydrogels, highlighting its advantages and limitations as a green solvent in hydrogel synthesis.

Chapter 4 extends this investigation by exploring Cyrene's role in dispersing graphene for advanced hydrogel nanocomposites. Carbon-based hydrogel nanocomposites have a hydrophobic nature with strong π - π interaction that makes them prone to agglomeration and difficult to disperse in water.^{21,22} Over the years, many strategies have been implemented to improve the water dispersibility of these carbon-based materials, including surface modification polymer grafting chemical treatments, and the use of biological macromolecules or surfactants.²³⁻²⁸ These methods come with significant limitations and drawbacks on their own, including high energy requirements, additional expenses, and the generation of toxic waste.²⁹⁻³²

The production of organogel precursors, followed by solvent exchange with water, offers in this scenario a simple and effective method for creating hydrogels nanocomposites structures. Cyrene is a new and potentially advantageous green solvent that can be incorporated into an organogel matrix to produce safe and environmentally friendly nanocomposite hydrogel materials. With its planar

aromatic structure, Cyrene can enable stable π - π interactions that keep graphene layers separate and well-dispersed in solution.³³ Therefore, graphene and Cyrene were shown to have close surface energies, making possible the formation of a steric or electrostatic barrier around each graphene sheet, effectively minimizing re-aggregation.^{34,35} Additionally, Cyrene's high polarity and hydrogen bonding capacity stabilizes graphene in suspension, as its oxygen-containing functional groups interact with graphene's surface to form hydrogen bonds and intermolecular forces.³⁶ As a result, high concentrations of graphene can be achieved using Cyrene as a solvent, with no need for surfactants, shortens processing time, reduces waste, and lowers energy consumption making it a more sustainable approach to graphene dispersion.

Cyrene's compatibility with carbon-based nanomaterials, particularly graphene, were evaluated, providing enhanced dispersibility and stability without the need for toxic surfactants or chemical modifications. The findings of chapter 4 indicate that Cyrene enables the production of highly concentrated and stable graphene dispersions, which can be effectively incorporated into hydrogel matrices to improve mechanical strength, electrical conductivity, and functional performance. This highlights the potential of Cyrene-based graphene hydrogels for applications in flexible electronics and biosensing.

By integrating green chemistry principles with advanced fabrication techniques, the objective of this thesis is to develop more sustainable, high-performance hydrogel materials. Through this study, we seek to bridge the gap between sustainability and high-performance material development, providing insights into the future of environmentally friendly hydrogel technologies.

Chapter 2 Literature Review

2.1 Hydrogels

2.1.1 Synthesis, Types, Properties, and Applications

Hydrogels are three-dimensional structures composed of hydrophilic polymer networks in a highly aqueous environment.¹ They are created by interconnecting, or cross-linking polymer chains in a water-based medium, generated by physical entanglement of polymer strands, ionic interactions, and covalent cross-linking.³⁷ These diverse cross-linking processes result in a characteristic 3D mesh-like structure, which allows hydrogels to retain a large amount of water while maintaining their structural integrity.

Based on their cross-linking mechanisms, hydrogels can be defined as either physically or chemically crosslinked.³⁷ Physically crosslinked hydrogels are composed of reversible bonds, such as entanglements between chains, hydrogen bonding, or hydrophobic interactions, which can be altered or broken under specific conditions.³⁷ In contrast, chemically crosslinked hydrogels are formed by permanent covalent bonds, most commonly created through a free radical mechanism.³⁸ Understanding this classification is crucial in hydrogel synthesis, as the type and degree of crosslinking directly influence key properties such as swelling behavior, mechanical strength and elongation, viscosity, among others.²

The choice of polymer source also plays a crucial role in defining the hydrogel's properties. Hydrogels can be synthesized from either natural or synthetic polymers.¹ Natural polymers are biodegradable, biocompatible, and environmentally friendly; however, they can often suffer from batch-to-batch variability, and have limited tunability of properties.^{39,40} Synthetic polymers, on the other hand, offer greater versatility and precise control over their properties, but are often non-biodegradable, raising environmental concerns.⁴⁰ The choice between natural and synthetic polymers depends on the specific application, as each type presents unique characteristics that influence their performance and applications.

Therefore, depending on their type or material, hydrogels possess unique sets of properties. Their high water content and biocompatibility allow them to mimic natural tissues, making them ideal for biomedical uses.⁴¹ The tunable mechanical strength and flexibility of hydrogels enable their use in tissue engineering and flexible electronics.^{3,42} Their ability to respond to stimuli such as pH,

temperature, and ionic strength makes them suitable for controlled drug delivery systems and soft robotics.^{4,43} The adhesive properties of some hydrogels make them valuable for wound dressings and wearable sensors.^{5,44} Additionally, the porosity of hydrogels makes them good adsorbent materials for removing contaminants, such as heavy metals, organic pollutants, and microplastics, from waste water.^{45,46} These diverse properties enable hydrogels to address a wide range of challenges in biomedicine, advanced materials development, and environmental engineering.

There are many other ways to classify hydrogels that can be found in literature, however, for the purposes of this thesis, the most relevant classifications and their range of applications are presented in Figure 1 below.

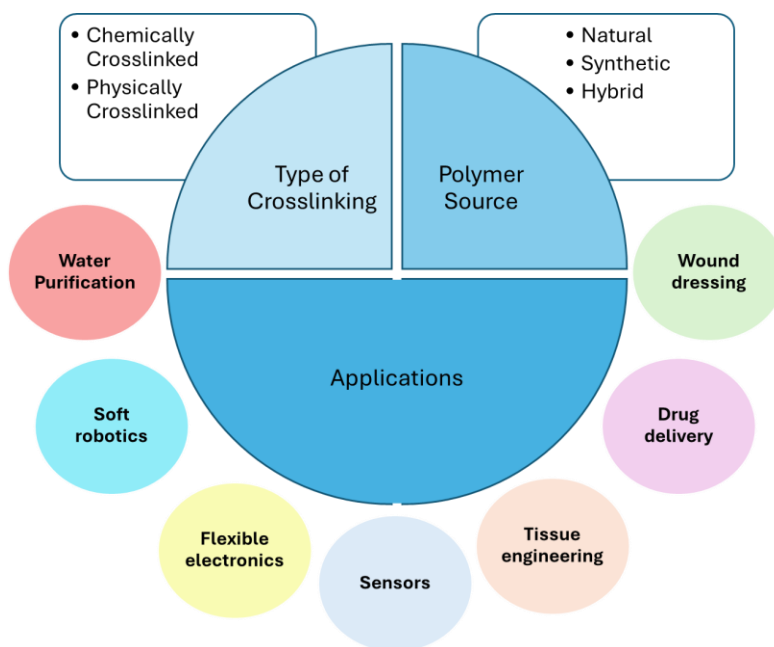


Figure 1. Hydrogel classification along with applications

2.1.2 Enhancing Hydrogel Properties

While hydrogels exhibit remarkable intrinsic properties, such as high-water content and biocompatibility, the development of advanced functionalities remains a key research focus. Many common hydrogels suffer from limited mechanical strength, poor electrical conductivity, or slow responsiveness, posing challenges for their use in advanced applications.³⁷ To address these limitations, researchers have explored the incorporation of fillers and nanofillers into hydrogel matrices.⁴⁷ By integrating reinforcing agents such as nanoparticles, carbon-based materials, or biocompatible

ceramics, hydrogels can achieve enhanced mechanical integrity, improved electrical conductivity, and tailored responsiveness to environmental stimuli.^{10–12} A comparison of the mechanical enhancements resulting from the incorporation of nanofillers into the hydrogel matrix is presented in Table 1.

Table 1. Comparison of mechanical properties of pristine and nanocomposite hydrogels.

Hydrogel Matrix	Nanoparticle ID	Concentration (%)	Strength (kPa)	Elongation at break (%)	Reference Number
PEGDA_{Mw10,000}	Laponite clay	0	142.6	68.8	48
		10 (w/v)	3728.7	94.9	
PEG_{Mw12,000}	Silica	0	~8	~310	49
		10	~34	~300	
PEG_{Mw400}	Carbon Nano Tubes	0	65	296	50
		1.4	375	659	
PVA_{Mw92,000}	Graphene Oxide	0	~1500	~102	51
		0.8 (wt)	~3300	~148	
PVA_{Mw146,000}	CNT	0	1060	237.5	52
		0.5	2060	267.6	
PVA	Bacterial Cellulose (BC)	0	3200	~370	53
		27	7200	~50	
PAAm	Graphene Oxide	0	~10	~100	54
		4	~250	~152	
PAAm	Carbon Nanodots	0	~17	~200	55
		1	~48	~3000	
PAAm	Layered Double Hydroxides	0	7	154	56
		3	45.8	4361	
PNIPAm	Graphene Oxide	0	~200	~45	57
		2	~260	~160	
PNIPAm	Starch nanoparticles	0	30	47	58
		1	8440	89.3	
PNIPAm	Laponite XLG	0	NA	NA	59
		46	51.3	1280	

*The table acronyms are the following: Mw (Molecular weight), PEGDA (Polyethylene glycol diacrylate), PEG (Polyethylene glycol), PVA (Polyvinyl alcohol), PAAm (Polyacrylamide), and PNIPAm (Poly(N-isopropylacrylamide)).

Fillers and nanofillers can be incorporated into hydrogels through various techniques. Common methods involve the direct mixing of nanofillers with the monomer solution before polymerization/gelation to create nanocomposite hydrogels, the introduction of nanoparticles into pre-formed gel matrices by swelling, and in situ growth of nanoparticles within the gel matrix by using nanoparticle precursors and reduction agents.^{60,61} The choice of incorporation method depends on the specific properties of the nanofillers and hydrogels, as well as the intended application of the composite

system. Therefore, a wide range of different nanofillers, such as silica, carbon-based materials (graphene, and carbon nanotubes (CNTs)), metal-organic framework, and metallic-based nanoparticles can be added.^{62–66}

However, the incorporation of nanofillers, particularly carbon-based nanofillers, into hydrogels presents several challenges. A primary concern is achieving uniform distribution of nanoparticles within the continuous phase, as poor dispersion can result in weak mechanical properties.^{21,22} The hydrophobic nature of some carbon-based nanomaterials, such as graphene, limits their use in water-based systems and hydrophilic compounds like hydrogels. This necessitates surface functionalization, polymer grafting, chemical treatments, or the use of biological macromolecules or surfactants.^{23–28} Despite their effectiveness, these methods come with significant limitations and drawbacks on their own, including high energy requirements, additional expenses, and the generation of toxic waste.^{29–32} Furthermore, they often introduce defects in the original nanoparticles, compromising their pristine properties and reducing stability.⁶⁷

2.2 3D Printing of Hydrogels

With the growing interest in hydrogels for various applications, 3D printing has emerged as a powerful technique to fabricate complex and customized hydrogel-based structures. Nozzle or extrusion-based printing methods emerged as the most popular technique for building hydrogel-based scaffolds.⁶⁸ This was followed by the development of inkjet printer-based systems, which offered non-contact printing of droplets onto a material platform.⁶⁹ As the field progressed, more sophisticated approaches were introduced, such as photopolymerized 3D printing, which enables high-resolution patterning and precise control over hydrogel crosslinking and architecture.⁷⁰

Among the photopolymerized 3D printing techniques, stereolithography (SLA) printing has gained significant attention due to its ability to produce highly detailed hydrogel structures with exceptional accuracy.⁷¹ SLA employs layer-by-layer solidification of liquid photocurable hydrogels using computer-controlled UV lasers, offering high structural resolution and good surface finishes (Schematic available in Figure 2).^{72,73} As an example, SLA's high precision can achieve resolutions down to 150 μm .⁷⁴ However, challenges remain in terms of mechanical integrity, as many hydrogel inks are extremely soft and unable to support their own weight, leading to unwanted deformation during printing.⁸ The limited range of photocurable hydrogels and the requirement for support structures are major drawbacks of SLA techniques in hydrogel fabrication.⁷⁵

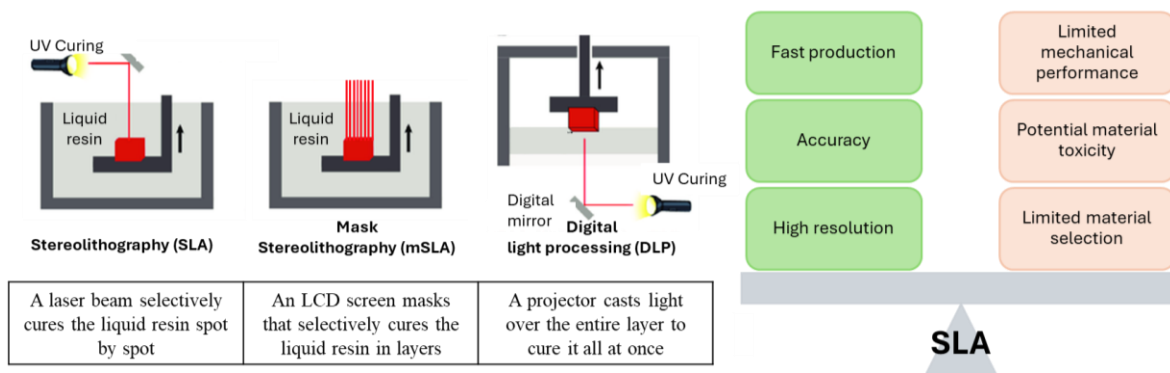


Figure 2. A schematic of photopolymerized 3D printing techniques is shown on the left, followed by the advantages of SLA on the right, highlighted in green, and the disadvantages of SLA, highlighted in red. (reproduced with permissions)^{76,77}

Despite these challenges, SLA remains a promising technique for applications requiring precise control over structure and material properties. The limitations highlight the need for ongoing research to develop hydrogel formulations that achieve an optimal balance of printability, mechanical integrity, and biological functionality for successful SLA 3D printing. Recent advancements in photopolymerized hydrogel fabrication techniques have significantly improved the design and functionality, particularly with the advent of stereolithography (SLA) 3D printing. These hydrogels were primarily synthesized through conventional methods, such as bulk polymerization or solution polymerization, limiting their structural complexity and application scope.^{78,79} SLA techniques have allowed for the development of highly stretchable and UV-curable polyacrylate-based hydrogels, such as poly(ethylene glycol) diacrylate (PEGDA) hydrogels, which can achieve water content up to 90 wt% and stretchability up to 1300%.⁸⁰

The synthesis of most photopolymerized hydrogels relies primarily on free radical polymerization using photoinitiators.^{81,82} The process involves initiation, where light-activated photoinitiators generate free radicals that react with monomers. During propagation, polymer chains grow rapidly as monomers are added to active sites. Simultaneously, cross-linking occurs, forming the hydrogel 3D network structure. Finally, the process ends with termination, interrupting chain growth. This method allows for controlled hydrogel synthesis, which is suitable for applications using SLA 3D printing.^{83,84}

The use of a photoinitiator in this context enables precise spatial and temporal control over the polymerization process, where selecting the appropriate photoinitiator is crucial to achieving an optimal polymerization rate and material functionality.⁸⁵ However, a major challenge in hydrogel 3D printing is the limited availability of water-soluble photoinitiators, as most traditional photoinitiators are hydrophobic and designed for non-polar environments, making them inherently water-insoluble.⁹ This limitation has driven the development of water-soluble photoinitiators, but despite advancements in this area, their solubility remains restricted.⁸⁶ For instance, water-soluble photoinitiators such as Irgacure 907 has a solubility of 17.9 mg/L at 20 °C, while Irgacure 184 reaches 1108 mg/L at 25 °C.⁹ These constraints underscore the need for hydrogel synthesis methods that accommodate a broader range of photoinitiators, enabling greater flexibility in material design and performance. Table 2 below highlights common photoinitiators used during photopolymerization, along with their respective water solubility.

Table 2. Common photoinitiators and their water solubility

Photoinitiator name	CAS No	Water solubility	References (i.e. SDS)
Irgacure 2959	106797-53-9	7.8 g/L at 25 °C	87
Irgacure 1173	7473-98-5	13.3 g/L at 20 °C	88
PTH	7152-42-3	NA (insoluble)	89
TPO-L	75980-60-8	0.0034 g/L at 20°C	90
Ru	50525-27-4	NA (soluble)	91,92
Ivocerin	1207515-90-9	NA (insoluble)	93
ZnTPP	14074-80-7	NA (insoluble)	94
Eosin Y	15086-94-9	0.8 g/L	95,96
Pheo A	15664-29-6	NA (insoluble)	97
OBN	2646-15-3	NA (insoluble)	98
AlPc	14154-42-8	NA (insoluble)	99
BChl a	17499-98-8	NA (insoluble)	100

**The table acronyms are the following: PTH (Phenyl Thiohydantoin), TPO-L(Ethyl (2,4,6-trimethylbenzoyl)phenylphosphinate), Ru (tris(2,2'-bipyridyl)ruthenium(II) chloride hexahydrate), ZnTPP (Zinc(II) Tetraphenylporphyrin), OBN (O-Benzoyl Oxime-N e), AlPc (Aluminum Phthalocyanine), and BChl a (Bacteriochlorophyll a). SDS (Safety data sheet).*

2.3 The Uses of Nanomaterials and Solvent in the Hydrogel SLA 3D Printing

Nanomaterials and solvents play crucial roles in enhancing SLA 3D printing outcomes, particularly in improving mechanical properties and expanding the range of printable materials.^{73,101} The addition of nanomaterials can for instance significantly enhance the final overall hydrogel properties, such as improved electrical conductivity, mechanical performance and tailored responsiveness to environmental stimuli.¹⁰⁻¹² Additionally, nanomaterials can modulate the absorption of UV or visible light, enhancing curing efficiency and print resolution, resulting in better crosslinking, reduced overcuring, and sharper feature definition in printed structures.¹⁰² For example, incorporating gold nanoparticles into hydrogel composites enabled high-resolution laser-based 3D printing, with nanoparticle concentration influencing critical energy requirements and printing speed.¹⁰³

As for the solvents, the selection of an appropriate solvent for SLA 3D printing is a key factor as it can not only improve the mechanical properties of printed structures but also facilitate the incorporation of functional additives or photoinitiators, ultimately enhancing the versatility and performance of SLA-printed hydrogels.¹⁰⁴ As mentioned above, a major challenge in hydrogel 3D printing is the limited availability of water-soluble photoinitiators due to the hydrophobic nature of most traditional options.⁹ Expanding the range of compatible photoinitiators through careful solvent selection can help overcome these solubility limitations, increasing the variety of printable materials.

Solvents can also play a role in improving the material's overall mechanical properties.^{105,106} It can impact molecular assembly, influence crosslinking density and distribution, and help maintain structural integrity in various environments.¹⁰⁷⁻¹⁰⁹ This flexibility in material design not only enhances the mechanical properties of printed constructs but also expands the potential applications of hydrogels. However, it is worthwhile to note that the use of solvents may compromise the inherent biocompatibility of hydrogels, potentially limiting their application in certain biomedical contexts.¹¹⁰ There is a trade-off that should be strategically explored to maintain biocompatibility while leveraging the benefits of solvent-assisted processing. For instance, the use of organic solvents instead of water creates a new class called organogels, which can expand the range of printable materials and potentially overcome some limitations associated with hydrogel-based SLA printing.¹³ The development of organogels as an alternative to hydrogels has gained increasing attentions as they can offer improved mechanical properties and stability in certain applications.¹¹¹ By instance, organogels can dissolve hydrophobic molecules, making them highly compatible with a broader range of functional materials,

such as conductive polymers, bioactive compounds, and hydrophobic drugs, increasing SLA material selection.¹³

Photopolymerized 3D printing techniques often lead to structure deformation or collapse due to the generation of mechanical stress between the moving plate and the reservoir in the printing process. Printing in organic solvents can increase intermolecular forces within the material and decrease the likelihood of structural disintegration.¹⁴ After 3D printing, a simple solvent exchange with water can result in the desired hydrogels, maintaining the advantage of biocompatibility along with complex structures produced by SLA 3D printing.¹⁵ This new approach of producing organogels followed by solvent exchange offers a promising solution to current challenges in SLA 3D printing, enabling the fabrication of mechanically stable structures while expanding the range of printable materials.

2.4 A Path Toward Sustainable Hydrogel Production

With increasing emphasis on sustainability, green chemistry has become integral to material synthesis by promoting environmental sustainability. It focuses on designing chemical products and processes that minimize or eliminate the use and generation of hazardous substances, contributing to energy savings, reduced toxicity, and decreased environmental impact.¹¹² The principles of green chemistry guide the development of using safer solvents and renewable feedstocks, and energy-efficient processes, leading to more sustainable material production.¹¹³

Recent research has unveiled exciting possibilities for synthesizing eco-friendly hydrogels in 3D printing. For instance, hydrogels made from nanocellulose and alginate have been successfully printed into architectural components using significantly less energy than traditional construction methods, demonstrating the transformative potential of biomaterials in large-scale applications.¹¹⁴ Similarly, studies on flame-retardant hydrogels derived entirely from biomass have shown that these materials can achieve exceptional fire resistance and mechanical strength when printed with low-intensity visible light.¹¹⁵ Current research primarily focuses on water-based hydrogels, which, while beneficial, can restrict the variety of printable materials and their potential applications as mentioned.^{9,104} Exploring bio-based alternatives like organogels offers promising opportunities, but selecting the right solvent is critical. The solvent not only affects properties such as solubility, reactivity, and viscosity, but also plays a pivotal role in determining the structural integrity and biocompatibility of the resulting hydrogels after solvent exchange.^{116–118}

To address environmental and safety concerns, researchers are exploring alternative pathways that minimize or eliminate hazardous solvents. Green solvents, designed to reduce toxicity, minimize environmental impact, and conserve energy, have emerged as a promising solution.¹¹⁹ Often derived from renewable sources like agricultural crops, these solvents offer a safer and more sustainable option for organogel synthesis. Some widely adopted eco-friendly alternatives, such as supercritical fluids and deep eutectic solvents (DESs), have been investigated for green polymer synthesis. Supercritical CO₂ (scCO₂) have emerged as an eco-friendly alternative to traditional organic solvents, offering solvent-free processing, recyclability, high yields, and minimal wastewater generation.¹²⁰ Similarly, DESs have attracted interest for their potential in green polymer synthesis, including applications in 3D printing.¹²¹ However, these alternatives face challenges such as high equipment costs, complex industrial-scale setups, and limitations in solvent properties, along with potential toxicity concerns.^{16–19} While recent studies have demonstrated the potential of eco-friendly hydrogels and organogels, challenges such as solvent selection, toxicity, and processing limitations must still be further addressed. Advancing these alternatives could pave the way for more sustainable, versatile, and high-performance 3D-printed structures.

2.5 Cyrene: A Bioderived Solvent for Green Synthesis

Cyrene, also known as dihydrolevoglucosenone, is a bioderived solvent obtained from renewable cellulose sources that offers significant potential for sustainable hydrogel synthesis.¹²² Structurally, it features a bicyclic, chiral, seven-membered heterocyclic cycloalkanone structure.¹²³ Chemically, it can be classified as a dipolar aprotic solvent with moderate polarity, similar to N-methyl pyrrolidone (NMP), positioning between highly polar water and nonpolar solvents.¹²⁴ This intermediate polarity enables Cyrene to dissolve a broad range of polymers and hydrophilic compounds.¹²⁴ Additionally, its unique structure of two fused rings forming a cyclic acetal contributes to its stability in basic conditions while showing reactivity towards strong reducing and oxidizing agents.¹²⁵

Compared to conventional organic solvents, Cyrene offers several advantages. First, its low toxicity and high biodegradability (99% within 14 days) make it a safer alternative to conventional solvents like dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF), and NMP.^{126,127} Second, Cyrene's production from cellulose-based biomass aligns with principles of green chemistry, promoting waste valorization and significantly reducing the carbon footprint associated with solvent production.¹²⁴ Third, it is non-mutagenic, not toxic to microorganisms in receiving waters or aerobic wastewater

treatment plants, and has an acute toxicity reported to be ten times lower than caffeine.^{124,126,128,129} Fourth, Cyrene's ability to decompose into CO₂ and H₂O at the end of its life cycle minimizes hazardous waste generation and environmental impact.¹²⁴

Cyrene also presents advantages over other green solvents (see details in Table 3). It offers better stability and easier handling compared to scCO₂, as well as more favorable physical properties than deep eutectic solvents (DESs).¹²⁴ Additionally, Cyrene has several advantages over other bio-derived green solvents, such as γ -Valerolactone (GVL) and dimethyl isosorbide (DMI). Its stability under normal laboratory conditions, high biodegradability, and high boiling point (227°C) make it a superior choice for refluxing and microwave-assisted reactions.^{124,127} Furthermore, it has also demonstrated potential in enhancing reaction rates and improving product purity in certain reactions.¹³⁰

Table 3. Comparison of Green Solvent Alternatives

Property	Cyrene^{131,132}	GVL^{133,134}	DMI^{135,136}	scCO₂^{137,138}	DESs^{139,140}
Chemical structure	Dipolar aprotic	Dipolar aprotic	Dipolar aprotic	Non-polar	Varies (often dipolar)
Renewable source	Cellulose waste	Cellulose waste	Starch	Captured CO ₂	Bio-based sources
Biodegradability	99% in 14 days	Readily	Not readily	N/A (inorganic)	Often readily
Toxicity	Low acute oral toxicity	Low acute oral toxicity	Very low toxicity	Non-toxic	Varies, often low toxicity
Mutagenicity	Non mutagenic	Non mutagenic	Non mutagenic	Non mutagenic	Varies
Polarity	Moderate	Moderate	Moderate	Low (tunable)	Varies (often high)
Boiling point	227°C	207°C	244°C	N/A	Varies (160-270°C)
Density (25°C)	1.25 g/mL	1.11 g/mL	1.18 g/mL	Tunable (liquid-like)	Varies (1-1.4 g/mL)
Viscosity	1.7 cP	1.8 cP	4.5 cP	Low (gas-like)	Often high (30-400 cP)
Water miscibility	Moderate	High	High	Low	Often high

Research has demonstrated Cyrene's effectiveness in various material systems. Its successful use as a medium has been reported for organic reactions such as HATU(1-Hydroxy-7-azabenzotriazine) amide synthesis, as well as Suzuki-Miyaura and Sonogashira couplings.^{141,142} Reactions conducted with

Cyrene blends as solvents gave comparable isolated yields to those obtained from DMF, and NMP, suggesting that Cyrene can effectively replace these traditional solvents without compromising reaction efficiency. Furthermore, Cyrene blends have shown stability at both room temperature and elevated temperatures (40°C) over extended periods, highlighting their practicality for chemical synthesis.

Cyrene also shows promise in the synthesis of various nanomaterials, including metal-organic frameworks (MOFs), graphene, and CNTs. Zhang et al. demonstrated Cyrene's successful application in synthesizing four different types of MOFs, with the HKUST-1 MOF synthesized using Cyrene exhibiting a superior Brunauer-Emmett-Teller (BET) surface area compared to its DMF-synthesized counterpart.¹⁴³ For single-walled CNTs, Cyrene enabled higher concentration dispersions (0.038 mg mL⁻¹) compared to NMP (0.013 mg mL⁻¹), with improved stability over time, all without the need for surfactant additives.¹⁴⁴ Similarly, in graphene production, Cyrene has proven to be an excellent solvent for liquid phase exfoliation of graphite.¹⁴⁵ Gharib et al. reported a sixfold increase in graphene concentration using Cyrene compared to NMP and DMF.¹⁴⁵

In the field of biomedicine, Cyrene has emerged as a promising green alternative to DMSO for antibacterial drug discovery against ESKAPE pathogens.¹⁴⁶ It offers comparable solvation properties without the protective effects against reactive oxygen species that DMSO exhibits. A study suggests that Cyrene could potentially replace DMSO in other biomedical applications where DMSO's radical scavenging properties may be undesirable.¹⁴⁶ Cyrene's unique properties and eco-friendly profile make it a promising solvent for developing sustainable hydrogel systems for biomedical applications. Its use in hydrogel synthesis can pave the way for innovative, high-performance materials with broad applications across various industries. The potential synergy between Cyrene's biomedical advantages and its effectiveness in hydrogel production opens exciting possibilities for future research and development in fields such as drug delivery systems and tissue engineering.

2.6 Challenges and Opportunities for Cyrene-Based Hydrogel Nanocomposites

Even though Cyrene holds great promise as a green solvent and has been successfully utilized in various organic synthesis applications, its use in material science, particularly in polymer chemistry, remains in its early stages. Studies have explored its use in polymer dissolution, polymerization, and membrane fabrication.¹⁴⁷ For example, Milesescu et al. demonstrated that Cyrene can successfully dissolve and process polymers such as poly(amide-imide) (PAI) for wire enamels, producing coatings with enhanced adhesion strength and chemical resistance compared to conventional solvents.

Additionally, Cyrene was successfully utilized in membranes synthesis without the need for additional pore-forming additives. In supramolecular chemistry, Cyrene has been investigated as a medium for self-assembling dipeptide-based supramolecular gels.¹⁴⁸ Sugiura et al. demonstrated that Cyrene-enabled gel formation was comparable to that achieved using DMF and DMSO while maintaining environmental benefits. These findings highlight Cyrene's potential as a sustainable alternative in polymer processing and advanced material design.

Cyrene has also demonstrated significant potential in the production of high-quality graphene inks, further expanding its applications in material science. Tkachev et al. successfully achieved highly concentrated dispersions of few-layer graphene flakes (up to 3.70 g L⁻¹) using a combination of tip-sonication and high-shear mixing.¹⁴⁹ Pan et al. advanced this work by developing an environmentally friendly graphene-based ink in Cyrene, reaching concentrations of 10 mg mL⁻¹, and up to 70 mg mL⁻¹ with the addition of a stabilizing agent.¹⁵⁰ These Cyrene-based graphene inks have exhibited superior performance in various applications, such as screen-printed electrodes, with electrical conductivity of 7.13×10^4 S.m⁻¹, the highest reported to date.¹⁵⁰ These findings further establish Cyrene as a high-performance, sustainable solvent for advanced material applications.

Despite its potential, Cyrene faces challenges, including high viscosity, reactivity, and a high boiling point.^{127,144} Its lower solubility for certain compounds compared to traditional solvents like DMSO and DMF can limit its applicability in some material systems.^{127,151} These challenges are particularly relevant in hydrogel and organogel synthesis. Cyrene's high viscosity can complicate handling and processing, potentially affecting the concentration and uniformity of organogel precursors, which in turn impacts the final gel properties.¹⁴⁴ Its reactivity may interfere with polymerization or cross-linking reactions, leading to unintended side reactions or alterations in material properties.¹²⁷ Furthermore, its high boiling point reduces the efficiency of solvent recovery, making it less economically viable and environmentally sustainable.¹²⁷ Lastly, the limited understanding of polymer solubility in Cyrene presents an additional challenge, as it could affect the formation and consistency of organogels, further influencing their performance and applicability.

Thus far, Cyrene has demonstrated significant potential for dissolving and processing polymers, with unique properties such as moderate polarity, biodegradability, and low toxicity, making it an attractive option for sustainable material development. However, optimizing its interactions with

different polymer systems, ensuring compatibility, and fully understanding its effects on material properties remain critical areas for further research. As studies progress, Cyrene could become a key player in sustainable polymer processing, serving as a viable alternative to both conventional and other green solvents. The challenges associated with Cyrene highlight the need for continued research and optimization to unlock its full potential for organogel synthesis and related applications. In organogel fabrication, Cyrene offers an opportunity to enhance biocompatibility while maintaining the advantages of non-aqueous but sustainable systems. More broadly, Cyrene stands out as a promising bioderived solvent for hydrogel synthesis, striking a balance between sustainability, performance, and environmental safety. Future research on Cyrene-based nanocomposites and hydrogel systems could drive innovations in sustainable material design, expanding its applications across various industries.

Chapter 3 3D Printing Organogels with Bioderived Cyrene for High-Resolution Customized Hydrogel Structures¹

3.1 Introduction

This chapter focuses on the exploration of Cyrene's role in hydrogel formulation, printability, and mechanical performance. Specifically, it investigated Cyrene's application in mask stereolithography (mSLA) 3D printing of organogels to produce high resolution hydrogel structures.

Mask stereolithography (mSLA) is a subclass of photopolymerization-based 3D printing techniques. It utilizes a liquid crystal display (LCD) mask to generate objects layer-by-layer into three-dimensional structures, most commonly by a bottom-up system, .^{153–155} Despite its advantages, mSLA printing of hydrogels presents its own set of limitations. mSLA printing of hydrogels often leads to structure deformation or collapse due to the generation of mechanical stress between the moving plate and the reservoir in the printing process.¹⁴ Organogels, which are gels composed of a 3D crosslinked network within an organic liquid phase, are used to address this challenge.¹⁵⁶ Utilizing organogels during 3D printing can increase intermolecular forces within the material compared to using water and can decrease the likelihood of structural disintegration.¹⁴ After 3D printing, a simple solvent exchange with water can be introduced, and hydrogels can be obtained as the final product, maintaining the advantage of biocompatibility along with complex structures produced by mSLA 3D printing.¹⁵

In the synthesis of organogels, the selection of a solvent becomes a critical factor. In particular, solvent toxicity and safety are critical considerations for applications involving contact with living systems or the environment.¹⁵⁷ To address environmental and safety concerns, researchers are exploring alternative pathways that minimize or eliminate the use of hazardous solvents, such as dimethyl sulfoxide (DMSO), which can deeply penetrate the skin and generate hazardous by-products.^{157,158} One such alternative is dihydrolevoglucosenone, or Cyrene, a bio-based solvent derived from cellulose.¹²⁴

¹ This chapter was partially reproduced from Ramirez, A. B., Bauman, L. A. & Zhao, B. 3D Printing Organogels with Bioderived Cyrene for High-Resolution Customized Hydrogel Structures. *Langmuir* 41, 646–653 (2025).¹⁵²

Our recent study has begun exploring Cyrene as a solvent for organogel synthesis and 3D printing. The chemistry behind the synthesis of conductive supramolecular hydrogels using a crosslinking strategy with poly(ionic) salts in Cyrene has been reported in a separate work.²⁰ In light of the previous work, this study hypothesized that the choice of solvent during organogel synthesis can have a significant influence on the hydrogel mechanical properties. Experimental studies of 3D printing of organo-hydrogels in different solvents – Cyrene and DMSO were conducted to reveal the solvent-dependent mechanical and physical characteristics of 3D printed hydrogels and further explore the potential of Cyrene as a sustainable alternative for hydrogel synthesis.

3.2 Materials and Methods

3.2.1 Materials

Chitosan quaternary ammonium salt (CD bioparticles), Amberlite IRA 458 resin (Rohm and Haas), Amberlite a26 resin (Sigma Aldrich), SSTC60 resin (Purolite), 2-Hydroxyethyl acrylate (Sigma Aldrich), 3-Sulfopropyl acrylate potassium salt (Sigma Aldrich), Dimethyl sulfoxide (Sigma Aldrich), Irgacure 819 (BASF), Cyrene (Sigma Aldrich), sodium hydroxide (Sigma Aldrich), phosphoric acid 85% (Sigma Aldrich).

3.2.2 Synthesis of the Neutralized Quaternized Chitosan Hydroxide Salt (QCh:SPA)

The synthesis was performed following previous work.²⁰ First, 50 g of quaternized chitosan chloride salt was homogenized in 3000 mL of ultrapure water (UPW). The solution underwent an ion exchange process with Amberlite IRA 458 and Amberlite a26 cationic ion exchange resins, which were regenerated with sodium hydroxide solution. Meanwhile, a solution of 3-sulfopropyl acrylate acid was prepared by mixing 8 g of 3-sulfopropyl acrylate potassium salt into 50 mL of UPW. This solution also underwent an ion exchange process using Purolite SSTC60 resin, which was regenerated with 10 wt.% phosphoric acid. Neutralization was then performed by gradually adding the 3-sulfopropyl acrylate (SPA) solution to the quaternized chitosan hydroxide solution. The resulting neutral product, labeled QCh:SPA, was dropped into a bath of liquid nitrogen and freeze-dried for 24 hours at -30°C.

3.2.3 Synthesis of Pure Hydroxyethyl Acrylate (HEA)

The monomer, 2-Hydroxyethyl acrylate (HEA), was purified through a liquid-liquid extraction process using a 1000 mL separating funnel. A 100 mL solution of HEA (1 HEA: 3 UPW) was extracted

with four rinses of 400 mL of hexane to remove ethylene glycol diacrylate (EGDA) crosslinker. The aqueous phase was then salted with 60 g of sodium chloride and extracted with two rinses of 400 mL diethyl ether to remove residual acrylic acid. The organic phase was dried by adding 10% v/v calcium sulphate. After 1 hour, the solution was filtered, and the diethyl ether was removed by rotary evaporation (Buchi AG EL131) at 40 °C under vacuum to obtain the final solution of pure HEA. Care was taken to avoid excessive heating of the HEA to prevent thermal polymerization, as the inhibitors were removed during purification.

3.2.4 Synthesis of 3D printable organogels

A 3D printable resin was prepared by homogenizing 2.03 g of QCh:SPA salt in 50 g of purified HEA at 10,000 rpm for 5 minutes. Then, 6.33g of solvent (DMSO or Cyrene) was added to the blend and mixed for an additional 5 minutes. The solution was subsequently sonicated for 5 minutes at 100% amplitude (Q125 ¼” probe, Qsonica, USA), followed by the addition of 0.5 g Irgacure 819. The resin was then mixed and degassed successively in an ARE-310 (Thinky, USA). Resins produced with Cyrene were labeled as resin_C while those produced with DMSO were labeled as resin_D.

Using a Phrozen 4K Sonic XL 3D printer (Phrozen, Taiwan), 3D objects were printed with a layer thickness of 50 µm and an exposure time of 8.5 s per layer. After printing, the samples were washed with water, followed by isopropyl alcohol, dried for 1 hour, and then subjected to a 30-minute post-curing process in a 300 W UV chamber. Subsequently, the samples underwent a solvent exchange process with UPW for 72 hours to remove unreacted monomers and to replace the organic solvent with water. The efficiency analysis of the solvent exchange can be found in the supplementary information of this article. Finally, the resulting samples were labeled as hydrogel_C and hydrogel_D, according to the resin used for their production.

3.2.5 Characterization

The optimal exposure time of the resin was determined by curing 15 regions with incremental exposure starting at 0.5 s and increasing by 0.5 s until 12 s (Figure 4a). After curing, the thickness of different regions was measured using a digital micrometer.

Viscosity measurements for resin_C and resin_D were conducted at 22°C with 200 µL of resin and a hold of 60 seconds, using a CAP 2000+ viscometer (Amtech Brookfield, USA) and a 1H spindle. Environmental scanning electron microscopy (ESEM, FEI Quanta Feg 250) was performed on hydrogel

samples that were frozen in liquid nitrogen and then broken. Tensile, compression, and fatigue tests were carried out using a Universal Material Tester (CETR-UMT, Bruker, USA). For tensile tests, printed coupons were stretched at 2 mm/s until failure; for compression tests, printed cube specimens were subjected to a force of 100 N until noticeable crack propagation; for fatigue tests, cube specimens were subjected to 25 cycles of compression at low ($25\pm5\%$), medium ($45\pm5\%$), and high ($65\pm5\%$) strain levels, followed by release with a rate of 2 mm/s. Swelling and dehydration tests were performed using 3D printed cubes consisting of hydrogel_C. For the swelling test, a dry cube was immersed in UPW at 25°C, and the mass and volume changes were measured over time. For the dehydration test, hydrated 3D printed cubes were kept at 25°C with relative humidity (RH) of 35%, and 85%. Mass and volume changes of the samples were monitored over time.

3.3 Results and Discussion

3.3.1 Microstructural transition during the 3D printing process

The schematic of the printing process along with the details of the internal structure are illustrated in Figure 3 below.

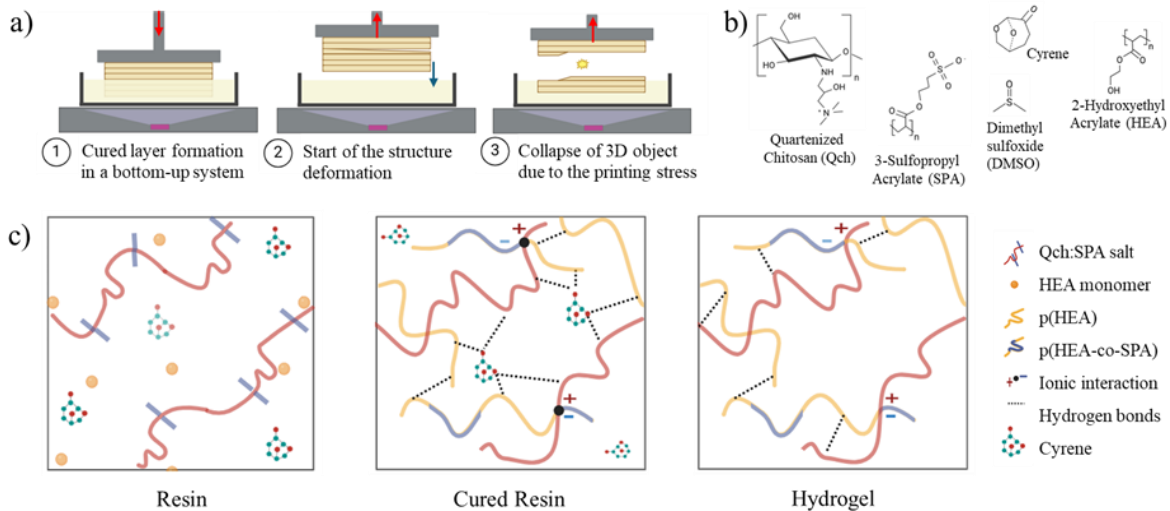


Figure 3. a) Representation of the mSLA 3D printing process and the structure collapse due to the formation of opposite forces between the building plate and the layer of cured resin. b) Chemical structures of resin components. c) Illustration of the gel microstructure before 3D

printing and polymerization (resin), after 3D printing (cured resin), and after solvent exchange with water (hydrogel).

In bottom-up SLA printing techniques, the movement of the print stage is known to generate opposing forces between the cured resin layer and the reservoir interface.¹⁴ These opposing forces can potentially cause deformation or collapse of the final structure, as illustrated in Figure 3a. To resist this stress and prevent structural disintegration, the cured resin must exhibit adequate mechanical properties, typically provided by the crosslink density of the polymeric structure.¹⁵⁹ However, a higher crosslink density also increases rigidity and reduces swelling capacity, which may be undesirable for certain applications.¹⁶⁰

Previous studies have introduced a novel polymeric salt-based crosslinker using a system of QCh and SPA to form poly(ionic) salts.²⁰ This approach seeks to create pseudo-chemical crosslinkers with HEA polymer, that enhances the stretchability of hydrogels without compromising their mechanical strength during printing. The chemical structures of all components of the resin are illustrated in Figure 3b.

The microstructural transition during the 3D printing process can be described by the resin composition and blending, the resin polymerization, and the organogel solvent exchange (Figure 3c). The resin is mainly composed of HEA monomers, which are blended with solvent, the associated salt (QCh:SPA), and a photoinitiator. During 3D printing, this blend is exposed to UV-light, where polymerization starts, and a network is first formed by SPA monomers that are incorporated into growing HEA chains. Meanwhile, the SPA monomers are also associated with QCh, forming the secondary network. At this stage, the lack of water prevents the salt from dissociating, showcasing the importance of an organic solvent. The association of ammonium groups in QCh with the sulfonic acid groups in SPA form strong ionic interactions between the networks, which are responsible for keeping the resin stable while 3D printing, overcoming the stresses from printing. This strong interaction during the resin polymerization enables this resin to produce detailed structures using the mSLA 3D printing technique. Finally, following polymerization, solvent exchange can be performed to replace the organic solvent with water, creating hydrogels as the final product.

Cyrene and DMSO are both strong hydrogen bond acceptors, with Cyrene having two ketone groups capable of forming hydrogen bonds, and DMSO having one sulfoxide group; thus, when analyzing the cured resin microstructure it is possible to point out that polymer-solvent hydrogen bonds

will tend to be more preferable than the formation of polymer-polymer hydrogen bonds.^{161–164} This increases solubility between the precursors and the chosen solvent. Finally, after the solvent exchange with water, the QCh:SPA salt dissociates along with its ionic bonds, resulting in the final hydrogel entirely composed of ionic crosslinks. This presence of ionic crosslinks provides hydrogel with enhanced stretchability performance, which is a characteristic difficult to achieve when using mSLA printing techniques.

3.3.2 Organogels printing parameters and resin printability

Equation 1 was used to study the exposure time necessary to generate a certain layer thickness of cured resin, where D_p is the depth of penetration, E_c is the critical energy, C_d is the measured thickness of cured resin, and E_0 is the exposure dose.¹⁶⁵

$$C_d = D_p \ln (E_0/E_c) \quad (1)$$

To determine E_c and D_p (illustrated in Figure 4a), which are two primary parameters of the resin for SLA 3D printing, a plot of the natural logarithm of the light energy (light intensity (2.8 mWcm^{-2}) multiplied by the exposure time against the thickness of the different exposure regions was used (Figure 4b). The plot along with Equation 1 gives the values of E_c and D_p for resin_C (8.82 mJcm^{-2} , and 0.33 mm), and resin_D (7.49 mJcm^{-2} , and 0.53 mm). Using these values, an exposure time of approximately 4 s for Cyrene and 3 s for DMSO was calculated considering a desired layer thickness of $50 \text{ }\mu\text{m}$.

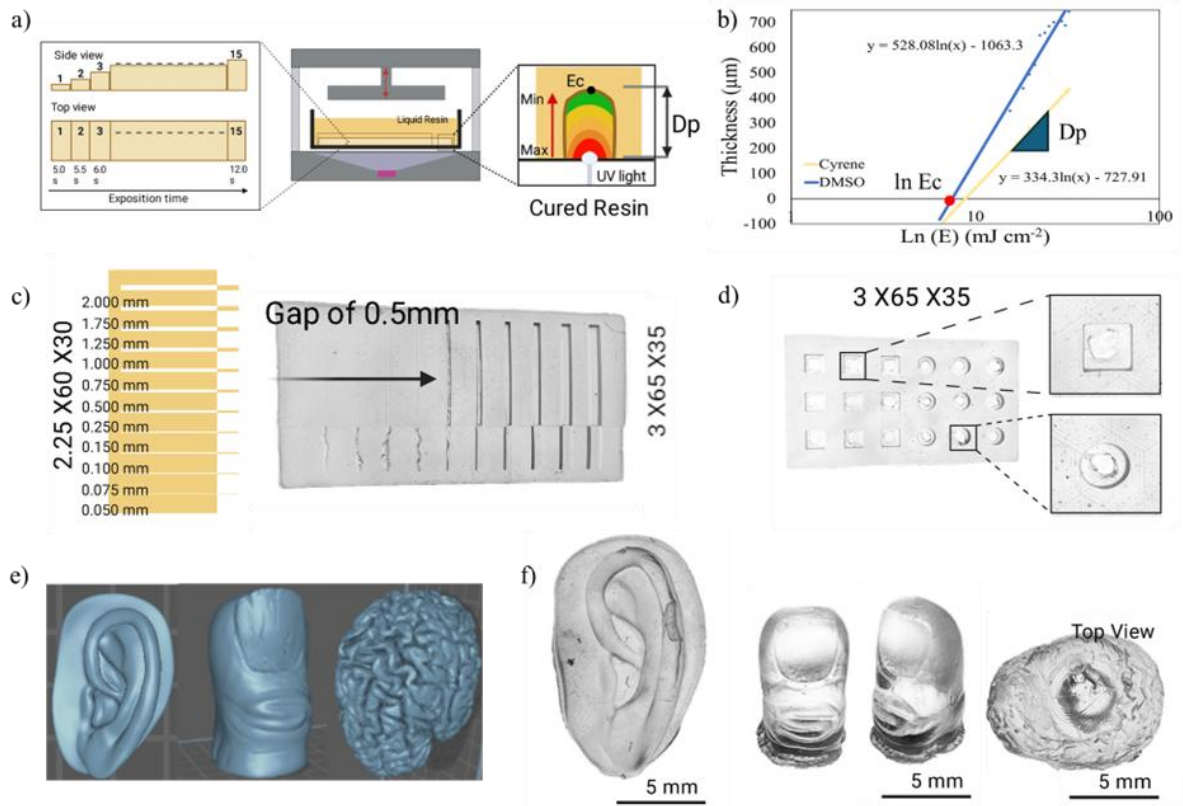


Figure 4. a) Schematic of 15 regions with different exposure times is shown on the left, and on the right is shown a schematic of the resin depth of penetration and critical energy after the UV light hits the resin and polymerization begins. b) Plot of $\ln(E)$ and thickness from the exposure time measurement for Cyrene and DMSO resins. c) 3D printed object produced to demonstrate the printing resolution. d) 3D printed object produced to demonstrate the accuracy of printing different shapes. e) Biomedical-related software 3D models (ear, brain, and toe). f) Photograph of 3D printed bio-inspired human brain, toe, and human ear.

While the samples could theoretically be printed at these exposure times, this represents the lower limit of the curing time. The stress generated during 3D printing is not accounted for in the formulation, which leads to delamination of the cured resin from the build plate at shorter exposure times. Our previous work reported similar findings, where curing the resin at varying exposure times with the build plate in constant motion revealed that the optimal layer exposure time for resin_C was 8.5 s.²⁰ Furthermore, since HEA is the primary component of the resin and largely determines the polymerization kinetics, the printing parameters established for resin_C are also appropriate for

resin_D. Consequently, an exposure time of 8.5 s was consistently used throughout the remainder of the study for both resins.

With the optimal exposure time established, the printing resolution was evaluated using three models. The first resolution model, Figure 4c, was chosen to evaluate the resin's ability to separate small details. The printing resolution of this resin could reach gaps greater than 0.25 mm of separation, and a resolution of 0.75 mm was achieved for printing accurate strength lines without warping due to swelling of the structure during printing. The second model, Figure 4d, was designed to evaluate the resin's ability to accurately print different shapes, specifically squares and circles. While circles demonstrate high fidelity to the model, with clear circle formation without any discontinuity, the sharp corners of the squares demonstrate a loss in accuracy, showing more rounded edges than sharp ones. These variations in the print fidelity were attributed to possible overpolymerization in the XY direction, since the oxygen depletion zone expands during longer exposure times. The third model, Figures 4e-f, demonstrates the resin's capability for details and accuracy with real-life objects by showcasing biomedical-inspired structures. The resin shows an extensive amount of detail and remarkable accuracy with real-life objects of the printed human parts, being able to recreate a human ear, brain and toe. However, after multiple uses, the resin starts failing to print taller objects, demonstrated by the lack of the top part of the human brain. This phenomenon occurred because after repeated printing cycles, the resin tends to increase considerably in its viscosity (Figure 5a labeled “old Cyrene resin”). Due to this high viscosity of the resin, it tends to migrate to the corners of the bath during printing, unable to return and thus failing to achieve printability of taller objects. Therefore, fresh resin should always be used when printing taller objects to guarantee successful printing.

3.3.3 Effect of 3D printing solvents (DMSO vs Cyrene) on the hydrogel mechanical properties

To understand the effect of the solvent on the hydrogel mechanical properties, we first checked the effect of solvent on the swelling behavior of hydrogels. Even though both solvents (Cyrene and DMSO) were found to have similar properties in the literature, after solvent exchange, the degree of swelling of Cyrene organogels was noted to be greater than that of organogels prepared in DMSO, with a volume increase of 374% and 187% for Cyrene and DMSO, respectively (Figure 5d).¹⁶⁶ This difference in swelling was attributed to the differences in compatibility between the two solvents and the QCh:SPA salt.

It was observed that Cyrene has higher compatibility with QCh. While Cyrene and DMSO are both strong hydrogen bond acceptors, Cyrene has two ketone groups capable of forming hydrogen bonds while DMSO only has one sulfoxide group.^{163,164} This increase in the degree of polymer-solvent bonds can contribute to the enhanced solubility, due to changes to the density of the polymeric network's microstructure.^{162,167} Viscosity measurements verify the difference in solubility. Figure 5a shows the comparison between Cyrene and DMSO organogel's viscosity. Cyrene exhibits an increase in viscosity of 102.7% when compared with DMSO solution; this increase in viscosity can be attributed to better solvation since improved polymer solvation leads to greater polymer chain expansion and stronger interaction with the solvent, consequently hindering the solution flow and increasing the solution viscosity.^{168,169} This solvation effect was shown to result in greater swelling behavior in hydrogel_C, which also demonstrates higher stretchability.

To examine how the solvent affects hydrogel morphologies, ESEM measurements were performed on hydrogel_C and hydrogel_D (Figures 5g-h). Both hydrogels were found to have a porous network, where hydrogel_C displayed a more homogeneous dispersion of the pores when compared with hydrogel_D. Clusters of the ionic salt can be observed at 500x and 2000x magnification from the hydrogel_D (Figure 5g); the presence of such clusters and particles may act as crosslinks, which can potentially influence the material's mechanical properties. In contrast, an excellent solubilization of the QCh:SPA salt was observed on hydrogel_C, which is in accordance with our previous work.

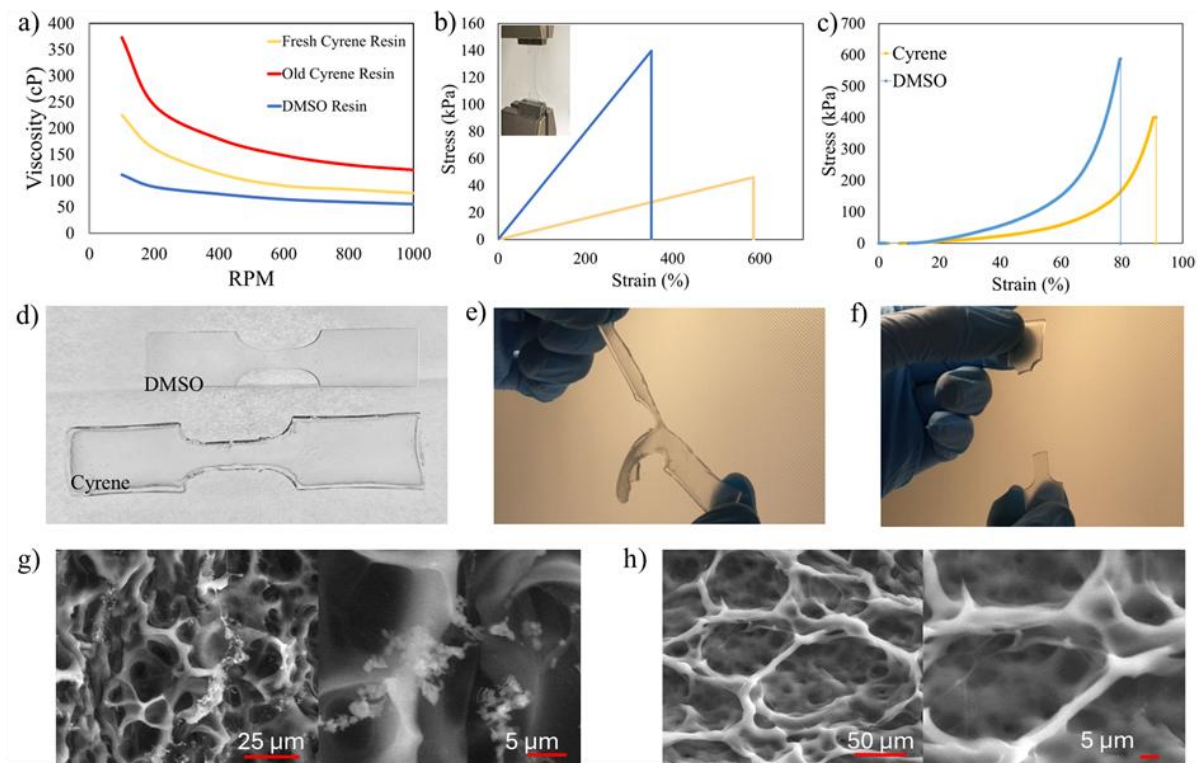


Figure 5. Hydrogel properties for solvent comparison. a) Viscosity measurements for fresh resin_C, old resin_C (after multiple 3D printing cycles), and fresh resin_D. b) Tensile test of hydrogel_C and hydrogel_D, c) Compression test of hydrogel_C and hydrogel_D. d) Equilibrium swelling of hydrogel_C (bottom) and hydrogel_D (top). e) Hydrogel_C photograph showing a brittle failure of the hydrogel network with increased stretchability. f) Hydrogel_D photograph demonstrating a brittle failure of the hydrogel network with poor stretchability. g) ESEM images of frozen hydrogel_D cross-section taken at 500x, and 2000x magnification. h) ESEM images of frozen hydrogel_C cross-section taken at 500x, and 1000x magnification.

Figures 5b-c show the tensile and compression properties, respectively, for hydrogel_C and hydrogel_D. Hydrogel_C had a considerably lower tensile strength but higher stretchability with a failure elongation of 674%, which is almost double the failure elongation of hydrogel_D, which is 320%. The degree of swelling at equilibrium is closely related to the material's tensile strength as the overall polymer content is inversely proportional to the degree of swelling; at the same time, the free volume and low polymer content enhances the hydrogel's stretchability, due to a lower network density and the presence of ionic crosslinks. As a result, the hydrogel_C demonstrated high stretchability with

brittle failure indicative of a sacrificial ionic network (Figure 5e), while a brittle failure with lower stretchability was observed in hydrogel_D indicative of a more rigid structure (Figure 5f). This variation may be attributed to the compatibility of the QCh:SPA salt within the resin; as the polymer solubility increases, the chains create entanglements and interactions between polymer and solvent molecules, which can further contribute to better stress dissipation. Similar to the tensile results, the ultimate compressive strength had an inverse relationship to the maximum strain achieved; hydrogel_C showed a higher strain (94%) but lower strength (500 kPa) compared to the compressive strain and strength of hydrogel_D (79%, and 700 kPa respectively), shown in Figure 5c.

3.3.4 Cyrene-based 3D printed hydrogel investigation for potential applications

The properties of hydrogel_C were further characterized to evaluate its potential applications. Fatigue tests were performed first due to the importance of cyclic compression for hydrogel materials.^{170–172} The 3D-printed hydrogel was submitted to three different loading strains, each with 51 steps, which were submitted to cyclic compression and decompression (Figure 6a). In the end, the hydrogel showed full recovery of its shape (Figure 6b), even after high strain of 70%,. A slight decrease in compressive strength was observed, which is expected due to the disruption of ionic crosslinks between the polymer chains caused by repeated compression, weakening the hydrogel. At 25%, 45% and 65% strains, the hysteresis was almost negligible, as the compressive stress stayed relatively constant after the 25 cycles. The maximum stress curves showed a linear slope of 0.01, 0.05, and 0.07, respectively, in response to increasing applied strain (Figure 6c).

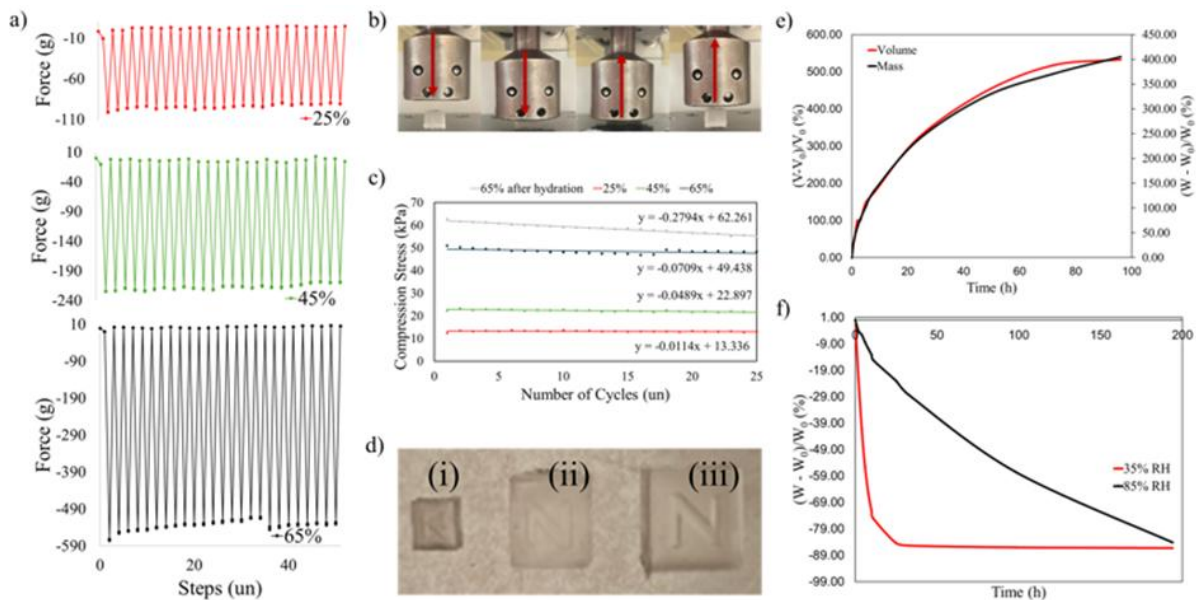


Figure 6. a) Relationship between applied force for 25, 45, and 65% strain at the different steps of the fatigue test. b) Photographs of the hydrogel during the last stage of the cyclic fatigue test demonstrating the hydrogel does not experience permanent deformation. c) Fatigue test performed in 25 cycles of compression for 25, 45, and 65% strain. For 65% strain the curve shows the results for loading before and after hydration. d) Hydrogel size difference after 24h of dehydration, showing the equilibrium dehydration (i), 35% RH (ii), and 85% RH (iii). e) Hydration test showing the volume change over time in the left vertical axis and the mass difference over time in the right vertical axis. f) Dehydration test performed at ambient conditions with 35% RH, and 85% rh. All measurements were performed at 25°C.

We measured the weight loss during the loading cycles and noticed a decrease of 10% in weight after the completion of 25 cycles with $65 \pm 5\%$ strain, along with a decrease in hydrogel stress by 7.95%. To check the stress recovery, the hydrogel sample was immersed in water for 1 hour after compression cycles, and re-tested. Soaking the hydrogel in water caused it to increase in mass by 7.8%, recovering 78% of the mass lost during the test. After rehydration, the hydrogel was able to fully recover its initial stress, thus the loss of the hydrogel strength can be considered reversible.

The swelling and dehydration characteristics of Hydrogel_C were further analyzed. After 72h of swelling in UPW, the hydrogel reached a final swelling degree of 408%. The swelling increased over time, stabilizing at an equilibrium state after this maximum swelling period (Figure 6e). This swelling

behavior is beneficial for 3D printing, as it allows for faster printing of larger structures compared to hydrogel resins printed at their equilibrium state, making up for the time needed for swelling. Additionally, the swelling time also serves as a leaching step, removing harmful compounds remaining in the resin from unreacted monomers. This step is necessary for all photopolymerization-based hydrogels, as the conversion of monomer to polymer is never 100%.

Regarding the dehydration, the size difference after 24 h for the different conditions is demonstrated in Figure 6d. As expected, the dehydration rate increased significantly with decreasing RH (curve in Figure 6e). At lower RH, a greater driving force is present for water molecules to diffuse to the atmosphere; at higher RH, the ambient air is more saturated, mitigating the loss of water to the environment. At 35% RH, a cubic hydrogel sample had a loss of 85% of its mass after 24 h, while at 85% RH, this mass loss required 194h (8 days).

3.4 Summary

In summary, the choice of solvent during organogel synthesis significantly impacts the mechanical properties of the resulting hydrogels. Cyrene-based hydrogels demonstrate greater swelling capacity and stretchability compared to DMSO-based hydrogels, attributed to differences in solvent-polymer interactions. The investigation of printability and fatigue resistance of hydrogels produced with Cyrene revealed its ability to maintain structural integrity under cyclic loading conditions, and a remarkable ability to print details as small as 0.5 mm.

A series of experimental characterizations were performed, and Cyrene hydrogels exhibit excellent stretchability, good printability, and fatigue resistance, making them suitable for applications requiring higher strain, durability, and the use of complex geometry. Furthermore, due to their biocompatibility, hydrogels find extensive utilization in biomedical applications. Additionally, as demonstrated by the printability test, 3D printing along with current technologies can produce a customized material that can be used for bone injuries or bone deformations treatment.

These research findings provide a new understanding of solvent effects on hydrogel properties and demonstrate the potential of Cyrene as a safer, environmentally friendly, and versatile solvent for 3D printed hydrogels, which are important for the development of innovative hydrogel-based materials with enhanced mechanical performance and reduced environmental impact.

Chapter 4 Cyrene-Driven Graphene Dispersion for Advanced Hydrogel Nanocomposites

4.1 Introduction

In this chapter we investigate Cyrene as a potential green solvent to produce graphene dispersion for advanced hydrogel nanocomposites. In recent years, hydrogel nanocomposites integrated with nanoparticles, such as metallic-based, carbon-based, and polymer-based nanoparticles, enabled the production of multifunctional materials with enhanced mechanical, thermal, and electrical properties.^{173–175} The synthesis of hydrogel nanocomposites in aqueous media presents inherent limitations that can affect material properties, stability, and overall functionality.^{176,177}

Over the years, many strategies have been implemented to improve the water dispersibility of carbon-based materials, such as graphene, including surface modification polymer grafting chemical treatments, and the use of biological macromolecules or surfactants.^{23–28} Despite their effectiveness, these methods come with significant limitations and drawbacks on their own, including high energy requirements, additional expenses, and the generation of toxic waste.^{29–32} Furthermore, they often introduce defects in the original nanoparticles, compromising their pristine properties and reducing stability.^{67,178}

The production of organogel precursors, followed by solvent exchange with water, offers a simple and effective method for creating hydrogels.¹⁶² Cyrene can be incorporated into an organogel matrix to produce at once, safe and environmentally friendly hydrogel materials.¹²⁴ Cyrene has a planar aromatic structure that is highly compatible with graphene's nanosheets, enabling stable π - π interactions that keep graphene layers separate and well-dispersed in solution.¹⁷⁹ Salavagione et al. demonstrated Cyrene as the most promising solvent, with the most favorable dispersion outcomes, during graphite exfoliation process.³³ Graphene and Cyrene were shown to have close surface energies, making the intermolecular forces between the different components similar to the forces within each component.^{34–36} This enables Cyrene to form a steric or electrostatic barrier around each graphene sheet, effectively minimizing re-aggregation. As a result, high concentrations of graphene can be achieved using Cyrene as a solvent, with no need for surfactants, shortens processing time, reduces waste, and lowers energy consumption making it a more sustainable approach to graphene dispersion.

In this work, we produced a Cyrene-based organogel composed of quaternized chitosan ionically crosslinked with 2-hydroxyethyl acrylate and graphene nanosheets. We hypothesized that the dispersion of graphene nanosheets in Cyrene can enhance the organogel properties, such as conductivity and strength, in addition to the overall energy cost reduction achieved in this new production of hydrogel nanocomposites. The produced organogel was subsequently immersed in water to produce hydrogel structures that could be tested for applications in flexible electronics. This method combines eco-friendly solvent use, enhanced material performance, and energy efficiency, making it a promising approach for sustainable hydrogel nanocomposite production.

4.2 Materials and Methods

4.2.1 Materials

Quaternary chitosan ammonium salt (CD bioparticles), Amberlite IRA 458 resin (Rohm and Haas), Amberlite A26 resin (Sigma-Aldrich), SSTC60 resin (Purolite), 2-hydroxyethyl acrylate (Sigma-Aldrich), 3-sulfopropyl acrylate potassium salt (Sigma-Aldrich), dimethyl sulfoxide (Sigma-Aldrich), Irgacure 819 (BASF), Cyrene (Sigma-Aldrich), sodium hydroxide (Sigma-Aldrich), and phosphoric acid 85% (Sigma-Aldrich), graphene nanoflakes (ACS materials).

4.2.2 Synthesis of the Neutralized Quaternized Chitosan Hydroxide Salt (QCh:SPA)

As described in Chapter 3, Section 3.2.2.

4.2.3 Synthesis of Cyrene-graphene dispersions

Graphene dispersions were prepared by adding 0.25% pure graphene nanoflakes to different solvents (Cyrene, DI water, acetone, hexane, ethanol, and DMSO). To ensure homogeneity, the mixtures were sonicated at 80% amplitude (Q125 ¼” probe, Qsonica, USA) for 10 minutes, with 2-minute rest intervals after every 1 minute of sonication. To assess the stability of the dispersion, the solutions were centrifuged at 10,000 rpm for 10 minutes following sonication, where the supernatant was observed.

4.2.4 Synthesis of the Cyrene Organogel-graphene Ink

A 3D-printable organogel ink was prepared using Cyrene as solvent, as described in Chapter 3, Section 3.2.4. After homogenization, different amounts of graphene nanoflakes were added to the

blend, followed by sonication at 80% amplitude for 10 minutes, with 2-minute rest intervals after every 1 minute of sonication.

For the curing process, samples were cast in polydimethylsiloxane (PDMS) molds and UV-cured for 1 minute using a UV probe, followed by a 10-minute post-curing process in a 300 W UV chamber. Subsequently, the samples underwent a solvent exchange process with UPW for 72 hours to remove unreacted monomers and replace the organic solvent with water before testing.

4.2.5 Characterization

Rheological measurements were conducted using a frequency sweep test from 0.01 to 100 Hz on a dynamic shear rheometer (MCR 702e Rheometer, Anton Paar). The test was performed at a strain rate of 1% and a normal force of 1 N, maintaining a constant temperature of 25 °C. A cone-plate geometry with a 25 mm diameter and a 1.0-degree angle was used. Environmental scanning electron microscopy (ESEM, FEI Quanta Feg 250) and Scanning Electron Microscopy (SEM-Zeiss Merlin High-resolution) analysis were performed on hydrogel samples that were frozen in liquid nitrogen and then broken, as well in dried Cyrene-graphene dispersions.

Conductivity measurements were performed in Cyrene dispersions and in the hydrogel, using a SP-300 potentiostat (BioLogic Scientific Instruments, France) attached with a 4-point holder (Cascade Microtech, USA) and C4S 57/1S 3716 probe (Cascade Microtech, USA). Raman spectroscopy (Bruker Senterra-2) was employed to analyze the dispersibility of Cyrene-graphene solutions using a green line laser (wavelength 532 nm). Tensile tests were carried out on the synthesized hydrogels after solvent exchange, using a Universal Material Tester (CETR-UMT, Bruker, USA), where printed coupons were stretched at 2 mm/s until failure.

4.3 Results and Discussion

4.3.1 Dispersibility of Graphene Nanosheets in Various Solvents

The dispersibility of graphene nanoflakes was evaluated by dispersing the flakes in various solvents without surfactants, under identical conditions. Figure 7a presents a visual comparison of dispersibility in Cyrene, water, acetone, hexane, ethanol, and DMSO. Among these, Cyrene demonstrated superior dispersion, forming a uniform and stable suspension. In contrast, other solvents,

including water, acetone, hexane, ethanol, and DMSO, exhibited poor dispersibility, with noticeable aggregation and sedimentation of graphene flakes.

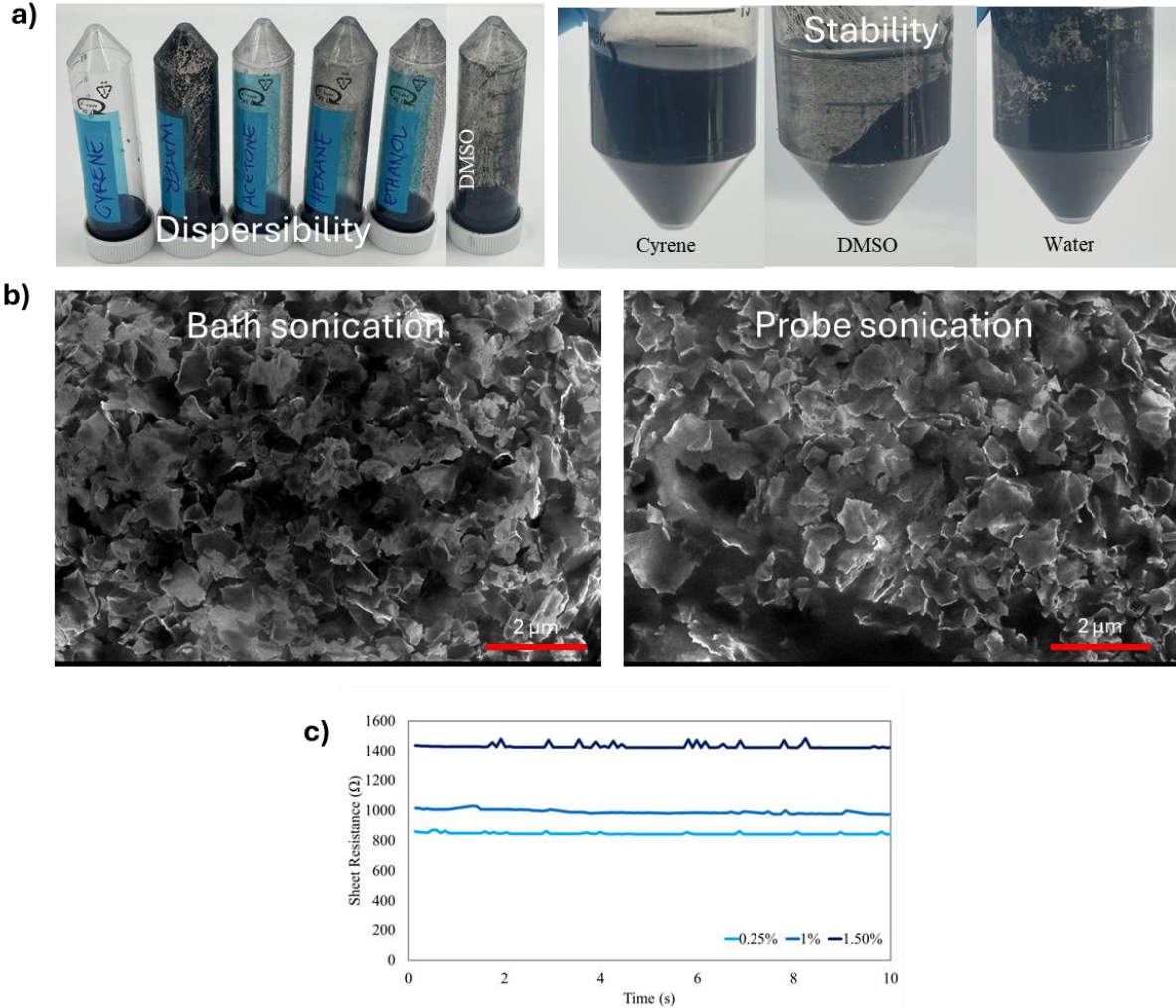


Figure 7. Graphene Dispersibility. (a) Visual representation of graphene dispersibility and stability in various conventional solvents. The left size image displays solutions in Cyrene, DI water, acetone, hexane, ethanol, and DMSO, respectively. (b) SEM images of 1% graphene dispersion at 10,000x magnification, comparing probe sonication and 8 hours of bath sonication. (c) Resistance measurements of Cyrene-graphene solutions.

Dispersion stability was assessed after 10 minutes of centrifugation. As shown in Figure 7a, solutions in DMSO and water displayed precipitation after sonication, indicating low stability. In contrast, Cyrene-based dispersions remained stable. The enhanced stability of graphene nanoflakes in

Cyrene is likely attributed to its high polarity and hydrogen bonding capacity, which facilitate interactions between oxygen-containing functional groups and the graphene surface, forming hydrogen bonds and intermolecular forces.³⁶

SEM images illustrate the dispersibility of graphene nanoflakes in Cyrene. A comparison of different homogenization methods, including bath and probe sonication, was conducted to ensure that high sonication amplitude and duration did not damage the flakes. As shown in Figure 7b, probe sonication resulted in better dispersibility while maintaining similar flake sizes, indicating that the chosen process preserves the integrity of the graphene flakes along with efficiency dispersibility.

Following, to confirm the good dispersibility and stability of Cyrene dispersions, conductivity measurements were performed using thin films of Cyrene solutions with graphene at concentrations of 0.25%, 1%, and 1.5%. The results show an increase in sheet resistivity as the graphene concentration increases, with an 18% rise between 0.25% and 1%, and a 70% increase between 0.25% and 1.5%. This suggests that as the concentration of graphene increases, conductivity decreases, likely due to higher agglomeration of graphene and loss of the percolation network. The highest conductivity values achieved were approximately $118 \pm 7 \text{ S.m}^{-1}$, $101 \pm 13 \text{ S.m}^{-1}$, and $70 \pm 16 \text{ S.m}^{-1}$ for 0.25%, 1%, and 1.5% graphene solutions, respectively.

4.3.2 Cyrene-Based Nanocomposite Hydrogels Characterization

Initial rheology measurements were performed in the pure Cyrene-based organogel and 0.1%Gr organogel, to understand the nature of the gel (Figure 8a). Both G' and G'' increase with frequency, indicating that the gel becomes stiffer and more resistant to deformation at higher frequencies. Throughout the frequency range, G' remains higher than G'' , suggesting that the organogel ink primarily exhibits solid-like behavior. The rheology data indicate that the organogel ink possesses strong viscoelastic properties, with a dominant elastic response ($G' > G''$) at almost all frequencies. The increasing moduli with frequency demonstrates that the ink can maintain its structural integrity under dynamic conditions, making it ideal for extrusion-based 3D printing or similar applications. Additionally, the graph of viscosity shows a decrease of viscosity with angular velocity, indicating a shear thinning behavior, that is desirable for extrusion base inks.

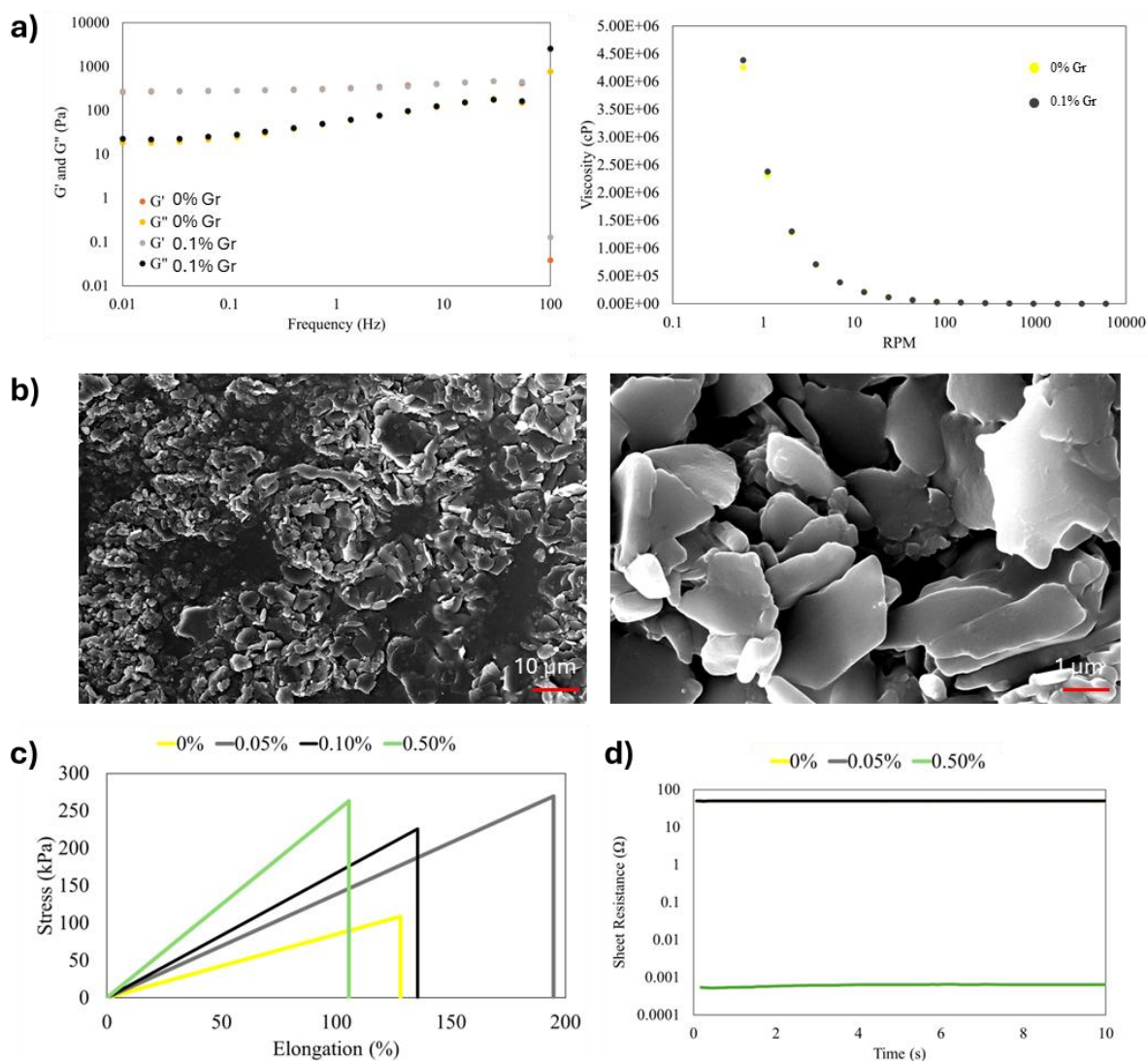


Figure 8. Cyrene-Based Nanocomposite Hydrogels Characterization. a) Frequency sweep test of Cyrene-based organogel ink from 0 to 100 Hz, illustrating the relationship between G' and G'' as a function of frequency (left) and viscosity versus angular velocity (right). b) SEM images of 0.1% graphene hydrogel after solvent exchange at 1,000x and 10,000x magnification (left to right). c) Tensile test of hydrogel nanocomposites. d) Resistance measurements of Graphene hydrogel nanocomposites, with the curve in black being an overlap of 0% and 0.05% graphene concentration.

Following the characterization of Cyrene-based organogels, initial experiments were performed with graphene dispersion in the solvent being incorporated into the rest of the ink blend to

assess its effects on mechanical and electrical properties. For this, a 1% graphene solution in Cyrene was prepared, homogenized, and then added to the gel matrix, creating dispersions ranging from 0% to 0.1% w/v graphene. Initial SEM images of the hydrogel cross-section containing 0.1% w/v graphene demonstrated a uniform distribution of graphene flakes within the gel matrix, a crucial factor for achieving consistent material properties (Figure 8b).

Tensile tests, reported in Figure 8c, revealed that the incorporation of graphene significantly enhanced both tensile strength and elongation, with the 0.05% graphene sample achieving the best balance between strength (a 150% increase) and elongation at break (a 52% increase). The improved mechanical performance of the hydrogels can be attributed to graphene's ability to reinforce the polymer matrix and enhance load distribution during deformation. At lower concentrations (0.05%), graphene is uniformly dispersed within the matrix, contributing to both strength and elongation at break. However, conductivity measurements showed that graphene concentrations up to 0.1% did not significantly enhance electrical properties (Figure 8d).

The electrical conductivity remained unchanged, indicating that the graphene content was insufficient to establish a percolating conductive network. These findings suggest that while graphene positively influences mechanical properties, its impact on electrical conductivity at low concentrations is minimal. To achieve substantial improvements in electrical properties, higher graphene concentrations or optimized formulations are required. Therefore, to overcome the challenge of incorporating higher graphene concentrations into the organogel matrix while maintaining Cyrene's efficiency as a dispersing solvent, an alternative approach was explored.

Instead of pre-dispersing graphene in Cyrene, graphene nanoflakes were directly introduced into the homogenized gel, followed by 10 minutes of probe sonication until visual homogenization was achieved, as described in Section 4.2.4. Initial results indicate that this method significantly improved hydrogel conductivity, which increased from $14 \text{ S}\cdot\text{m}^{-1}$ to $8.22 \times 10^5 \text{ S}\cdot\text{m}^{-1}$. While tensile strength remained relatively unchanged, with a maximum value of 263 kPa, elongation at break decreased by 17%. It is suggested that at higher concentrations, elongation slightly decreased, likely due to reduced flexibility caused by graphene aggregation or the formation of a stiffer network.

However, despite these promising improvements, long-term stability remained a concern, particularly with respect to conductivity. Over time, significant reductions in conductivity were observed (Conductivity of 14.55 S m^{-1} after 2 days), likely due to the reorganization of the graphene

network within the hydrogel matrix. Graphene flakes may have aggregated or settled, disrupting conductive pathways and diminishing electrical performance. These findings highlight the need for stabilization strategies to ensure the long-term reliability of conductive hydrogels.

4.4 Potential Applications and Challenges of Graphene Organogel Nanocomposites

The development of graphene-based inks and/or graphene hydrogel nanocomposites presents exciting opportunities across multiple fields. The high electrical conductivity, mechanical reinforcement properties, and printability of graphene make it a promising material for next-generation technologies. However, as it was possible to see in the previous discussions, several challenges remain in optimizing these materials for real-world applications.

Initial observations were made to evaluate the potential of the produced graphene-organogel ink for SLA 3D printing. The first step involved investigating the curing time and depth of this new material. The 0.1% graphene-organogel ink demonstrated a curing time of 6.74 seconds with a curing depth of 0.04 mm, as calculated using the method described in Chapter 3.3.2., and plotted in Figure 9a. Subsequently, the resin was tested in an mSLA printer, using a thicker layer of 0.02 mm and an exposure time of 14 seconds per layer. However, the resin failed to successfully print structures, resulting in printing failures (Figure 9b).

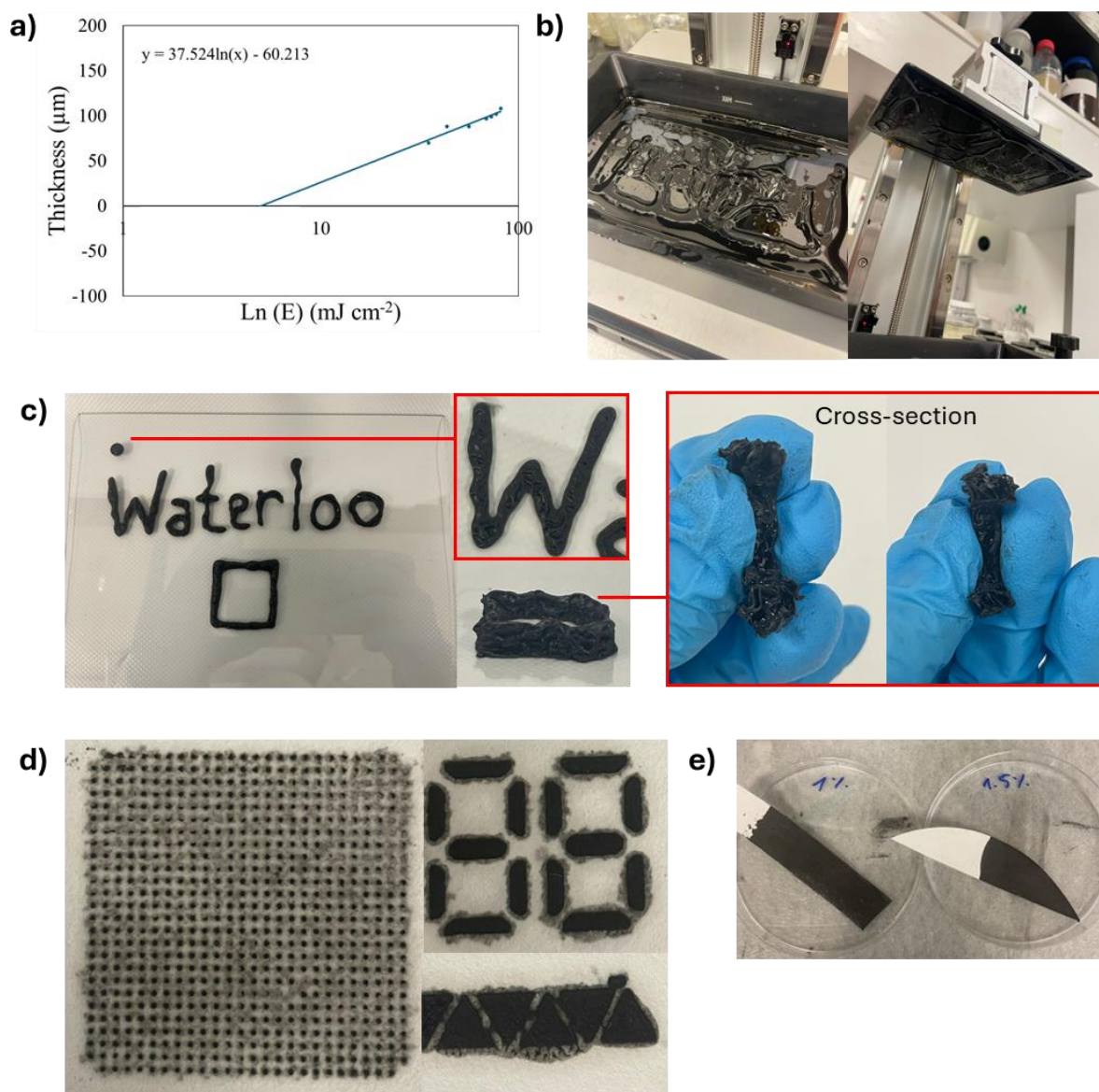


Figure 9. Potential Applications of Graphene-Organogel Inks. a) Plot of $\ln(E)$ versus thickness derived from exposure time measurements for graphene-organogel ink. b) Photograph illustrating printing failure in mSLA 3D printing for the 0.1% graphene-organogel ink. c) Demonstration of extrusion-based 3D printing, showcasing freeform writing and the fabrication of a 10-layer-high structure with a visible cross-section. d) Organogel casting in various patterns on paper. e) Cyrene-graphene dispersion cast onto paper at concentrations of 1% and 1.5%.

The primary reason for these failures is likely related to graphene's strong light absorption properties. Graphene absorbs UV and visible light, which interferes with the photopolymerization process by significantly reducing the penetration depth of UV or laser light into the resin.^{180,181} This limits the degree of polymerization, leading to incomplete curing and poor layer adhesion, ultimately compromising the structural integrity of the printed parts. These findings highlight a critical challenge in adapting graphene-based inks for SLA 3D printing. Alternative 3D printing techniques such as extrusion-based printing could be explored, as it is less dependent on light penetration.

Following the applicability investigation, the produced organogel ink was extruded through a needle with a nozzle size of 1.6 mm (external diameter), allowing for efficient creation of printed material layers after UV curing. This ink was successfully used for writing and constructing a structure with 10 layers, with each layer cured for 10 seconds before adding the next, as shown in Figure 9c. The cured material exhibited a wave-like pattern, which could have been caused likely due to curing dynamics during the photopolymerization process.^{182–184} Graphene's strong light absorption properties reduce the penetration depth of UV or laser light into the resin, causing localized variations in curing.

A potential application for this type of material involves using graphene organogel ink to print directly onto textiles or flexible substrates, enabling the creation of wearable electronic devices such as health monitoring sensors for heart rate or body temperature.^{185–187} The ability to print graphene ink onto fabrics, paper, or polymer films facilitates the development of flexible and stretchable electronics. Initial tests were performed involving casting graphene organogel and Cyrene dispersions onto paper substrates, as shown in Figure 9d and 9e. The organogel was successfully casted, demonstrating the potential for creating conductive paper. This development could lead to future applications in creating conductive patterns for smart surfaces, further expanding the versatility of graphene-based materials in innovative technologies.

4.5 Conclusion

This study highlights the potential of graphene organogel inks by leveraging Cyrene as a green solvent for dispersing graphene nanoflakes. Initial experiments demonstrate the superior dispersibility of graphene in Cyrene compared to other solvents, achieving uniform and stable suspensions without the need for surfactants. The incorporation of graphene into the organogel matrix enhanced mechanical properties, such as tensile strength and elongation. However, improvements in electrical conductivity were limited at low graphene concentrations and exhibited poor long-term stability. Challenges arose

in SLA 3D printing due to graphene's strong light absorption, affecting the curing process. In contrast, extrusion-based techniques proved successful, enabling the fabrication of layered structures with UV curing. Additionally, casting onto paper substrates demonstrated potential for wearable electronics and conductive paper applications. Future research should focus on optimizing graphene dispersion stability, developing effective curing protocols, and implementing stabilization strategies to maintain conductivity over time.

The development of graphene organogel inks presents exciting opportunities for advanced material formulations. To effectively utilize graphene organogel for creating smart textiles or conductive paper, detailed studies involving several key steps are necessary. First, the optimization of the dispersion of graphene within the organogel matrix needs to be reevaluated to ensure uniformity and high conductivity. This may require the use of stabilizers to prevent graphene aggregation and maintain dispersion stability over time. Additionally, the development and optimization of curing and drying protocols are needed to ensure the stability and conductivity of the graphene organogel on substrates like textiles or paper. The use of stabilizers or adjustments to the organogel formulation can help overcome issues with conductivity changing over time as discussed previously. By addressing these challenges, graphene can rise as a new material in smart textiles and conductive paper applications. Overall, this work contributes to the broader understanding of graphene-based materials for emerging applications. The use of Cyrene as a green solvent aligns with the growing demand for sustainable and environmentally friendly materials, positioning it as a promising candidate for future advancements in printable electronics and wearable devices.

Chapter 5 Conclusion and Future Work

5.1 Thesis Conclusion

This thesis explored the use of Cyrene, a bioderived and environmentally friendly solvent, to advance sustainable fabrication of hydrogels by 3D printing organogels, followed by solvent exchange with water. Extensive investigations were conducted on the synthesis and properties of organogels, demonstrating this method as a promising strategy to overcome challenges associated with direct hydrogel synthesis.

In Chapter 3, we demonstrated Cyrene's effectiveness in enhancing hydrogel properties such as stretchability, swelling behavior, and structural stability, while also addressing environmental concerns related to traditional solvents like DMSO. Cyrene-based hydrogels exhibited superior mechanical properties compared to DMSO-based hydrogels, emphasizing the critical role of solvent-polymer interactions in determining hydrogel performance. This study is among the first to report the fabrication of organogels followed by solvent exchange. Furthermore, it highlights the benefits of this technique in producing highly flexible hydrogel structures without the risk of structural disintegration when using SLA. Most SLA-produced hydrogels are rigid, leaving a gap in the production of flexible yet high-precision structures. This research fills that gap and provides new insights for future studies on these materials across various applications.

Chapter 4 builds on the research, investigating graphene dispersion, demonstrating Cyrene's ability to stabilize graphene nanoflakes without the need for surfactants or chemical modifications, leading to uniform and defect-free dispersions. The incorporation of graphene into Cyrene-based hydrogels improved mechanical properties such as tensile strength and elongation, although enhancements in electrical conductivity were limited at low graphene concentrations. Challenges such as long-term conductivity stability and nanoparticle aggregation were identified, underscoring the need for further optimization. Additionally, we observed that extrusion-based techniques and casting can also successfully fabricate layered structures and conductive patterns on substrates like paper. These findings highlight the versatility of Cyrene-based hydrogels for applications in wearable electronics, flexible sensors, and smart surfaces.

Overall, the research findings contribute to the broader understanding of Cyrene's potential as a green solvent for advanced hydrogel systems. The key novelty of this study lies in introducing and

validating a new synthesis pathway, using green solvents like Cyrene for organogel fabrication, followed by solvent exchange to achieve desirable hydrogel properties. This approach expands the application range of SLA-printed hydrogels, providing a foundation for further advancements in biomedicine, soft robotics, and flexible electronics.

5.2 Future Work

To build upon the findings of this thesis, several directions for future research can be proposed. First, optimizing graphene dispersion stability is essential to ensure uniformity and high conductivity in Cyrene-based hydrogels. This could involve exploring the incorporation of stabilizing agents to prevent graphene aggregation and maintain stability over time. Additionally, long-term stability studies are necessary to evaluate the mechanical performance and conductivity of Cyrene-based hydrogels under various environmental conditions and repeated usage scenarios. Such studies would provide deeper insights into potential instabilities and degradation mechanisms affecting these materials.

A potential improvement could be the investigation and incorporation of different nanomaterials within the hydrogel network to enhance mechanical properties, such as cellulose nanocrystals (CNC). The addition of CNC could lead to better dispersibility and mechanical reinforcement without compromising the ability to perform mSLA 3D printing. Finally, it is crucial to assess the biocompatibility of Cyrene-based hydrogels for biomedical applications. Hydrogel safety for long-term use in contact with living tissues, particularly the possibility of any residual monomers or solvent remaining after solvent exchange needs to be evaluated to ensure that these hydrogels do not elicit adverse biological responses or toxicity.

Furthermore, while the current synthesis process utilizes a green solvent like Cyrene, it still relies on synthetic polymers such as HEA, which may introduce less sustainable aspects and potential toxicity due to residual monomers. To address this, we propose modifying the polymeric structure of the hydrogel by replacing HEA with gelatin methacryloyl (GelMA). Gelatin is a natural polymer and can be modified to GelMA introducing acrylate groups and enabling photopolymerization and copolymerization with acrylate salts, as previously described for HEA and SPA. The investigation of different hydrogel matrices could also be investigated to identify how changing monomers, such as HEA, have an impact on the solvent-dependent analyzed previously.

This future work could pioneer the use of mSLA 3D-printed hydrogels, an area with limited research due to the hydrogel's inherently weak structure. The presence of acrylate salts and the organogel state of the gel could potentially improve structural integrity, allowing it to retain its shape and prevent printing failures. By integrating these features, strong materials could be developed for a wide range of applications.

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3D Printing Organogels with Bioderived Cyrene for High-Resolution Customized Hydrogel Structures

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Appendix A - Supporting Information¹

1. Solvent exchange analysis

To evaluate the efficiency of the solvent exchange method, a study using a gas chromatograph (GC) was performed. After 3D printing, five printed cubes with a volume of 0.125 cm³ were submerged in a container with 200 mL of ultrapure water (UPW) for 24 hours. A sample of the water content was then removed and labeled "soak 1". The cubes were subsequently placed in a second container filled with fresh UPW, which was sonicated in a bath for an additional 24 hours. After sonication, a sample of the water content was removed and labeled "soak 2".

Gas chromatography was performed using an Agilent 6890 instrument, with a HP-Wax Bonded polyethylene glycol column and Helium as the carrier gas at a constant flow rate of 8.9 mLmin⁻¹. A flame-ionization detector (FID) was used, set to 230°C and 1 psi, operating in splitless mode with a 5 mLmin⁻¹ purge for three minutes. The injection volume of the samples was set at 0.2 µL, with six pre-injection washes using UPW and four post-injection washes with methanol. A temperature gradient was applied, starting at 100°C (held for 1 minute), followed by an increase of 30°C/min until 190°C, then a second increase of 10°C/min until the final temperature of 230°C, which was held for 5 minutes to ensure complete removal of the sample from the column.

A calibration curve was created using a Cyrene/UPW solution (50, 200, and 500 mgL⁻¹) for the quantitative analysis of residual solvent before measurements (Figure S10). The curve shows a good linear relationship between the concentration and peak area ratio, being described by the equation $Y = 1.6035x + 21.67$, with $R^2 = 0.9951$, and average retention time of 11.23 min (n=3).

¹ This chapter was partially reproduced from Ramirez, A. B., Bauman, L. A. & Zhao, B. 3D Printing Organogels with Bioderived Cyrene for High-Resolution Customized Hydrogel Structures. *Langmuir* 41, 646–653 (2025).¹⁵²

The corresponding gas chromatograms of the samples are presented in Figures S11-S12. The chromatogram of sample one shows that at the initial 24 h the kinetics of exchange is considerably elevated, as the peak area of Cyrene is equal to 1997.26, which is equivalent to a concentration of 1.23 gL⁻¹. After the first 24 h, the sample is transported to another container with fresh water. This sample shows that the presence of Cyrene in the 3D printed object has considerably decreased in 48 h of solvent exchange, achieving a concentration of 72.40 mgL⁻¹, which is 6% of the concentration present in soak 1. Therefore, it is possible to conclude that the solvent exchange mostly is present at the first 24 h of swelling, confirming the experiment steps of leaving the hydrogel hydrate for at least three days, to have a complete solvent exchange before testing it.

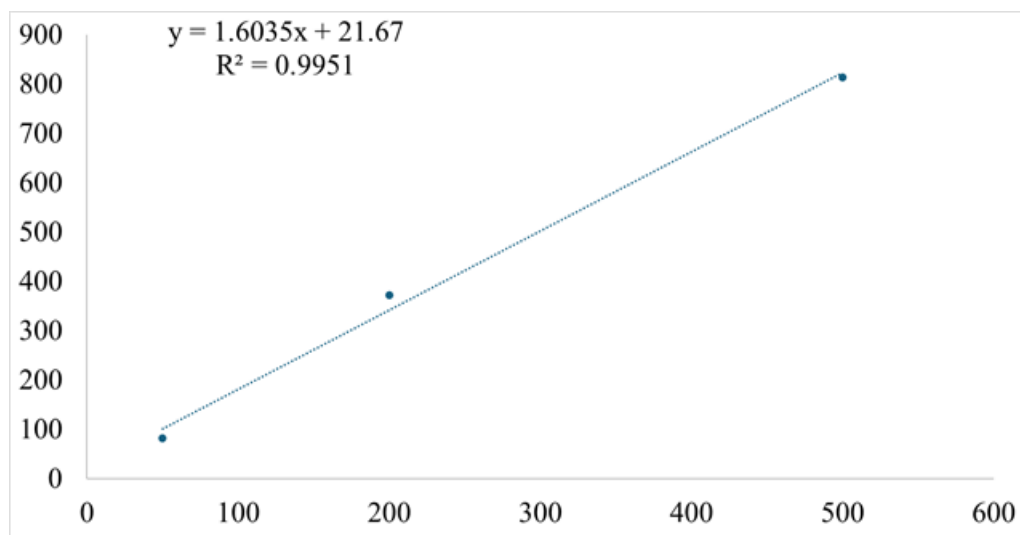


Figure S10. Calibration curve of Cyrene in water solution performed in gas chromatography.

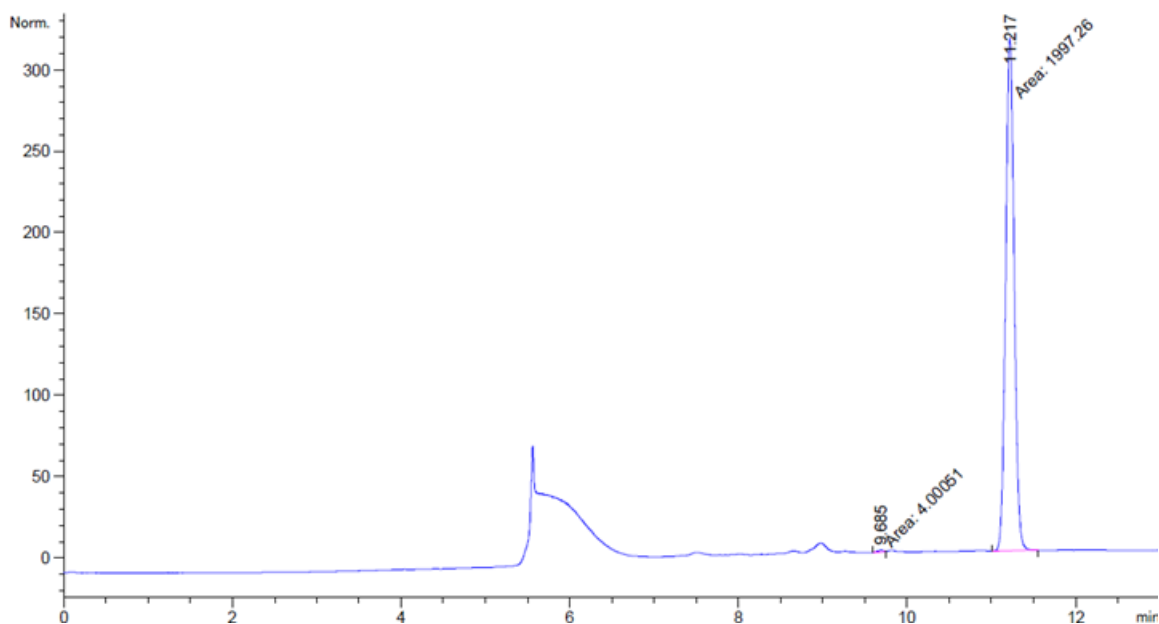


Figure S11. Chromatogram of sample one, labeled soak 1. The HEA residual monomer peak is detected at 9.685 min, and the Cyrene residual solvent is detected at 11.217min.

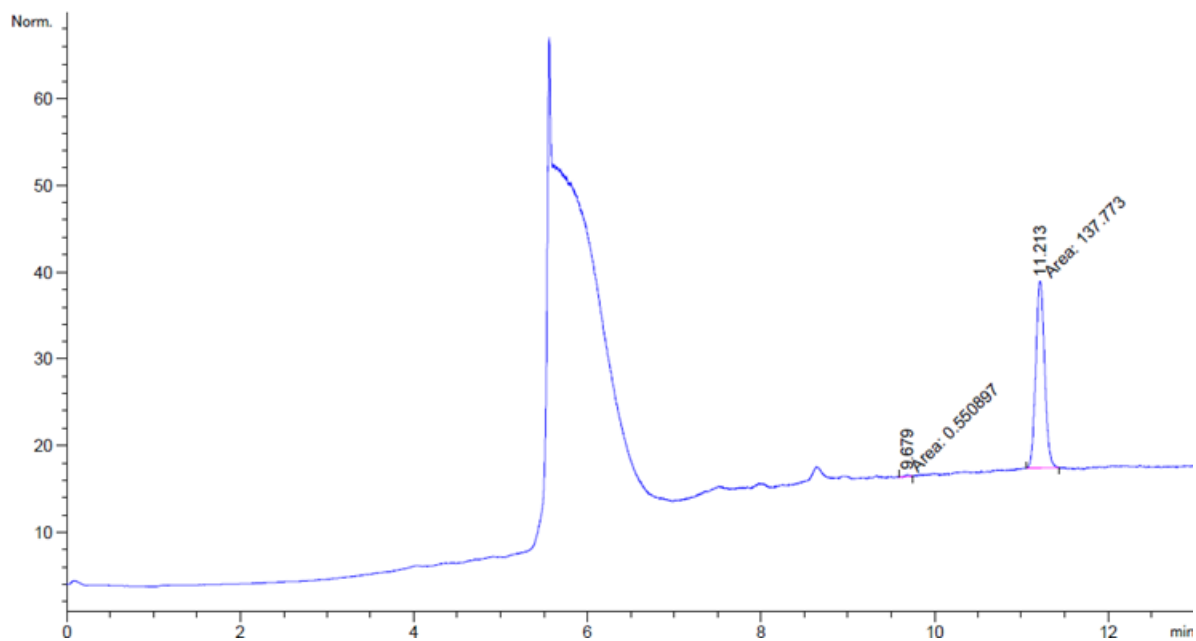


Figure S12. Chromatogram of sample two, labeled soak 2. The HEA residual monomer peak is detected at 9.679 min, and the Cyrene residual solvent is detected at 11.213 min.