



Title	Preparation of Novel Bio-Based Composite Films Reinforced with Poly(vinyl alcohol) and Their Practical Applications
Author(s)	Yao, Ying
Citation	大阪大学, 2025, 博士論文
Version Type	VoR
URL	https://doi.org/10.18910/105265
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The University of Osaka

Doctoral Dissertation

**Preparation of Novel Bio-Based Composite Films
Reinforced with Poly(vinyl alcohol) and Their Practical
Applications**

ポリ（ビニルアルコール）によって強化された
新規バイオマス複合フィルムの開発とその応用展開

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September 2025

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The University of Osaka

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General Introduction

Background

Sustainable environmental governance has become a pressing global concern, with greenhouse gas (GHG) emissions representing one of the most critical challenges.^[1] Current statistics indicate that approximately 75% of global GHG emissions originate from energy-related activities, widely recognized as the primary drivers of global climate warming and ecological degradation.^[2] With accelerating population growth and industrialization, global energy demand continues to rise, exacerbating the negative environmental impacts of CO₂ and other GHG emissions.^[3] For instance, global energy consumption in 2017 increased by 2.1% year-on-year, significantly higher than the 0.9% growth in 2016 and the five-year average of 0.9%, indicating a rapidly accelerating consumption trend.^[4] Against this backdrop, the fossil fuel-based energy structure—primarily reliant on coal, oil, and natural gas—is increasingly challenged by resource depletion and environmental pollution.^[5,6] Fossil fuels, being non-renewable, release substantial amounts of GHGs during use, further intensifying environmental burdens.^[7] Therefore, accelerating the transition of the global energy mix by incorporating a higher share of renewable energy has become a key pathway to achieving low-carbon development.^[8,9] Ongoing global research efforts are now focused on developing alternative energy sources to reduce dependency on fossil fuels and mitigate GHG emissions at their source.^[10]

Among various renewable energy options, biomass energy is increasingly regarded as one of the most promising alternatives to conventional fossil fuels due to its inherent renewability, carbon neutrality, and environmental compatibility.^[11,12] Biomass refers to renewable organic resources derived from plant tissues, animal metabolic by-products, and microbial activities.^[13,14] These resources offer notable advantages, including abundant

availability, diverse sourcing routes and high renewability.^[15] Moreover, their unique biochemical structures allow for substantial molecular design flexibility.^[16] Leveraging their functional group tunability, biomass feedstocks can be converted via multistep physical processing or chemical transformation into high-value, multifunctional derivatives.^[17,18] Compared to fossil fuels, biomass energy provides superior environmental benefits by enabling efficient resource recycling and reducing ecological impacts, thereby aligning well with sustainable development and green low-carbon goals (**Figure 1**).^[19,20] From the strategic perspective of global energy restructuring and climate change mitigation, advancing renewable and biodegradable biomass-derived energy and functional materials is of paramount importance.^[21] It not only alleviates pressures related to resource depletion but also plays a vital role in reducing GHG emissions and fostering an eco-friendly energy paradigm.^[22]

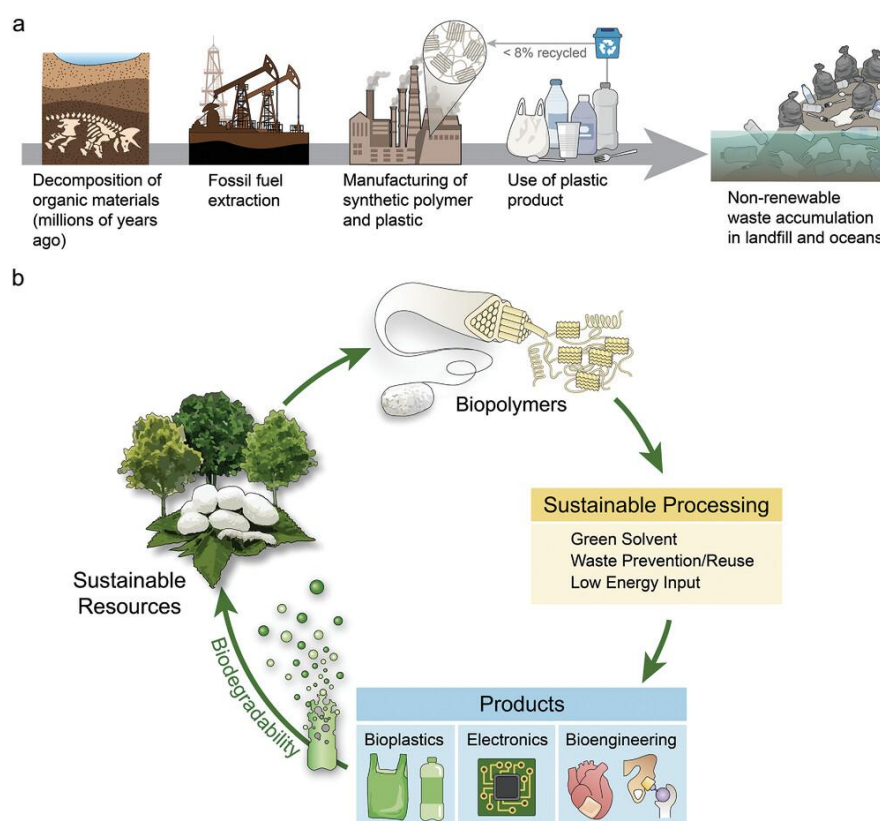


Figure 1. Schematic illustration of sustainability strategies: (a) Linear life cycle of conventional synthetic polymers and (b) circular life cycle of bio-based polymers enabling resource renewal and environmental compatibility.^[23]

Biopolymer

Biomass, owing to its abundant resource base and excellent renewability, plays a pivotal role in the development of sustainable materials and energy systems.^[24] The diverse origins of biomass confer significant structural variability, making it suitable for a wide range of processing methods and applications.^[25,26] Raw biomass can typically be obtained directly from nature and further processed through physical or chemical means to yield small molecules or macromolecular units such as polysaccharides, polyphenols, and proteins—collectively referred to as biopolymers (**Figure 2**).^[28] Based on their origin and synthesis method, biopolymers are generally categorized into two major groups: natural polymers and synthetic bio-based polymers.^[29] Natural polymers can be further classified by source into plant-derived (e.g., starch, cellulose), animal-derived (e.g., chitosan, gelatin), and microbial-derived polymers (e.g., polyglutamic acid and polyhydroxyalkanoates).^[30–32]

Biomass		
Plant wood, grass, algae ...	Polysaccharides Polyphenols Proteins Others	Polyphenolic derivatives
Animal silk, wool, shells, feathers, bones...	Polysaccharides Proteins Others	
Microbial bacteria strains, bacteria cellulose, fungi...	Polysaccharides Proteins Others	Oligo/mono-saccharides Amino acids Others

Figure 2. Sustainable production of smart functional materials can be achieved using biomass from various hierarchies, including plants, animals, microbes, and their derived molecular components.^[27]

Plant-derived biopolymer

Plant-derived biopolymers hold a prominent position in the development of green materials due to their abundant availability, diverse functionality, and strong renewability.^[33] Common representatives include starch, cellulose, pectin, lignin, and soy protein (**Figure 3**).^[33,34] Starch, widely distributed in plant roots, stems, and seeds, is composed of amylose and amylopectin, which consist of glucose units linked by α -1,4 and α -1,6 glycosidic bonds.^[36,37] Its excellent biodegradability and film-forming ability make it a promising candidate for sustainable material applications.^[38] Cellulose, the most abundant natural polymer on Earth, is primarily found in plant cell walls and comprises pyranose glucose units linked via β -1,4 glycosidic bonds, exhibiting high crystallinity and water insolubility.^[39,40] Its hydroxyl groups can undergo esterification, etherification, or nitration to produce derivatives such as cellulose acetate and ethyl cellulose, which are widely used in pharmaceuticals, packaging, and consumer products.^[41,42] Pectin, a natural heteropolysaccharide primarily composed of α -1,4-D-

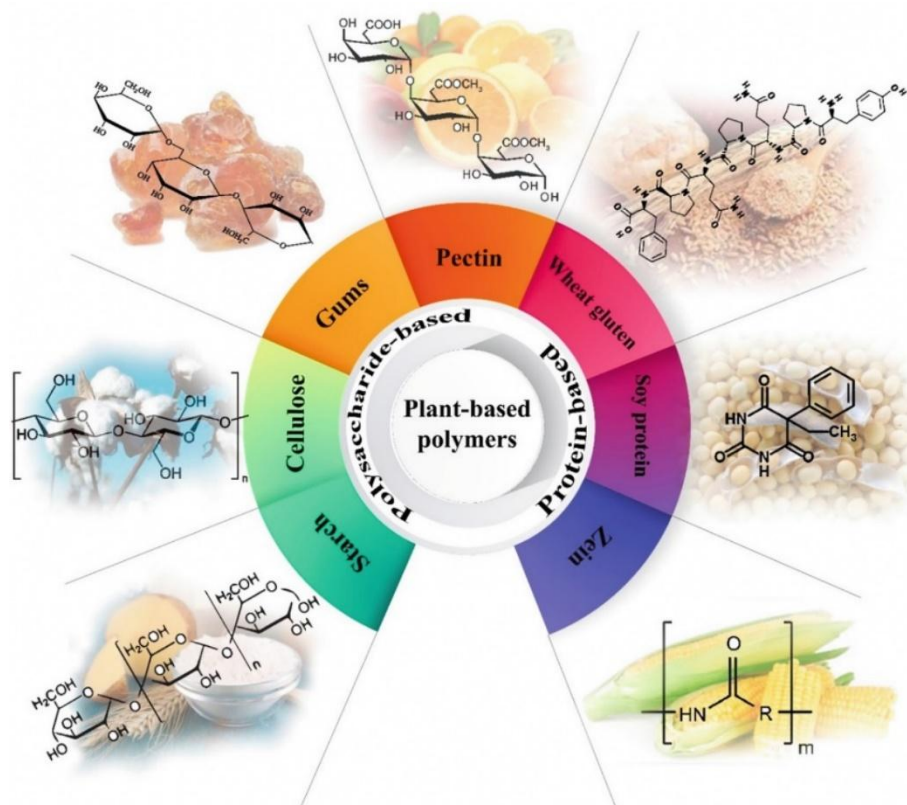


Figure 3. Major Types of Plant-Based Biopolymers.^[35]

galacturonic acid, is also abundant in plant cell walls.^[43] It features methyl-esterified regions, neutral sugar side chains, and multiple functional groups that afford high reactivity and structural tunability.^[43,44] Through appropriate modifications, pectin has been applied in food additives, drug delivery systems, and functional film materials.^[45,46]

Microbial-derived biopolymer

Microbial-derived biopolymers have emerged as a vital component in sustainable materials research due to their controllable biosynthesis pathways and outstanding functional properties.^[47] Representative examples include alginate, poly(γ -glutamic acid) (γ -PGA), polyhydroxyalkanoates (PHA), and polylactic acid (PLA).^[48,49] Alginate is a linear, water-soluble polysaccharide primarily extracted from brown algae or certain bacterial strains and can form stable gel networks via ionic crosslinking with metal ions.^[50] Owing to its excellent biocompatibility and high affinity for cations, alginate is widely used in dye adsorption, heavy metal removal, and tissue engineering.^[51] γ -PGA is a naturally occurring polyamide synthesized by *Bacillus* species through fermentation, consisting of glutamic acid units linked via amide bonds between γ -carboxyl and amino groups.^[52] As a water-soluble anionic polymer containing both carboxyl and amide groups, γ -PGA exhibits outstanding water retention, biodegradability, thermal stability, and antioxidant capacity, enabling broad applications in agriculture, food, drug delivery, and functional films.^[53] Polyhydroxyalkanoates (PHAs) are microbial polyesters produced by various microorganisms under specific carbon-rich conditions as intracellular carbon and energy storage compounds.^[54] The PHA family comprises over 150 monomer variants, offering tunable thermoplasticity, mechanical strength, and electrical properties, making them promising candidates to replace petroleum-based plastics.^[55,56]

Animal-derived biopolymer

Animal-derived biopolymers have become essential components of functional and sustainable materials due to their large-scale availability from animal by-products (**Figure 4**). Representative examples include gelatin, chitosan, and keratin.^[58] Gelatin, a hydrolyzed form of collagen primarily obtained from animal skin, bones, and connective tissues, exhibits excellent gelling and film-forming properties and is widely used in the food industry, pharmaceutical coatings, and cosmetic formulations.^[59,60] Chitosan, derived from the deacetylation of chitin found in the exoskeletons of crustaceans, is one of the few naturally occurring cationic polysaccharides.^[61] It offers strong antimicrobial activity, biodegradability, and ion-exchange capacity, making it suitable for applications in tissue engineering, environmental remediation, and food packaging.^[62] Keratin is a cysteine-rich structural protein

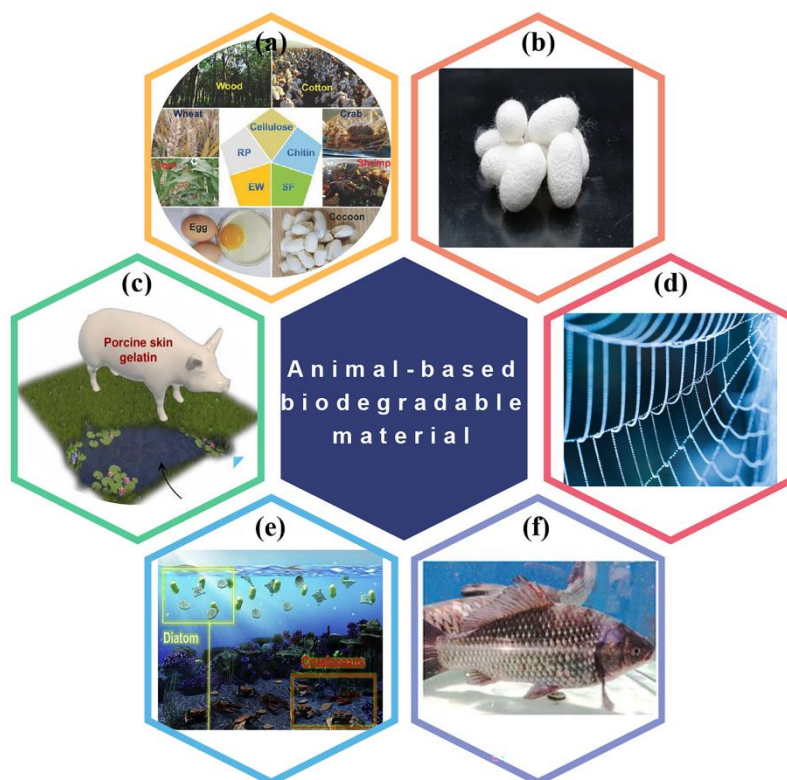


Figure 4. Some representative animal-based biodegradable polymers. (a and b) Biopolymers derived from silk fibroin, egg components, and chitosan; (c) Gelatin; (d) Recombinant spider silk fibroin; (e) Biocompatible chitosan; (f) Fish gelatin.^[57]

with diverse conformations (e.g., α -helix and β -sheet) and can be processed into hydrogels, films, sponges, and scaffolds.^[63,64] It demonstrates excellent biocompatibility and mechanical stability, supporting its widespread use in biomedical applications.^[65] In summary, animal-derived polymers are gaining increasing attention as versatile and processable building blocks for the development of high-performance biomaterials.

Bio-based composite films

In recent years, the development of bio-based polymers derived from renewable agricultural resources and biomass has emerged as a vital strategy for constructing environmentally friendly and sustainable material systems.^[66,67] These materials offer excellent biocompatibility and biodegradability, demonstrating significant advantages in the field of green materials and showing potential to replace conventional petroleum-based polymers.^[68,69] However, the inherent limitations of bio-based polymers, such as poor mechanical strength, low thermal stability, and inadequate moisture resistance, have restricted their widespread adoption and high-performance applications in engineering fields.^[70,71] To address these challenges, bio-based composite materials have been proposed as an effective approach to enhancing the performance of biopolymers.^[72] These composites typically consist of biopolymeric matrices combined with either biodegradable or non-biodegradable polymeric substrates, selected based on the requirements of specific application scenarios.^[73]

Petroleum-based polymers have long dominated the membrane materials market due to their favorable cost-performance ratio and excellent thermal and chemical stability.^[74] However, growing environmental concerns have driven researchers to explore more sustainable alternatives, positioning bio-based composite films as promising candidates for the next generation of high-performance membranes.^[75,76] By integrating natural polymers with reinforcing components through rational design, their physical, chemical, and mechanical

properties can be significantly improved, resulting in enhanced structural stability and functional tunability.^[77–79] This composite strategy not only improves the applicability of biopolymers but also lays a foundation for the scalable development of functional film materials.^[80]

Physical and Chemical Interactions in Biopolymer Composite Films

In the construction of bio-based composite films, the interaction mechanisms between different components play a pivotal role in determining their microstructure, interfacial adhesion, and overall macroscopic performance.^[81,82] These interactions are generally categorized into physical interactions and chemical crosslinking, with the former providing the structural foundation and the latter offering enhanced stability and tunability.^[83,84] Physical interactions primarily include hydrogen bonding, electrostatic forces, and van der Waals interactions, etc.^[85] In contrast, chemical crosslinking involves the formation of covalent bonds between polymer chains, constructing highly stable and controllable network structures.^[86] Permanent covalent crosslinking forms dense three-dimensional networks that endow the films with excellent mechanical strength, thermal and dimensional stability, and resistance to chemical and environmental degradation.^[87] However, the irreversible nature of such structures—being insoluble, infusible, and difficult to recycle—poses significant limitations in the context of sustainability. Recently, the emergence of covalent adaptable networks (CANs), mediated by dynamic covalent bonds (DCBs), has offered promising solutions.^[88] These systems maintain structural integrity while allowing reversible crosslinking, enabling properties such as self-healing, recyclability, and reprocessability, and expanding their potential in the field of green and intelligent materials.^[89] Overall, rational design of both physical and chemical interaction pathways allows for the synergistic optimization of structure and performance in bio-based composite films.

Preparation technology of composite films

In laboratory settings, solvent casting is a fundamental and widely employed technique for fabricating polymer films.^[90] This method involves pouring a polymer solution onto a substrate, followed by solvent evaporation through oven drying, vacuum drying, or ambient conditions, ultimately yielding a freestanding film that can be peeled off from the mold.^[91] Commonly used solvents include water, alcohols, and various organic solvents.^[92] The method is favored for its simplicity, accessibility of equipment, low energy consumption, and cost-effectiveness, making it particularly suitable for processing a wide range of bio-based polymers.^[93,94]

In addition to solvent casting, electrospinning has emerged as an important technique for the fabrication of functional films, especially those with nanofibrous structures and porous morphologies.^[95] This method employs a high-voltage electric field to draw polymer solutions into continuous fibers, during which the solvent rapidly evaporates, forming solid nanofibers with high surface area, elevated porosity, and excellent adsorption capacity.^[96,97] Compared to conventional film-forming methods, electrospinning offers ease of operation, low cost, and strong drug-loading capacity, while its cleanliness and safety largely depend on the choice of solvent.^[98–100] The increasing adoption of water as a green solvent in recent years further supports the advancement of sustainable electrospinning techniques.^[101]

Polyvinyl alcohol (PVA)

Polyvinyl alcohol (PVA) is a water-soluble and non-toxic synthetic polymer known for its excellent biocompatibility, film-forming ability, and high tensile strength.^[102,103] It has been widely applied in bio-based composite films, where it serves as an effective reinforcing component, significantly enhancing the overall material performance.^[104,105] As illustrated in **Figure 5**, PVA is produced through the hydrolysis of polyvinyl acetate (PVAc). During the

alcoholysis process, partial hydrolysis of PVAc yields PVA, whose physicochemical properties depend primarily on the degree of polymerization and, most critically, the degree of hydrolysis. By precisely controlling the preparation process, PVA resins with varying hydrolysis and polymerization degrees can be synthesized. Common commercial grades include PVA-105, PVA-117, and PVA-217, where the first digit denotes the hydrolysis level and the following two digits indicate the degree of polymerization.^[107]

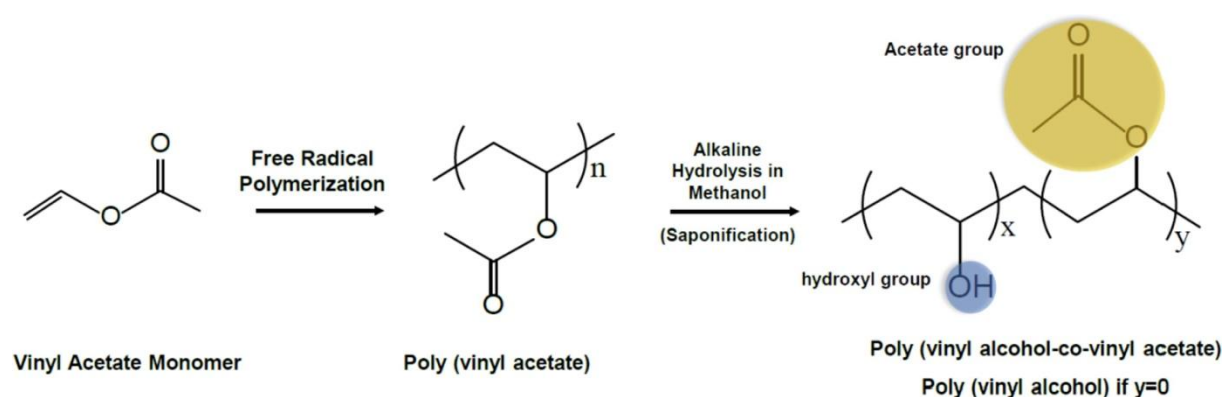


Figure 5. Schematic illustration of the synthesis route and chemical structure of PVA.^[106]

Moreover, the abundant hydroxyl groups in PVA confer strong polarity and chemical reactivity, enabling it to form stable composite structures with various natural polymers via hydrogen bonding or chemical crosslinking.^[108,109] These interactions not only significantly enhance the mechanical strength and water resistance of the resulting materials but also make PVA an ideal matrix for reinforcing the performance of natural macromolecules.^[110–112] The synergistic integration of PVA with biopolymers effectively compensates for the structural and functional limitations of the latter, facilitating the development of sustainable, high-performance materials for critical applications such as smart packaging, biodegradable plastics, and biomedical devices.^[113,114]

Outline of this dissertation

In this dissertation, high-performance bio-based composite materials were developed by constructing composite systems reinforced with poly(vinyl alcohol). Three reinforcement strategies were employed: hydrogen bonding (Chapter 1), covalent crosslinking (Chapter 2), and a dual-interaction mechanism (Chapter 3), to achieve synergistic enhancement in mechanical properties, water stability, and functional performance. The practical potential of the resulting materials in packaging and biomedical applications was also evaluated.

Chapter 1

In Chapter 1, plant-derived starch was utilized to fabricate PVA/starch blend films. PVA with varying degrees of saponification (DS) and polymerization (DP) was combined with two types of modified starch—hydroxypropyl starch (HPS) and octenyl succinate starch (OctS)—via hydrogen bonding (**Figure 6**). The films were prepared using a solution casting method, and their overall performance was systematically assessed. The synergistic effects of PVA's DS and DP on blend compatibility were investigated, along with a comparative analysis of the film-forming behaviors of HPS and OctS. Additionally, the potential of these films for sustainable packaging was demonstrated, offering new insights into eco-friendly material development.

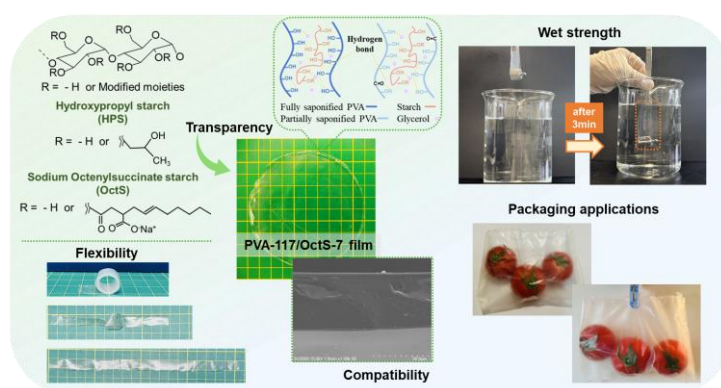


Figure 6. Graphical abstract of Chapter 1.

Chapter 2

In Chapter 2, microbially derived γ -polyglutamic acid (γ -PGA) was used to prepare γ -PGA/acetoacetylated PVA (AAPVA) composite films (**Figure 7**). γ -PGA was first grafted with adipic dihydrazide (ADH) to introduce reactive hydrazide groups, which subsequently formed stable acylhydrazone bonds with AAPVA via a Schiff base reaction, resulting in a recyclable thermoset film fabricated through solution casting. The resulting crosslinked network significantly improved the performance and environmental sustainability of γ -PGA-based materials, highlighting their potential as viable alternatives to conventional thermoset plastics.

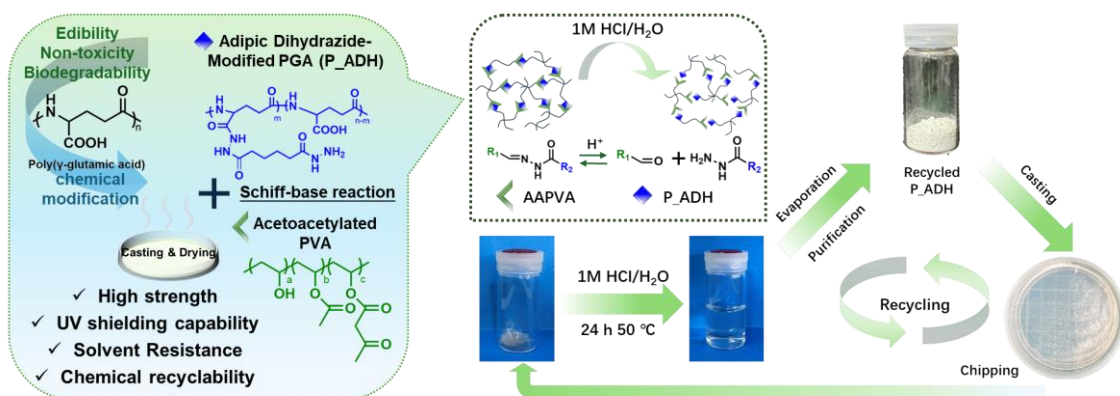


Figure 7. Graphical abstract of Chapter 2.

Chapter 3

In Chapter 3, animal-derived chitosan was crosslinked with AAPVA and combined with nitrogen-doped carbon quantum dots (CQDs) synthesized from hydroxypropyl starch (**Figure 8**). Using electrospinning technology, a pH-responsive, non-cytotoxic nanofibrous wound dressing was fabricated. The resulting CQDs/CS/AAPVA nanofibers exhibited high mechanical strength and wet-state stability, along with clear optical responsiveness across the pH range of 5–8, providing a smart and environmentally friendly platform for chronic wound management.

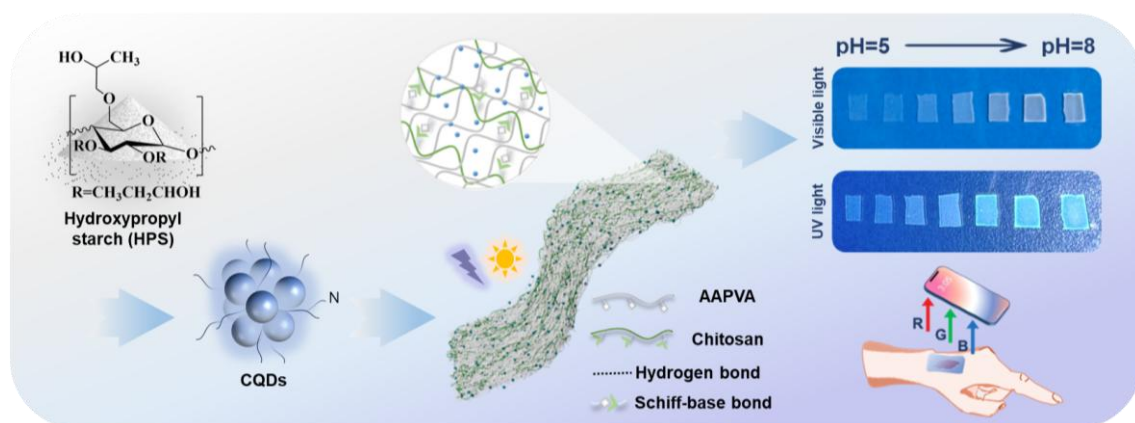


Figure 8. Graphical abstract of Chapter 3.

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Chapter 1

Enhanced physical properties and water resistance of films using modified starch blend with poly(vinyl alcohol)

1.1. Introduction

The petrochemical industry's development has resulted in significant waste production.^[1,2] It is imperative to explore renewable and biodegradable alternatives.^[3,4] To achieve this objective, researchers are dedicating efforts towards developing biodegradable polymers sourced from either natural resources or synthetic processes.^[5–7] However, materials composed solely of one type of biomass polymer often encounter practical limitations. In contrast, composites created by blending two or more polymers are emerging as promising substitutes for petroleum-based packaging materials.^[8,9] This approach improves physical properties, making composite materials more suitable for a variety of applications.^[10–12]

Starch is an extensively investigated and promising biomass material, owing to its cost-effectiveness, abundance, and biodegradability.^[13,14] Despite this, natural starch-based films' inadequate mechanical properties, high rigidity, and limited water resistance limit their application in materials engineering.^[15,16] Packaging films produced by natural starch paste possess weak water resistance, dissolving in minutes.^[17] It is crucial to address these issues urgently to develop viable starch films. PVA is a hydrophilic synthetic polymer that exhibits non-toxicity and distinguishes itself with remarkable tensile strength and superior film-forming capabilities.^[18,19] Research has shown that blending PVA and starch can enhance the physical characteristics of blend films.^[20,21] CA Gómez-Aldapa et al.^[22] demonstrated that incorporating PVA enhances the functional properties of blend films by forming intermolecular and intramolecular hydrogen bonds. However, the numerous hydroxyl groups present in the

structure of both PVA and starch contribute to the composites' poor water resistance, significantly limiting their potential applications in the packaging industry.^[23,24]

Common effective strategies for interface optimization in PVA/starch composites typically involve chemical or physical modifications during or after the blending process. These modifications may include acid treatments, crosslinking reactions, surface modifications, or the addition of nanoparticles to the blend.^[25,26] In previous studies on interface optimization of PVA/starch composites, researchers have employed glutaraldehyde, boric acid, and triethylamine to form chemical cross-linking structures with PVA and starch, thus enhancing mechanical strength and water resistance.^[27–29] However, potential hazards and toxicity have limited their large-scale application. Additionally, inorganic fillers like titanium dioxide, silver nanoparticles, and graphene oxide were utilized to enhance the characteristics of PVA/Starch composite films.^[30–32] Nonetheless, these fillers may introduce impurities and non-uniform structures, leading to increased light scattering and absorption, negatively impacting optical transparency and potentially compromising film safety. Thus, developing PVA/Starch blend films with excellent tensile strength, optical properties, flexibility, and water resistance is still a significant challenge.

The solubility, mechanical characteristics, crystallinity, and thermal properties of PVA are greatly influenced by its DS and DP, which consequently affects the blends' overall performance.^[33,34] N. Limpan et al.^[35] prepared and characterized biodegradable composite films made of fish myofibrillar protein and PVA, showing that PVA with higher molecular weight improved the tensile properties by enhancing intermolecular interactions with protein molecular chains. Similarly, J. Zanela et al.^[36] developed PVA/Starch sheets using five PVA with varying molecular weights and levels of hydrolysis via extrusion and calendering. The sheets made with highly saponified PVA were stiffer, while those produced with a high molecular weight PVA had greater durability. This suggests that the type of PVA can further influence its performance

in various applications. However, few studies have been conducted to investigate the synergistic effects of various DS and DP in PVA/Starch blends on the physical properties and water resistance of the films.

Besides, modified starch plays an essential role in developing starch-based films by introducing new functional groups that promote effective film formation and properties.^[37,38] HPS and OctS, widely utilized in industries, serve as representative examples.^{[77,78][39,40]} HPS, a stabilized starch obtained through the reaction between propylene oxide and starch granules under alkaline conditions, incorporates hydroxypropyl groups. These groups promote hydrogen bonding, which improves the interfacial adhesion of HPS, thereby increasing compatibility with PVA.^[41] On the other hand, OctS is produced by esterifying granular starch with octenyl succinic anhydride, introducing hydrophobic long-chain octenyl succinate groups onto the hydrophilic starch molecules. This modification confers surface activity and reduces the hydrophilicity of the starch, aiming to enhance the water resistance of the films through hydrophobic interactions with PVA.^[42,43] Notably, previous research has primarily focused on blends of a single type of PVA and starch. Therefore, while optimizing the synergistic effects of PVA with different DS and DP on PVA/starch films, we will also explore and improve the performance of the blend films using these two industrial starches with distinct substituent groups. Additionally, glycerol is added as a plasticizer to enhance compatibility and flexibility.^[44]

In this study, PVA/Starch blend films with varying ratios were fabricated by solution casting method, utilizing PVA with different DS and DP. For the first time, the physical properties and water resistance of HPS and OctS when blended with PVA were comprehensively evaluated. Various characterization tests, including mechanical tests, optical analysis, thermal analysis, water contact angle measurement, and swelling tests, were employed to identify optimal combinations, leading to blend films with superior overall performance.

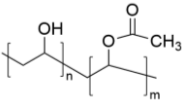
Moreover, the potential application of PVA/Starch blend films as packaging materials was demonstrated. This research aims to guide the selection of suitable PVA/Starch combinations, providing new insights for developing starch films with superior mechanical properties and water resistance.

1.2. Experimental Section

1.2.1. Materials

Four types of PVA with different DS (fully saponified and partially saponified) and polymerization were obtained from Kuraray Co. Ltd., Japan (details summarized in **Table 1-1**), namely PVA-105 (Grade: 5-98, purity $\geq 94\%$, Viscosity (4%, 20°C) $\text{mPa}\cdot\text{s} = 5.2 \sim 6.0$), PVA-117 (Grade: 28-98, purity $\geq 94\%$, Viscosity (4%, 20°C) $\text{mPa}\cdot\text{s} = 25.0 \sim 31.0$), PVA-205 (Grade: 5-88, purity $\geq 94\%$, Viscosity (4%, 20°C) $\text{mPa}\cdot\text{s} = 4.6 \sim 5.4$), PVA-217 (Grade: 22-88, purity $\geq 94\%$, Viscosity (4%, 20°C) $\text{mPa}\cdot\text{s} = 20.5 \sim 24.5$). Glycerol was purchased from Nacalai Tesque Inc., Japan. The HPS (propylene chlorohydrin compounds: less than 1.0 ppm, a degree of hydroxypropyl substitution of 20.0 %) and sodium octenyl succinate starch (residual octenyl succinic acid: 0.8% or less, a degree of octenyl succinic anhydride substitution of 3.0 %) were obtained from Matsutani Chemical Industry Co. Ltd., Japan.

Table 1-1. Properties of PVA used in this study.

	PVA type	Viscosity (4%, 20°C) $\text{mPa}\cdot\text{s}$	Purity %	DS (% mol)	DP	Molecular structure
Fully saponified	PVA-105	5.2 ~ 6.0	Above 94.0	98.0– 99.0	500	
	PVA-117	25.0 ~ 31.0	Above 94.0	98.0– 99.0	2800	
Partially saponified	PVA-205	4.6 ~ 5.4	Above 94.0	86.5– 89.0	500	
	PVA-217	20.5 ~ 24.5	Above 94.0	87.0– 89.0	2200	

1.2.2. Fabrication of the PVA/Starch blend films using different PVAs and modified starches

At first, 8 mL of deionized water at 85°C was used to dissolve PVA, which was constantly swirled until it was fully dissolved. To get a homogenous solution, starch was separately dispersed in 8 mL of deionized water and heated to 95°C for 30 minutes to fully gelatinize the starch particles, resulting in a homogeneous solution. After that, the mixture was cooled to room temperature and stirred. Then, by combining the PVA and starch solution in ratios of 3:7, 5:5, and 7:3 (w/w), several batches of starch and PVA composite solutions were created at 90°C while being stirred for 1 h. The high temperature stirring helps improve compatibility between PVA and starch, promoting better molecular interactions. Glycerol was used as the plasticizer, accounting for 30% (w/w) of the entire solid weight. The mixture was transferred into a Petri dish (Φ100 mm) while left overnight at room temperature. Ultimately, the combined mixture was dried for a full day at 50°C in an oven to produce dry blend films.

Figure 1-1 and **Table 1-2** represent the film preparation and composition.

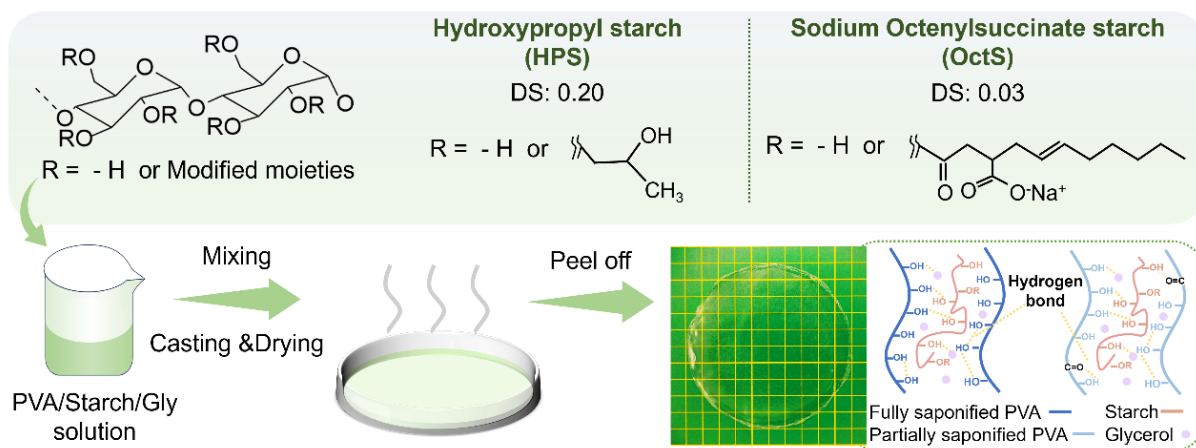


Figure 1-1. Illustration of film preparation and the hydrogen interaction formed by PVA, starch, and glycerol.

Table 1-2. Properties of PVA used in this study.

Sample	PVA (g)	Starch (g)	Glycerol (g)	Water (mL)
PVA	0.80	/	/	16.0
Starch	/	0.80	/	16.0
PVA/Gly	0.80	/	0.24	16.0
Starch/Gly	/	0.80	0.24	16.0
Starch/Gly	/	0.80	0.24	16.0
PVA/starch-3	0.24	0.56	0.24	16.0
PVA/starch-5	0.40	0.40	0.24	16.0
PVA/starch-7	0.56	0.24	0.24	16.0

1.2.3. Water resistance and dissolution behavior

Measurements of contact angles were made using a DropMaster DM300 contact angle meter (Drop Master DM300, Kyowa Interface Science, Saitama, Japan). On the surface of the sample, a 0.5 μ L droplet of deionized water was applied. For each sample, the contact angles were measured three times. Ten seconds after the droplets were deposited, the reported values were recorded.

The water resistance and dissolution behaviors of the films (~ 1 cm $\times$ 1 cm) were determined by immersion in 5 mL deionized water and different pH solutions (4, 7, and 9) at 25°C. After 24 h, the dissolved samples were carefully extracted with tweezers, and any extra water was wiped off via thin paper before weighing. After three tests were conducted on each film, the swelling ratio (SR) was computed by **Eq. (1-1)**.^[45]

$$SR = \frac{W_{ss} - W_{ds}}{W_{ss}} \times 100 \quad (1-1)$$

where W_{ds} and W_{ss} represent the weights of the original and swelling samples, respectively.

1.2.4. Characterization

FT-IR spectroscopy measurements of the materials in the 500-4000 cm⁻¹ wavelength were carried out with a Nicolet iS5 FTIR spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). For each spectrum, 20 scans were coadded.

Differential scanning calorimetry (DSC) was performed using a differential scanning calorimeter (Hitachi High-Tech Science Co., Tokyo, Japan) under a nitrogen flow at a heating rate of 10 °C/min over a temperature range of 0 to 250 °C. First, the sample (5 mg) was heated from room temperature to 250 °C at the selected heating rate and maintained at 250 °C for 2 minutes to eliminate thermal history. The sample was then cooled to 0 °C and reheated to 250 °C. Data were recorded during the cooling and second heating steps. The crystallinity degree of PVA was calculated as shown in **Eq. (1-2)**.^[46]

$$X_c = \frac{1}{W_{PVA}} \times \left[\frac{\Delta H_m}{\Delta H_0} \right] \times 100\% \quad (1-2)$$

where ΔH_m , is the melting enthalpy of the sample, ΔH_0 , the melting enthalpy of a 100% crystalline PVA sample (138.6 J·g⁻¹)^[47] and W_{PVA} , the mass fraction of PVA in the film.

A spiral micrometer was employed to measure the films' thickness with a precision of 0.0001 mm. Each sample's average thickness was determined by taking measurements at three randomly chosen locations.

A tensile test was conducted on the films to assess their mechanical properties. A 10-N loading cell was used in a universal testing machine (EZ Graph, Shimadzu Co., Kyoto, Japan) at the strain rate of 20 mm/min. Before the test, the film thicknesses were measured using a micrometer. Three different locations were used to collect measurements for each sample, and the average value was used for computation.

The transparency of the films used in this experiment was measured with a wavelength range of 200 to 800 nm by utilizing an ultraviolet spectrometer (U-2810, Hitachi, Tokyo, Japan).

The data were collected and evaluated using the UV solution 2.0 software. The film's transparency was calculated by **Eq. (1-3)**.^[48]

$$\text{Transparency value} = -\frac{(\log T_{600 \text{ nm}})}{d} \quad (1-3)$$

where the film thickness (mm) is represented by d and the transmittance at 600 nm is T_{600} . Lower transparency is indicated by a larger transparency value, and this is correlated with the tested film sample's lower transmittance.

The film's haze was measured through a haze meter (Haze meter NDH 4000, Nippon Denshoku Kogyo Co. Ltd.). During the experiment, the blank medium was the air.

The SU3500 scanning electron microscope (Hitachi High-Technologies Corp., Tokyo, Japan) was utilized to acquire the surface and cross-section morphologies of the films. The films were quickly frozen in liquid nitrogen when they were dry, broken up right away, and then vacuum-dried. In advance of observation, the samples underwent gold sputtering on the MSP-1S magnetron sputter (Vacuum Device Inc., Osaka, Japan).

With the use of a thermogravimetric analysis device (TGA, Hitachi TG/DTA7200, Tokyo, Japan), the samples' thermal stability curves were recorded. The samples were heated at a rate of 10°C per minute while being analyzed in a nitrogen atmosphere with temperatures ranging from 30 to 600°C.

1.3. Results and Discussion

1.3.1. Fabrication and characterization of PVA/starch blend films

The incorporation of PVA along with glycerol markedly enhanced film-forming capabilities, yielding transparent blend films characterized by smooth surfaces and increased flexibility (**Figure 1-2a**). FT-IR spectroscopy is used to characterize and analyze the properties of the blend films, revealing a significant impact from the interactions among PVA, starch, and plasticizer molecules. The asymmetric stretching vibrations of the -OH groups contribute to the broad bands seen in the $3600\text{--}3000\text{ cm}^{-1}$ range of the pure PVA FT-IR spectrum (**Figure 1-3**).^[49] Additionally, -CH₂ asymmetric and symmetric stretching vibrations, and C-H and C-O stretching vibrations are identified as absorption peaks around 2930–2960, 2905, 1430–1420, and $1080\text{--}1100\text{ cm}^{-1}$, respectively.^[50–52] For PVA-205 and 217, the presence of bands near 1730 cm^{-1} refers to carbonyl groups, indicative of residual acetate groups from partial saponification during manufacturing.^[53] The Starch/Gly spectra exhibit characteristic broad bands for OH

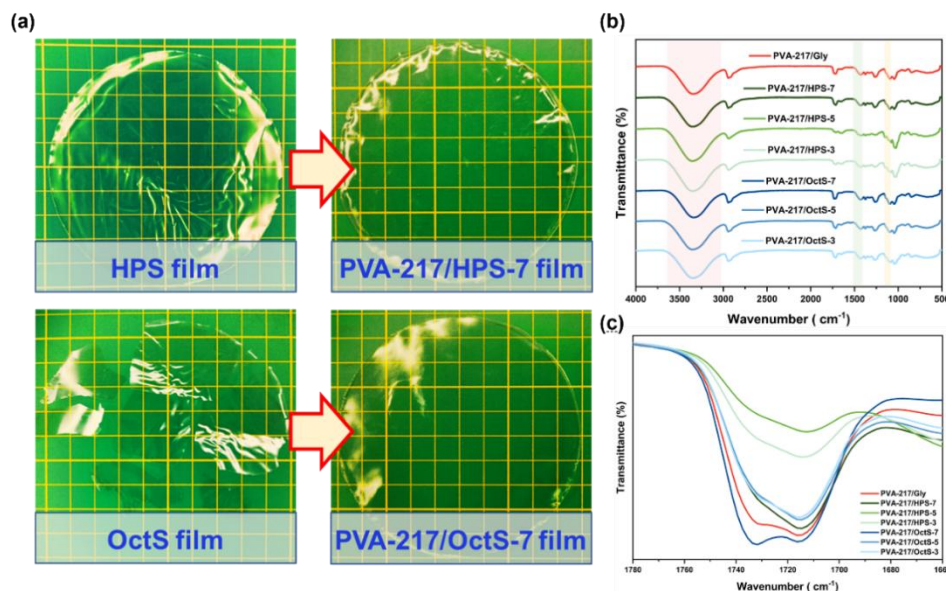


Figure 1-. (a) Digital photo comparison of pure starch and PVA/Starch-7 blend films. (b) FT-IR spectra of PVA-217 series films in the wavenumber range of 500–4000 nm; (c) the enlarged FT-IR spectra of PVA-217 series films in the wavenumber range of 1660–1780 cm^{-1} .

stretching vibrations around 3330 cm^{-1} and -OH deformation bending near 1640 cm^{-1} .^[54] The peaks at 1150 , 1080 , and 1022 cm^{-1} are distinctly associated with the C-O stretching vibrations of the glucose ring structure.^[55] Differing from HPS, OctS exhibits two unique bands at 1730 cm^{-1} and 1580 cm^{-1} , assigned to the carboxyl group resulting from starch modification.^[56] In this modification, the hydroxyl group on the starch molecule is substituted with a carboxyl group in OSA, leading to the observed C=O stretching vibration.^[57]

Taking PVA-217 as an example (**Figure 1-2b**), the stretching vibration at 3330 cm^{-1} moved to a higher wavenumber (3342 cm^{-1}) in this spectrum of PVA-217/Gly blend film compared to that of pure PVA-217. This shift implies the introduction of glycerol interferes with certain original hydrogen bonds between PVA molecules, resulting in the hydroxyl groups' absorption peaks shifting blue.^[58]

The spectra of the PVA-217/OctS blend film

revealed a shift in the bands corresponding to the characteristic peaks of -OH stretching with changes in starch and PVA content. At a composition ratio of 7:3, the concentration of hydroxyl groups within the PVA/Starch blend films increases, potentially resulting in enhanced intermolecular hydrogen bonding of PVA. In such cases, the hydrogen bonding network within PVA molecules could be denser compared to that between PVA and starch or glycerol. This more tightly-knit network of hydrogen bonding could trigger a redshift in the peaks corresponding to hydroxyl groups, indicating a decrease in vibrational energy for these groups.^[59] Furthermore, the absorption peak near 1730 cm^{-1} assigned to the carbonyl group in blend films exhibits a noticeable shift and intensity reduction (**Fig. 1-2c**), which corresponds to the observed shift in the hydroxyl group's characteristic stretching band. This phenomenon is

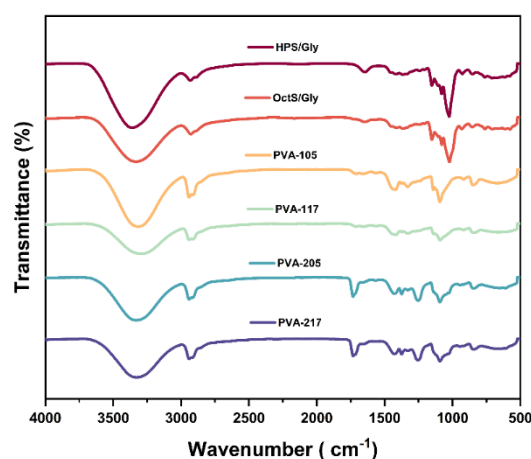


Figure 1-3. FTIR spectra curves of
(a) Starch/Gly and PVA films.

consistently observed across different formulations of PVA blend films, confirming interactive dynamics among PVA, starch, and glycerol.

1.3.2. Thermal stability of PVA/starch blend films

Figure 1-4 presents the DSC heating and cooling thermograms of the blends. The DSC thermogram of starch does not exhibit melting and crystallization peaks, whereas PVA and PVA/starch blends show clear melting and crystallization peaks. As the starch content in the blend increases, both the melting and crystallization enthalpies decrease along with the corresponding melting and crystallization temperatures. The crystallization temperature (T_c), crystallization enthalpy (ΔH_c), melting temperature (T_m), melting enthalpy (ΔH_m), and degree of crystallinity for the PVA/starch blends are summarized in **Table 1-3**. The melting peak of pure PVA (PVA-217 film) is prominent, indicating a relatively high degree of crystallinity. In the PVA-217/Gly film, compared to pure PVA-217, the exothermic peak is weaker, suggesting that glycerol may inhibit the crystallization of PVA. This is due to the plasticizing effect of glycerol, which reduces the interactions between PVA chains, making it more difficult for them to arrange into an ordered crystalline structure.^[60]

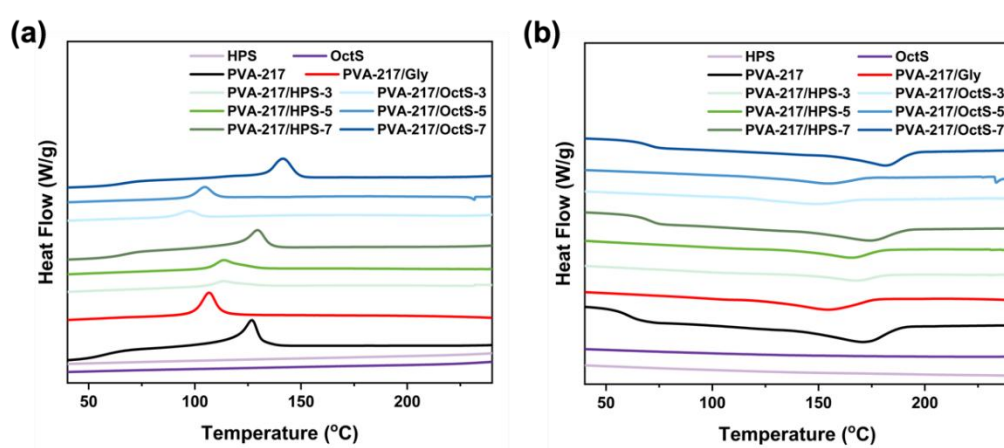


Figure 1-4. FTIR spectra curves of (a) Starch/Gly and PVA films.

With the addition of starch, certain trends in PVA's crystallization behavior are observed. As the starch content increases, the T_m decreases. The degree of crystallinity drops from 22.2% to 17.4% (PVA-217/HPS series films) and from 22.0% to 18.0% (PVA-217/OctS series films). This could be attributed to the inhibitory effect of starch on PVA crystallization, as the amorphous structure of starch disrupts the regularity of the polymer matrix. For films with similar starch and PVA ratios, a similar inhibitory effect of starch on PVA crystallization can be observed. Conversely, when the PVA content is highest, the degree of physical entanglement between the polymer chains is maximized, and the synergistic interactions between PVA and starch are stronger, leading to an increase in T_m and crystallinity.

Table 1-3. Surface area, product yield, and elemental composition of samples carbonized at different temperatures.

	T_g (°C)	T_c (°C)	ΔH_c (J g ⁻¹)	T_m (°C)	ΔH_m (J g ⁻¹)	X_c (%)
HPS	/	/	/	/	/	/
OctS	/	/	/	/	/	/
PVA-217	70.5	126.9	29.2	170.9	31.1	22.4
PVA-217/Gly	/	106.7	18.3	154.6	20.9	19.6
PVA-217/H-7	84.2	129.5	18.1	174.0	18.4	22.2
PVA-217/H-5	/	113.8	10.4	165.6	11.4	21.4
PVA-217/H-3	/	113.8	5.21	167.5	5.55	17.4
PVA-217/O-7	81.0	141.2	16.6	181.1	18.3	22.0
PVA-217/O-5	/	104.4	7.34	154.7	10.4	19.5
PVA-217/O-3	/	97.5	3.92	148.8	5.76	18.0

Regarding the glass transition temperature (T_g), the T_g of PVA-217/HPS-7 and PVA-217/OctS-7 blends is 84.2°C and 81.0°C, respectively, both higher than that of pure PVA-217 (70.5°C). This indicates that, at these ratios, the interactions between starch molecules and PVA are enhanced, resulting in a more pronounced blending effect. In contrast, for blends with higher

starch content, a clear T_g could not be observed, likely due to the amorphous and hygroscopic nature of starch [25].

To investigate the thermal stability and degradation behavior of PVA/Starch blend films at different ratios, thermogravimetric analysis was performed on pure PVA, PVA/Gly, and blend films. As shown in **Figure 1-5**, the thermal degradation diagrams of blend films demonstrated four distinct stages of degradation within the respective degradation ranges of $<120^\circ\text{C}$, $150\text{--}250^\circ\text{C}$, $250\text{--}350^\circ\text{C}$, and $>350^\circ\text{C}$. The loss of bound and adsorbed water in blend films is represented by the first weight-loss stage. The breakdown of the glycerol-rich phase is attributed to the following weight loss phase. Upon reaching a temperature of 250°C , a third stage of weight loss occurs primarily due to the thermal decomposition process involving PVA and starch, which are by-products, in addition to carbonaceous matter decomposition. PVA concentration in the film has an impact on weight loss; a higher PVA content causes less weight loss. The fourth stage, which happens at temperatures over 350°C , is the carbonization of the organic material. However, for the PVA/Starch-7 films, no distinct correlation between each PVA grade and the thermogravimetric curves was observed as all curves exhibited similarity. This observation indicates that despite the higher weight ratio of PVA compared to starch (7:3), variations in DS and DP have no significant effect on the thermal properties of films blended with HPS or OctS.

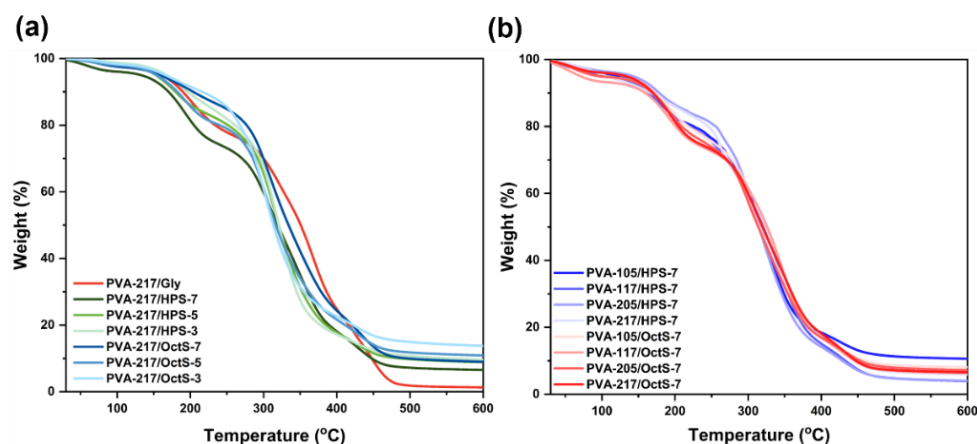


Figure 1-5. Thermal analysis of the (a) PVA-217 series films; and (b) PVA/Starch-7 blend films.

1.3.3. Mechanical properties of PVA/starch blend films

Through investigating the component ratios within the PVA/Starch blend films, the mechanical properties of the films were studied. PVA-217 was selected to evaluate how varying PVA concentrations influence the attributes of these blend films (**Figure 1-6a**). The TS and flexibility of HPS and OctS films, which are typically too fragile and brittle in practical usage, can be enhanced by incorporating PVA and glycerol into the film matrix, as demonstrated in **Table 1-4**. The integration of glycerol into the PVA-217/Gly films resulted in a transition towards more pliable and ductile mechanical properties, marked by a notable increase in elongation but a reduction in TS.^[61] This change was ascribed to increased molecular chain activity and the breaking of intermolecular hydrogen bonds between PVA molecules. When starch was added, the films' TS and EAB drastically decreased because the interactions between the polymer chains were reduced. From the morphological characteristics at the fracture sites in the strain-stress curves shown in **Figure 1-7**, it can be observed that the increase in PVA content significantly improves the ductility and plastic fracture behavior of the blend films. In both PVA/OctS-7 and PVA/HPS-7 blend films, smoother fracture surfaces were observed, indicating better ductility. Additionally, the higher crystallinity enhances the mechanical properties. As the starch content gradually increases, the fracture surfaces of PVA/HPS-5 and

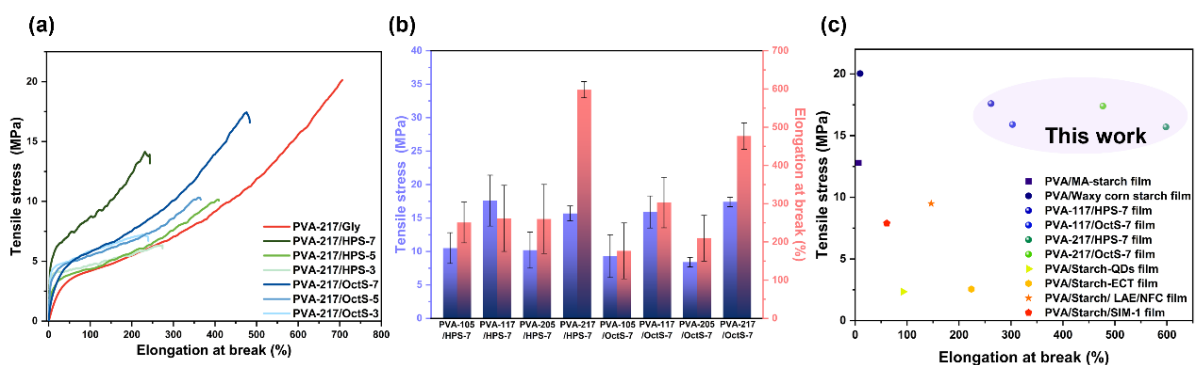


Figure 1-6. (a) Mechanical property by the strain-stress curve of the PVA-217 series films; (b) TS and EAB of PVA/Starch-7 blend films; (c) Ashby plot in terms of TS vs EAB of PVA/Starch-7 films and the previously reported PVA/Starch blend films.

PVA/HPS-3 films show more rough structures and cracks, indicating that the presence of starch restricts the material's ductility, reduces the crystallinity, and leads to weak mechanical properties.

In **Figure 1-6b**, the tensile properties of the blend films prepared from various components were compared, and the TS of the PVA/Starch films with various DS was equivalent. The tensile properties of the blend films prepared from various components were compared, and the TS of the PVA blend films with different DS was similar. Still, the EAB difference was larger, and the same trend as pure PVA and PVA/Gly, a series of films prepared with PVA with a large DP had a larger EAB. The influence of PVA on the overall tensile properties was smaller when the starch content was higher. At this ratio of PVA/Starch-7, PVA dominates, and the blend films prepared from PVA-105 and PVA-205 with the same DP exhibit different TS and EAB. Nevertheless, the differences are small, whereas from the TS of the blend films prepared from PVA-105 and 117, and PVA-205 and 217 with the same DS it can be seen

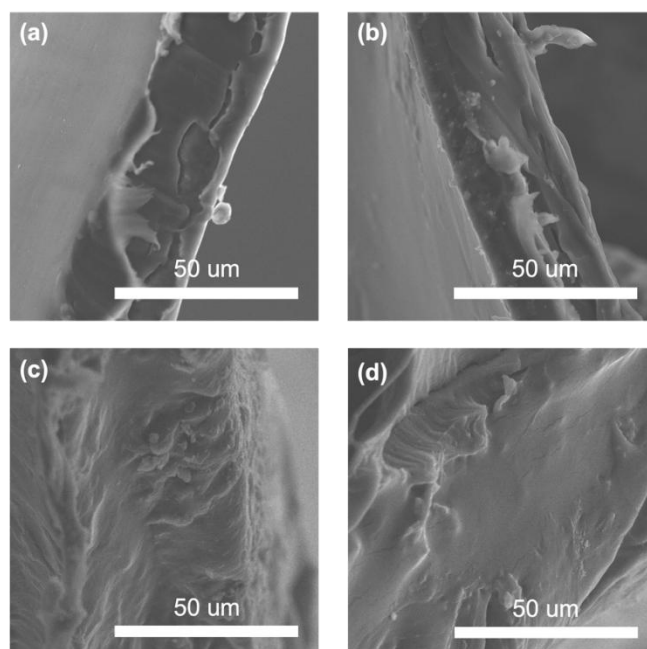


Figure 1-7. SEM micrographs of the cleavage sites for the strain-stress curves of (a) PVA-217/OctS-7 film; (b) PVA-217/HPS-7 film; (c) PVA-217/HPS-5 film; (d) PVA-217/HPS-3 film.

that the blends of PVA with higher DP (PVA-117 and 217) with starch have higher TS and EAB. In this case, the DP increases the relative molecular mass of PVA, thereby increasing the intermolecular forces between PVA and starch.^[23] Despite different substituents influencing their properties, the two types of starch exhibited similar tensile properties in the blend films. As seen in **Figure 1-6c**, it is noteworthy that the PVA/Starch-7 films studied here have tensile properties that are superior to the majority of PVA/Starch blend films documented in the literature that were produced by the solution casting method.^[62–67]

Table 1-4. Thickness(μm), Tensile strength (MPa), Elongation at break (%), Optical Transparency (%) (at 600 nm), and Haze values (%) of the PVA/Starch blend films.

Films	Thickness (μm)	Tensile strength (MPa)	Elongation at break (%)	Optical Transparency (%) (at 600 nm)	Haze values (%)
PVA-105/HPS-3	58.3	5.1 ± 0.5	55.1 ± 23.1	3.8 ± 0.4	51.8
PVA-117/HPS-3	62.8	6.4 ± 0.3	211.3 ± 84.7	3.3 ± 0.5	27.8
PVA-205/HPS-3	67.3	3.9 ± 0.6	99.7 ± 13.7	2.5 ± 0.3	39.2
PVA-217/HPS-3	60.8	6.1 ± 0.6^f	297.3 ± 41.9	3.0 ± 1.4	22.1
PVA-105/HPS-5	78.3	6.0 ± 1.0	32.9 ± 11.5	3.2 ± 0.3	37.1
PVA-117/HPS-5	78.7	10.8 ± 0.4	257.5 ± 4.6	2.7 ± 0.5	7.6
PVA-205/HPS-5	94.4	4.1 ± 0.5	110.0 ± 34.1	2.7 ± 0.2	46.5
PVA-217/HPS-5	71.8	9.8 ± 0.5	390.7 ± 44.2	3.2 ± 1.0	29.5
PVA-105/HPS-7	54.3	10.5 ± 2.3	250.8 ± 53.1	1.9 ± 0.2	7.3
PVA-117/HPS-7	59.0	17.6 ± 3.8	261.7 ± 86.5	2.4 ± 0.3	12.9
PVA-205/HPS-7	73.3	10.2 ± 2.7	260.0 ± 90.2	2.3 ± 0.5	20.4
PVA-217/HPS-7	61.8	15.7 ± 1.1	598.1 ± 21.2	2.1 ± 0.3	10.6
PVA-105/OctS-3	63.0	5.2 ± 1.6	68.2 ± 17.4	5.4 ± 1.0	39.2
PVA-117/OctS-3	61.2	4.8 ± 0.6	165.5 ± 82.1	4.4 ± 1.8	42.0
PVA-205/OctS-3	76.5	4.8 ± 0.8	58.6 ± 45.4	1.2 ± 0.1	10.2
PVA-217/OctS-3	71.5	6.4 ± 0.9	209.5 ± 70.0	1.5 ± 0.3	11.9
PVA-105/OctS-5	76.7	6.2 ± 0.9	121.1 ± 24.9	2.8 ± 1.1	37.1

PVA-117/OctS-5	88.5	8.6 $\pm$ 2.0	234.6 $\pm$ 62.9	2.3 $\pm$ 0.3	30.4
PVA-205/OctS-5	74.3	4.3 $\pm$ 0.2	168.8 $\pm$ 22.0	2.0 $\pm$ 0.1	29.2
PVA-217/OctS-5	73.6	10.0 $\pm$ 1.3	410.0 $\pm$ 60.6	1.5 $\pm$ 0.4	6.5
PVA-105/OctS-7	74.9	9.3 $\pm$ 3.2	176.6 $\pm$ 72.9	2.8 $\pm$ 1.3	16.2
PVA-117/OctS-7	84.0	15.9 $\pm$ 2.4	302.9 $\pm$ 66.1	3.0 $\pm$ 1.0	15.4
PVA-205/OctS-7	88.2	8.4 $\pm$ 0.7	209.7 $\pm$ 59.7	1.8 $\pm$ 0.4	46.3
PVA-217/OctS-7	66.1	17.4 $\pm$ 0.7	476.8 $\pm$ 34.5	1.8 $\pm$ 0.3	7.8

1.3.4. Optical properties of PVA/starch blend films

As illustrated in **Figure 1-8**, all films fabricated in this study had significant transparency and excellent appearance, and all the images covered by the films were visible, indicating that the films' transmittance was within acceptable limits. **Table 1-4** shows that the type and concentration of PVA affects the transparency of the films. For fully saponified PVA, an increase in PVA concentration correlates with enhanced transparency. For instance, in the case of PVA117/HPS blend films, transparency improved from 3.3 ± 0.5 to 2.4 ± 0.3 as the PVA concentration increased. Conversely, the transparency of blend films fabricated from partially saponified PVA exhibited less sensitivity to variations in concentration. **Figure 1-8a** presents the transmittance curves of the film materials ranging from 200 nm to 800 nm. Since transparency is affected by the film thickness, in conjunction with **Table 1-4**, it can be observed that when the starch content is at its lowest, the PVA-217/starch-7 film exhibits the highest transparency, indicating the best compatibility and the highest crystallinity between the two components. ^[68] As the starch content increases, crystallinity decreases, and the transparency values increase, indicating a reduction in transmittance. It is worth noting that the transparency values of PVA/starch-5 and PVA/starch-3 are similar. Among the two types of starch, films incorporating fully saponified PVA demonstrated greater transparency, evidenced by measurements of 1.9 ± 0.2 for PVA-105/HPS-7 and 2.4 ± 0.3 for PVA-117/HPS-7. In contrast,

partially saponified PVA achieves higher transparency when combined with OctS, indicating that due to the amphiphilic and interfacial properties of OctS, it can be better mixed with partially saponified PVA, reducing light scattering and resulting in higher transparency.^[69] The PVA-217/OctS blend film exhibits relatively high transparency and significantly reduced haze across all its components.

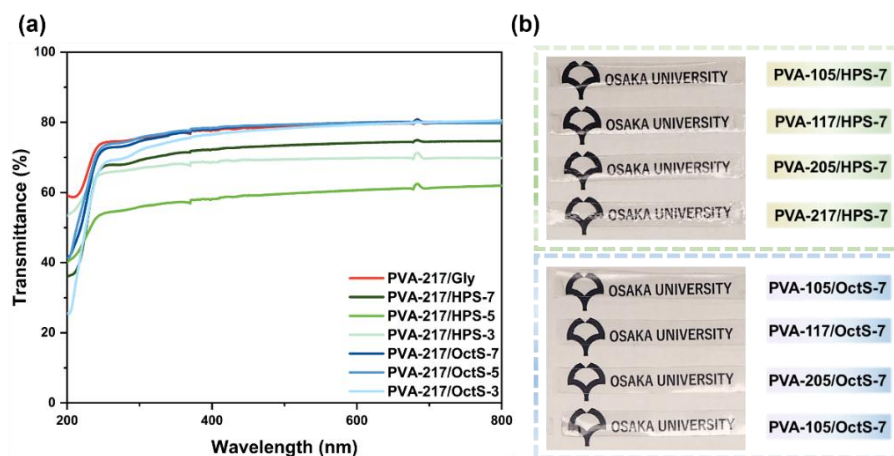


Figure 1-8. (a) Optical transmittance of films at 200–800 nm; (b) the digital photos of PVA/Starch-7 blend films.

1.3.5. Micro-morphological analysis of PVA/starch blend films

Scanning electron microscopy was applied to directly observe the microstructure of the blend films' surface and cross-section, as shown in **Figure 1-9**. The PVA-217/Starch series films exhibited smooth and continuous surfaces. As the amount of starch increases, the blend films displayed a flat and dense microstructure on both the surface and cross-section, devoid of any observable particles or voids (**Figure 1-10**). The efficient plasticization of PVA and starch made possible by glycerol and the hydrogen bonding that takes place between PVA and starch both contribute to the explanation of this phenomenon.^[70] The PVA/HPS-7 blend films showed phase separation, with clearly defined PVA-rich and starch-rich phases.^[71] In contrast, a cross-sectional structure of the OctS-based films appears more homogeneous, indicating improved

compatibility between OctS and PVA. Microstructural differences attributable to different grades of PVA used for blend films could not be discerned.

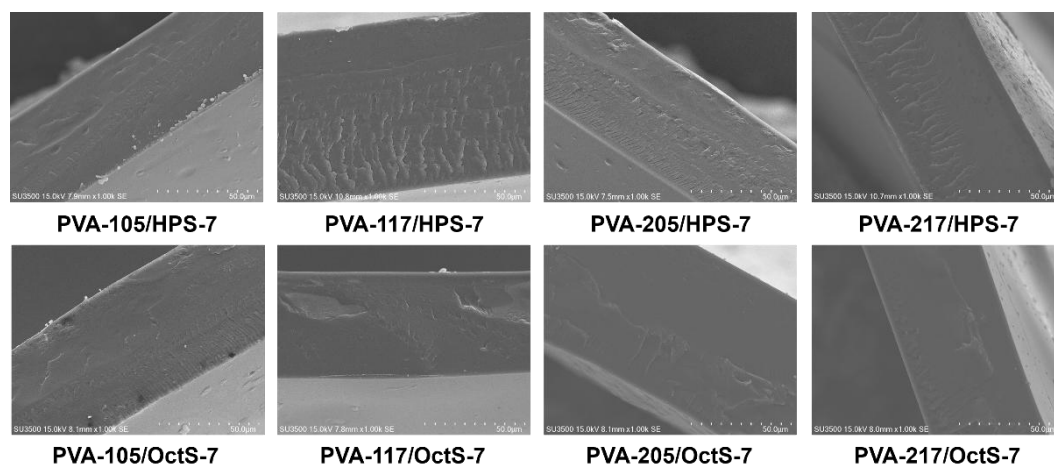


Figure 1-9. Cross-sectional SEM micrographs of PVA/Starch-7 blend films.

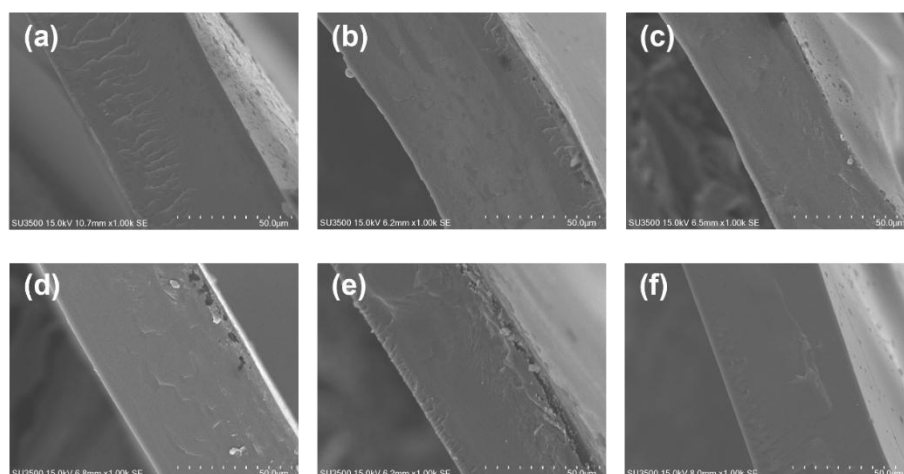


Figure 1-10. SEM micrographs of cross-section of (a) PVA-217/HPS-7 film; (b) PVA-217/HPS-5 film; (c) PVA-217/HPS-3 film; (d) PVA-217/OctS-7 film; (e) PVA-217/OctS-5 film; (f) PVA-217/OctS-3 film.

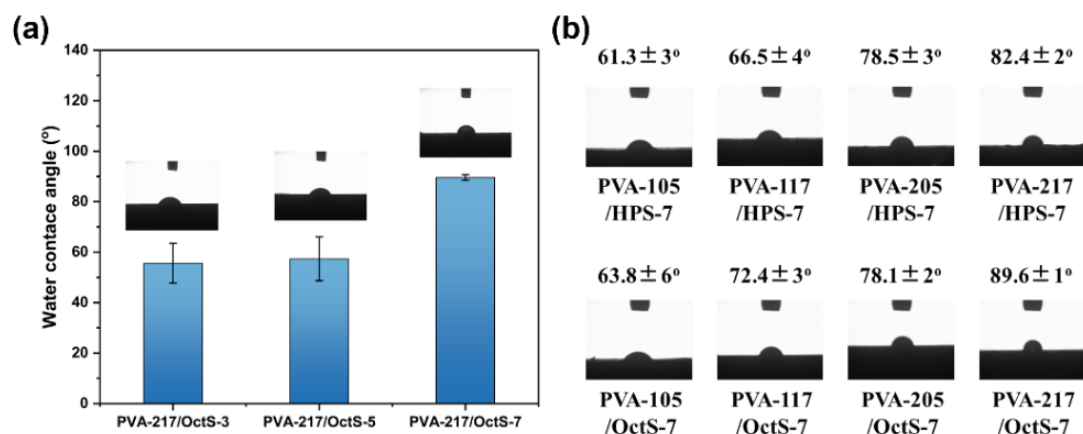
1.3.6. Water contact angles of PVA/starch blend films

The hydrophilic properties of PVA/starch blend films were measured through the measurement of their water contact angles, a critical characteristic for their practical applications. The direct application of PVA and starch is restricted due to their intrinsic

hydrophilic properties. The water contact angle measurements for pure PVA are presented in **Table 1-5**. Films fabricated from partially saponified PVA (PVA-217 and PVA-205) exhibited elevated water contact angles, attributed to the presence of residual hydrophobic acetyl groups resulting from saponification. However, the addition of glycerol reduced the film's water contact angles. Using the PVA-217/OctS blend film as an example (**Figure 1-11a**), the measured water contact angles of the PVA/OctS-3, PVA/OctS-5, and PVA/OctS-7 films were $55.6 \pm 7.9^\circ$, $57.4 \pm 8.7^\circ$, and $89.6 \pm 1.1^\circ$, respectively. The water contact angle is significantly influenced by the starch content in the films. When the starch content is high, the water contact angle is lower, indicating enhanced hydrophilicity of the film. This is because starch is hydrophilic, which increases the film's ability to adsorb water molecules, thus reducing the contact angle. Conversely, when the starch content is low, the contact angle increases, suggesting that the interaction between starch and PVA becomes stronger. Additionally, the inherent properties of PVA influence the hydrophilicity of the blend, making the film surface more hydrophobic under these conditions. This phenomenon is directly related to the hygroscopic nature of starch and its interaction with PVA in the blend. Consequently, the PVA/starch-7 blend film was selected for further testing. As illustrated in **Figure 1-11b**, the contact angles across the surface of blend films followed a similar trend to that of pure PVA, with the PVA-217/OctS-7 film achieving the highest water contact angle of $89.6 \pm 1^\circ$, indicating a significant reduction in film hydrophilicity. When comparing the PVA/OctS-7 blend film with the PVA/HPS-7 blend films, minimal variance was observed. Despite this, the OctS-based blend film exhibited a higher contact angle attributed to its inherent hydrophobic groups in comparison to modified HPS starch containing hydrophilic ether groups.^[72]

Table 1-5. Water contact angles of the PVA and PVA/Gly films.

Samples	PVA-105	PVA-117	PVA-205	PVA-217	PVA-105/Gly	PVA-117/Gly	PVA-205/Gly	PVA-217/Gly
Contact angles (°)	54.0±2	56.8±3	76.3±1	83.6±5	29.7±3	29.7±2	39.5±2	61.5±2

**Figure 1-11.** (a)The water contact angle of PVA/OctS blend films; (b) the water contact angle of PVA/Starch-7 blend films.

1.3.7. Water resistance and dissolution behaviors of blend films

The water resistance of PVA/starch blend films, particularly when incorporated with glycerol, has consistently posed a significant challenge. **Figure 1-12** illustrates the swelling of the films with a 7/3 ratio after immersion in water for 24 h. PVA molecules with high DS reduce the air permeability and water absorption by promoting inter- and intramolecular interactions with the -OH group, resulting in a less soluble network that increases the films' water resistance. [73,74] This explains why films prepared from PVA-105 and PVA-117 maintained their shape in water, particularly when using PVA-117 (higher DP). When PVA-117 was used in combination with the two starches, OctS exhibited superior water resistance compared to the HPS. This can be attributed to the fact that although the hydroxypropyl group is less hydrophilic than the native

hydroxyl group of natural starch, the presence of a hydrophilic ether group restricts interactions with PVA, allowing for easier utilization by water molecules and rendering the films more hydrophilic.^[41,75]

Building on these observations, the swelling rates of the PVA-117/OctS series films were evaluated after 24 h of immersion in freshwater (**Figure 1-13**). It was noted that

the swelling rates decreased from ~137.2% to ~108.6% as the PVA content increased, indicating an enhancement in water resistance.

The best water-resistant PVA-117/OctS-7 and PVA-117/HPS-7 films were tested after 24 h of immersion in solutions of different pH values (**Figure 1-14a**). The PVA-117/OctS-7 film showed clear and intact structures with a low SR of ~175.5% and ~156.6% in solutions of pH = 4 and 7, respectively (**Figure 1-14b**). In contrast, the other films became cloudy and were unable to be retrieved using tweezers.

As demonstrated in **Figure 1-14c**, in an alkaline environment at pH = 9, the hydroxyl groups are more readily ionized, thereby enhancing the water solubility. Concurrently, the film swells because of the deprotonation of the carboxyl groups in OctS, leading to the generation of anionic charges and subsequent electrostatic repulsions. High solubility in alkaline solutions also provides a further solution for its degradation. In contrast, at pH levels of 7 and 4, the hydrogen bonding network established among PVA-117, OctS, and glycerol exhibits enhanced

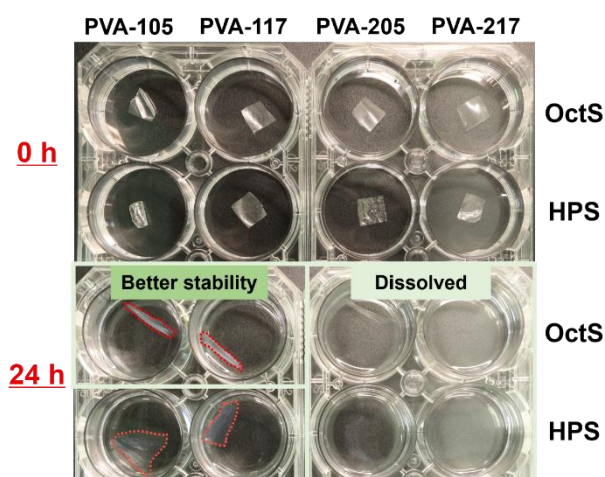


Figure 1-12. Photographs of PVA/Starch-7 blend films after soaking for 24 h.

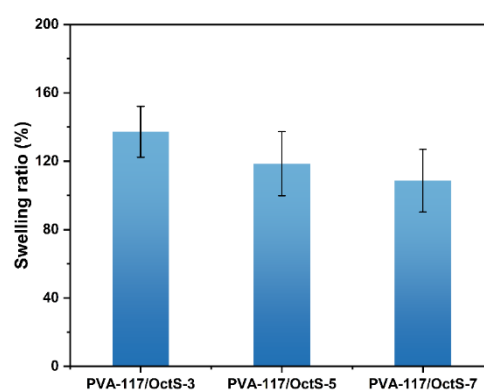


Figure 1-13. Swelling ratio of PVA-117/OctS blend films.

stability. Furthermore, under these conditions, the carboxyl groups are protonated, rendering the OctS component relatively hydrophobic owing to the presence of long alkyl chains which mitigate electrostatic repulsion, thereby contributing to the overall stability of the films. Therefore, selecting PVA types and modified starch had a significant impact on improving the water resistance of the blend films.

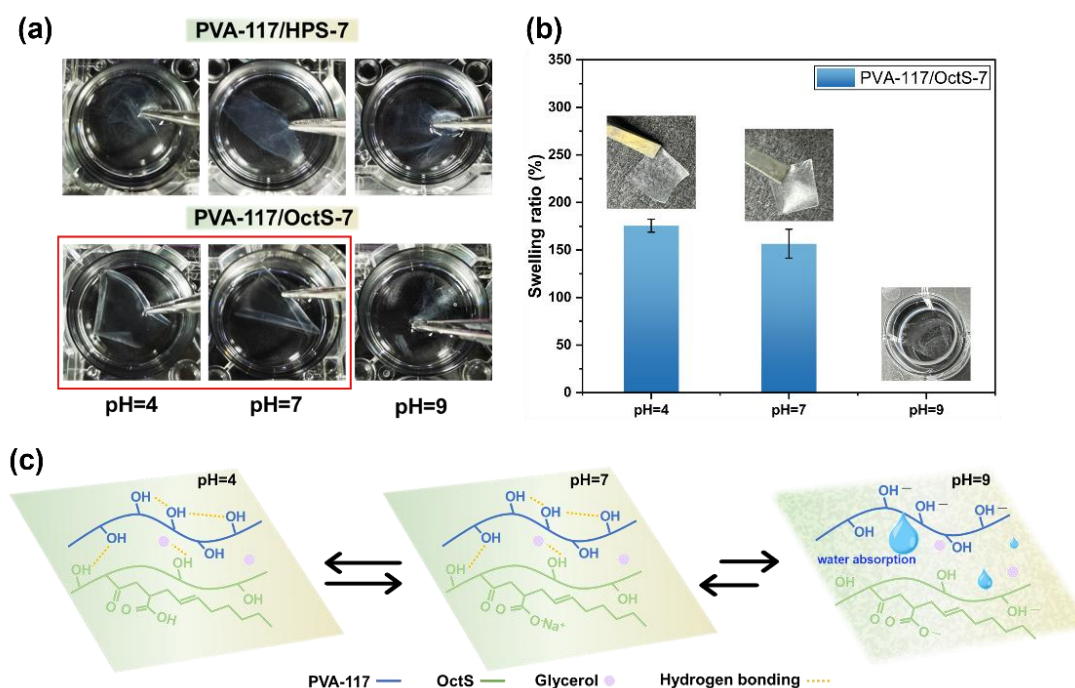


Figure 1-14. (a) Dissolution behaviors of PVA/starch-7 composite films in different pH solutions; (b) swelling ratio of PVA-117/OctS-7 blend film in different pH solutions; (c) mechanisms of changes in the interactions of the components of PVA-117/OctS-7 blend films under aqueous solution conditions with different pH values.

Due to its sufficient mechanical strength (~ 15.9 MPa) and high optical transparency, the PVA-117/OctS-7 film is deemed suitable for packaging applications. This film not only demonstrates excellent water resistance but also maintains certain wet strength. As shown in **Figure 1-15a**, three minutes after being submerged in water, the film swelled by 114.2% while remaining stable and capable of bearing weight. **Figure 1-15b** and **1-15c** illustrate the film's

potential for large-scale production and packaging use. Additionally, the PVA-117/OctS-7 film is highly flexible and extensible, capable of being knotted, curled, and reverting to its original shape after folding, allowing it to be shaped as needed for various applications (**Figure 1-15d**).

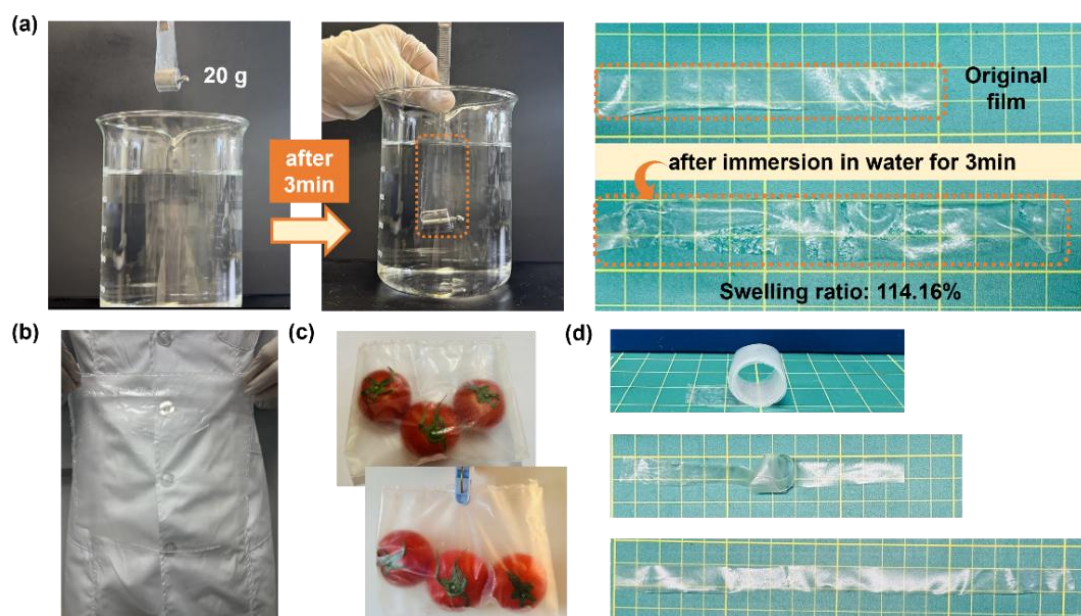


Figure 1-15. (a) PVA-117/OctS-7 film used for wet strength demonstration is highlighted by the orange square; (b) large-scale PVA-117/OctS-7 film; (c) tomatoes in packaging bags made of PVA-117/OctS-7 film; (d) demonstration of the flexibility of PVA-117/OctS-7 film.

1.4. Conclusions

To address the challenges of low mechanical strength and poor water resistance inherent in natural starch films, a comprehensive study was conducted on the effects of incorporating two different types of starch (HPS and OctS) into blends with four distinct grades of PVA. Films blended with a 7:3 ratio of PVA to starch (PVA/starch-7) exhibited superior transparency, mechanical properties, and thermal stability, especially when blended with OctS. Moreover, the DP of PVA had a major impact on the mechanical properties of the films, with higher DP values corresponding to better tensile properties. Notably, PVA-217/OctS-7 film demonstrated a TS of 17.4 MPa and the highest EAB of 460%. The hydrophilicity and water resistance of the films were also significantly influenced by the DS of PVA. Films derived from low DS PVA,

containing residual hydrophobic acetyl groups, exhibited reduced hydrophilicity. Conversely, blend films prepared from fully saponified PVA displayed enhanced stability in water, particularly the PVA-117/OctS-7 film, which also demonstrated varied dissolution behaviors across different pH solutions. Outstanding mechanical, wet strength, and water resistance characteristics of the PVA-117/OctS-7 films make them perfect options for use as packaging materials. Additionally, their responsive dissolution behavior in different pH solutions further broadens their application and degradation potential, particularly for innovative packaging solutions.

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Chapter 2

Schiff Base Crosslinked Poly(γ -glutamic acid) Composite Films with High Mechanical Strength and Remoldability

2.1. Introduction

In recent years, the persistent accumulation of plastic waste in natural ecosystems has become a global environmental concern. The low degradation efficiency of conventional plastics poses a major barrier to pollution mitigation.^[1] To address this issue, the scientific community has been actively investigating sustainable carbon-based feedstocks and advancing green synthesis strategies for the development of high-performance bio-based materials, with the ultimate goal of replacing petroleum-derived plastics.^[2]

Poly(γ -glutamic acid) (γ -PGA) is a water-soluble, biodegradable synthetic polypeptide composed of naturally occurring D- and L-glutamic acid units linked via amide bonds between the α -amino and γ -carboxyl groups.^[3] This biopolymer is primarily produced through microbial fermentation by *Bacillus subtilis* or *Bacillus natto* and is well known for its excellent biocompatibility and environmental sustainability.^[4] Due to these attributes, γ -PGA has been widely applied in fields such as biomedicine, agriculture, food, and environmental engineering.^[5] In recent years, increasing emphasis on green and sustainable development, γ -PGA has attracted increasing interest as it advances toward industrial-scale production and shows strong potential as a renewable alternative to petroleum-based plastics.^[6] The presence of abundant carboxyl ($-\text{COOH}$) functional groups along the molecular backbone offers versatile reactive sites for chemical modification.^[7,8] Various functional moieties such as allyl glycidyl ether, glycidyl methacrylate, cysteamine hydrochloride, furfurylamine, and tyramine have been grafted onto γ -PGA to tailor and improve its material properties.^[9–11] However,

despite these advantages, the high hygroscopicity and poor film-forming ability of γ -PGA remain significant limitations, especially in applications requiring structural integrity, such as packaging materials.

To address these limitations, the construction of stable covalently crosslinked networks has emerged as an effective strategy to enhance the performance of γ -PGA.^[12] For example, Yu et al.^[13] designed a covalent crosslinking system between γ -PGA and chitosan oligosaccharide embedded in bacterial cellulose, which simultaneously improved the mechanical strength and effectively regulated the swelling behavior. In another study, Chen et al.^[14] developed an adhesive hydrogel by crosslinking γ -PGA with amino-functionalized poly(ethylene glycol)-grafted poly(glycerol sebacate), which exhibited excellent wet adhesion and dynamic mechanical stability. These studies collectively demonstrate that the formation of covalently crosslinked structures can significantly improve the mechanical properties and functional stability of γ -PGA-based materials, offering promising potential for applications in biomedical and functional packaging.

However, poor film-forming ability remains a significant obstacle, limiting both processability and practical performance. Incorporating a polymer with superior film-forming properties and chemical reactivity has been recognized as an effective strategy to address this challenge.^[15] Poly(vinyl alcohol) is a nontoxic and water-soluble polymer that is widely used in medical dressings, food packaging, and biodegradable films.^[16] Owing to its excellent chain flexibility and film-forming capability, PVA can promote the formation of dense film structures and enhance interfacial cohesion, thereby improving the mechanical strength, durability, and surface uniformity of the resulting composite films.^[17]

Although conventional covalently crosslinked networks are effective in enhancing material strength and stability, their irreversible nature often compromises sustainability and raises concerns regarding secondary environmental pollution.^[18] In recent years, covalent

adaptable networks (CANs) based on dynamic covalent chemistry have received increased attention.^[19,20] By incorporating reversible covalent bonds, these networks maintain their structural integrity while introducing desirable features such as degradability and recyclability, which are particularly advantageous for the development of bio-based functional materials.^[21,22] Among the various dynamic covalent strategies, the Schiff base reaction has emerged as a powerful and widely adopted click chemistry approach owing to its mild reaction conditions, metal-free catalysis, and compatibility with aqueous environments.^[23] Recent studies have demonstrated the potential of this reaction for the fabrication of high-performance and sustainable polymeric materials. For instance, Khin et al.^[24] developed a bio-based packaging film through Schiff base crosslinking between gelatin and dialdehyde cellulose in aqueous solution, achieving excellent tensile strength (49.4 ± 4.5 MPa), water resistance, and gas barrier performance. Similarly, Zhou et al.^[25] designed a sustainable konjac glucomannan-based bioplastic using dynamic imine bond formation, which enabled hot-press processability, thermally triggered self-healing, and recyclability. These studies highlight the significant potential of Schiff base chemistry for advancing green synthetic strategies for high-performance degradable film materials and offer valuable insights into the design of next-generation sustainable polymer networks.

In this study, we report a novel recyclable thermoset film based on γ -PGA and PVA. The material was fabricated through a Schiff base reaction under mild conditions between hydrazide-functionalized γ -PGA (P-ADH) and acetoacetylated PVA (AAPVA), leading to the formation of a stable acylhydrazone ($-\text{C}=\text{NNH}-$) crosslinked network. This dynamic covalent structure endows the resulting bio-based thermoset film with excellent mechanical strength, ultraviolet (UV)-shielding ability, and chemical recyclability. The mechanical properties of these films were systematically evaluated and compared with those of several representative commercial packaging materials. The results demonstrated that the γ -PGA-based films

exhibited superior overall performance, confirming the feasibility and effectiveness of acylhydrazone crosslinking for the development of sustainable functional films. This work provides an effective strategy for enhancing the performance of γ -PGA-based materials and offers a promising foundation for designing next-generation, environmentally friendly, and recyclable bio-based plastic alternatives.

2.2. Experimental Section

2.2.1. Materials

Acetoacetylated polyvinyl alcohol (AAPVA, degree of hydrolysis: 99%, $M_w \approx 44,000$ g mol⁻¹, acetoacetyl substitution degree: 5%) was kindly supplied by The Nippon Synthetic Chemical Industry Co., Ltd. (Tokyo, Japan) and used without further purification. Poly(γ -glutamic acid) (PGA, $M_w = 1.6 \times 10^5$ g mol⁻¹) was provided by Kookmin University (Seoul, Korea). The reagents 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), adipic dihydrazide (ADH), and 4-amino-L-phenylalanine monohydrate (4-amino-Phe) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Additionally, 2-morpholinoethanesulfonic acid (MES) was procured from Sigma-Aldrich (St. Louis, MO, USA).

2.2.2. Synthesis of P_ADH

To synthesize P_ADH, 5.2 g of PGA was completely dissolved in 500 mL MES buffer (0.1 mol/L, pH 5.5). Subsequently, 4.6 g EDC and 2.76 g of NHS were added and allowed to react for 15 min to activate the carboxyl groups. Then, 3.5 g of ADH was added, and the reaction was carried out under continuous stirring at 25°C for 24 h. Upon completion, the reaction mixture was subjected to dialysis using a membrane with a molecular weight cut-off of 1×10^4 ,

first against a 0.1 mol/L NaCl solution for 3 days, followed by deionized water for 4 days. Finally, the purified P_ADH was obtained as a white, spongy solid by lyophilization.

2.2.3. Fabrication of the P_ADH/AAPVA films

The P_ADH/AAPVA films were prepared using the solution casting method. Initially, AAPVA was dissolved in 20 mL of deionized water at 90°C and then cooled to room temperature. Separately, P_ADH was dissolved in 20 mL deionized water at room temperature. The AAPVA solution was gradually poured into the P_ADH solution, followed by continuous stirring to ensure homogeneity. The resulting mixture was transferred into a Petri dish (Φ100 mm) and dried in an oven at 40°C for 2 days. The resulting films were designated as AAPVA, P_ADH, PGA/P-3, P_ADH/P-1, P_ADH/P-3, and P_ADH/P-5, as detailed in **Table 2-1**.

Table 2-1. Composition of AAPVA, P_ADH, PGA/P-3 and P_ADH/AAPVA films.

Group	Weight ratio	Molar ratio
AAPVA	0 : 1 (0 : 100)	/
P_ADH	1 : 0 (100 : 0)	/
PGA/P – 3	3 : 1 (75 : 25)	/
P_ADH/P – 1	1 : 1 (50 : 50)	2 : 1
P_ADH/P – 3	3 : 1 (75 : 25)	6 : 1
P_ADH/P – 5	5 : 1 (83 : 17)	10 : 1

2.2.4. Characterization

The ¹H nuclear magnetic resonance (¹H NMR) spectra of the samples were obtained using JNM-ECS400 (400 MHz, JEOL Ltd., Japan). The Fourier transform-infrared (FT-IR) spectra of the materials in the 500-4000 cm⁻¹ wavenumber range were measured using a Nicolet iS5 FTIR spectrometer (Thermo Fisher Scientific, Waltham, USA). The chemical compositions and structural features were confirmed using X-ray diffraction (XRD; SmartLab, Rigaku Co. Ltd., Japan) and X-ray photoelectron spectroscopy (XPS; KRATOS ULTRA2, Shimadzu Co.

Ltd., Japan). The mechanical properties of the films were investigated using a universal testing machine (EZ Graph, Shimadzu Co., Japan). A dynamic mechanical analyzer (DMA 7100, TA Instruments, USA) was used to determine the thermomechanical properties of the films. The transparency of the films was measured in the wavelength range 200–800 nm using an ultraviolet spectrometer (U-2810, Hitachi, Japan). The morphologies of the films were observed using scanning electron microscopy (SEM; SU5000, Hitachi High-Technologies Corp., Japan). Water contact angles were measured using a Drop Master DM300 (Kyowa Interface Science, Japan).

2.2.5. Swelling ratio of the films

The swelling ratio of the films ($\sim 1\text{ cm} \times 1\text{ cm}$) were determined by immersion in 10 mL deionized water at 25°C. After 24 h, the samples were carefully extracted with tweezers and the excess water was wiped off with a thin piece of paper and then weighed. After three tests were conducted on each film, the swelling ratio (SR) was computed by **Eq. (2-1)**.^[26]

$$SR = \frac{W_{ss} - W_{ds}}{W_{ds}} \times 100 \quad (2-1)$$

where W_{ds} and W_{ss} represent the weights of the original and swelling samples, respectively.

2.3. Results and Discussion

2.3.1. Synthesis and characterization of P_ADH/AAPVA films

In this study, a covalent adaptive network was constructed based on hydrazide-modified γ -polyglutamic acid (P_ADH) and acetoacetylated polyvinyl alcohol (AAPVA), leading to the synthesis of a sustainable, chemically degradable, and high-performance film material. The fabrication strategy involved chemical modification of γ -PGA followed by dynamic covalent crosslinking reactions with AAPVA (**Figure 2-1**).

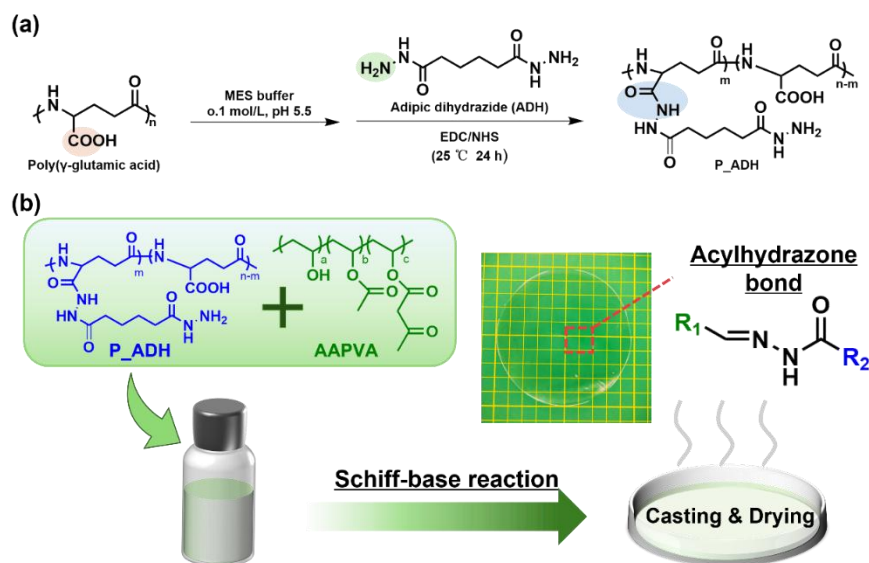


Figure 2-1. (a) Schematic illustration of the chemical synthesis route of P_ADH; (b) Fabrication process of the P_ADH/AAPVA film.

P_ADH was synthesized via EDC/NHS coupling using the synthetic pathway illustrated in Fig. 1a. The chemical structure and degree of substitution of the P_ADH polymer were characterized using ^1H NMR and FT-IR (**Figure 2-2**). In the ^1H NMR spectrum, a new proton signal was observed at 1.46 ppm, corresponding to the methylene protons in ADH, confirming the successful conjugation of ADH with γ -PGA.^[27,28] In the FTIR spectrum, the overlapping

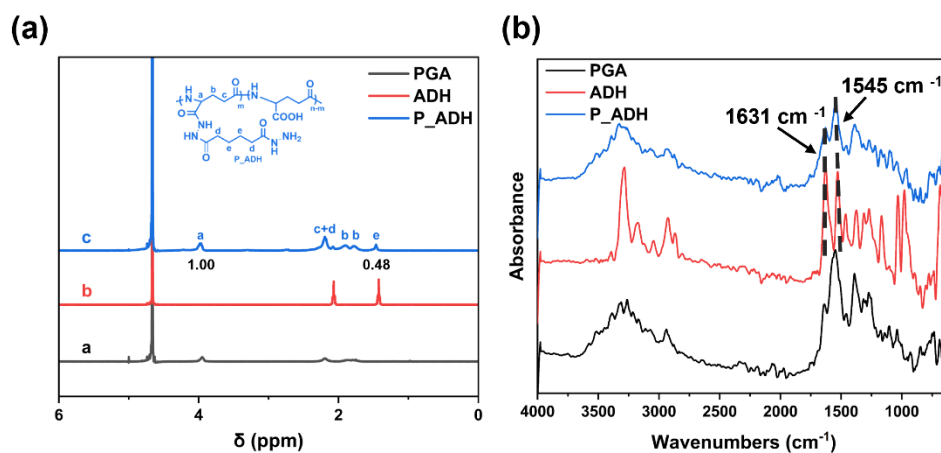


Figure 2-2. (a) ^1H NMR spectra of PGA, ADH, and P_ADH and (b) FTIR spectra of PGA, ADH and P_ADH.

vibrations of C=O, amide I, and amide II groups from γ -PGA and ADH resulted in the emergence of new peaks at 1545 cm^{-1} and 1631 cm^{-1} .^[29,30] The degree of substitution of P_ADH was determined to be 12% by comparing the integral area of the methylene protons in ADH (4H) with the α -protons of γ -PGA (1H) at 3.96 ppm.

Subsequently, P_ADH and AAPVA were crosslinked via a Schiff base reaction to form the P_ADH/AAPVA films. The FTIR spectra of the P_ADH, AAPVA, PGA/P-3, and P_ADH/AAPVA films are shown in **Figure 2-3a**. The characteristic peaks observed at 1735 cm^{-1} - 1705 cm^{-1} correspond to the acetoacetyl groups in AAPVA.^[31,32] After crosslinking, these peaks disappeared in the P_ADH/AAPVA films, while a new peak appeared at $1640\text{--}1643\text{ cm}^{-1}$, corresponding to the C=N bond of the hydrazone linkage.^[33] Additionally, the hydroxyl absorption band in the P_ADH/AAPVA films shifts to a lower wavenumber than that of AAPVA, indicating the presence of hydrogen bonding interactions.^[34,35] The XRD patterns of

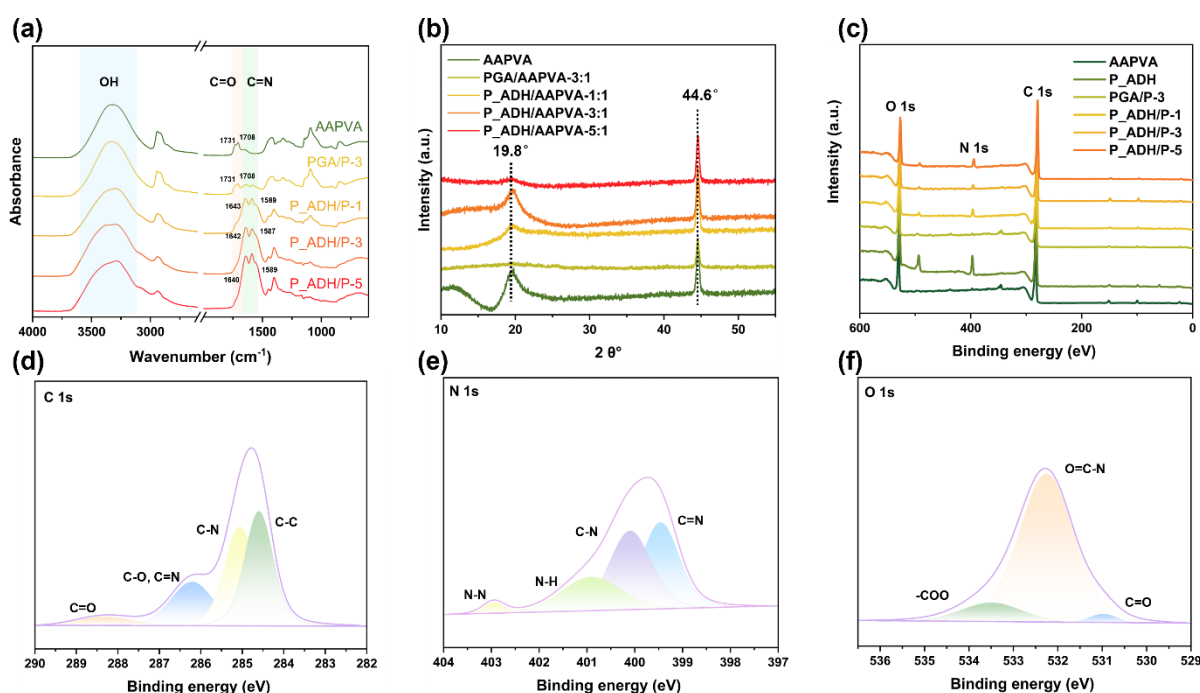


Figure 2-3. (a) FT-IR spectra of AAPVA and composite films; (b) XRD patterns of AAPVA and composite films; (c) XPS survey spectra of AAPVA, P_ADH, PGA/P-3 and P_ADH/AAPVA films; high-resolution XPS spectra of the P_ADH/P-3 film: (d) C 1s, (e) N 1s and (f) O 1s.

the AAPVA and modified films are shown in **Figure 2-3b**. The pristine AAPVA film exhibited a broad crystalline peak at $2\theta = 19.8^\circ$, attributed to intra- and intermolecular hydrogen bonding within AAPVA chains.^[36] As the P_ADH content increased and acylhydrazone bonds were introduced, the formation of hydrogen bonds was reduced, disrupting the crystalline structure and resulting in a reduced peak intensity in all P_ADH/AAPVA films. Furthermore, the chemical structures of the films were confirmed using XPS (**Figure 2-3c, 2-3d, 2-3e and 2-3f**), using the P_ADH/P-3 film as a representative sample. The appearance of new peaks at 286.3 eV (C=N) in the C 1s spectrum and 399.5 eV (C=N) in the N 1s spectrum provides strong evidence for the successful formation of acylhydrazone linkages within the crosslinked network.^[25,37]

2.3.2. Mechanical Properties of P_ADH/AAPVA Films

The mechanical properties of P_ADH/AAPVA films with different compositions were evaluated via tensile testing (**Table 2-2**). Compared to the pure AAPVA film (46.79 ± 5.82 MPa), P_ADH film (61.43 ± 2.16 MPa), and unmodified γ -PGA/AAPVA blend film (5.422 ± 2.44 MPa), the P_ADH/AAPVA series exhibited significantly enhanced tensile strength (**Figure 2-4a, 2-4b and 2-4c**). This improvement is attributed to the formation of acylhydrazone bonds within the polymer network, where both covalent crosslinking and hydrogen bonding contribute to the increased rigidity and reinforcement. The Young's modulus of the P_ADH/P-3 film (4.4 ± 0.02 GPa) was comparable to those of the P_ADH/P-1 and P_ADH/P-5 films. However, its tensile strength increased significantly to 123.5 ± 2.32 MPa, which was substantially higher than that of the P_ADH/P-1 (91.81 ± 3.91 MPa) and P_ADH/P-5 (95.37 ± 13.4 MPa) films, representing improvements of 34.5% and 29.5%, respectively. This enhancement was likely due to the optimal crosslinking density in the polymer matrix, indicating that either an excess or deficiency of P_ADH can compromise the mechanical

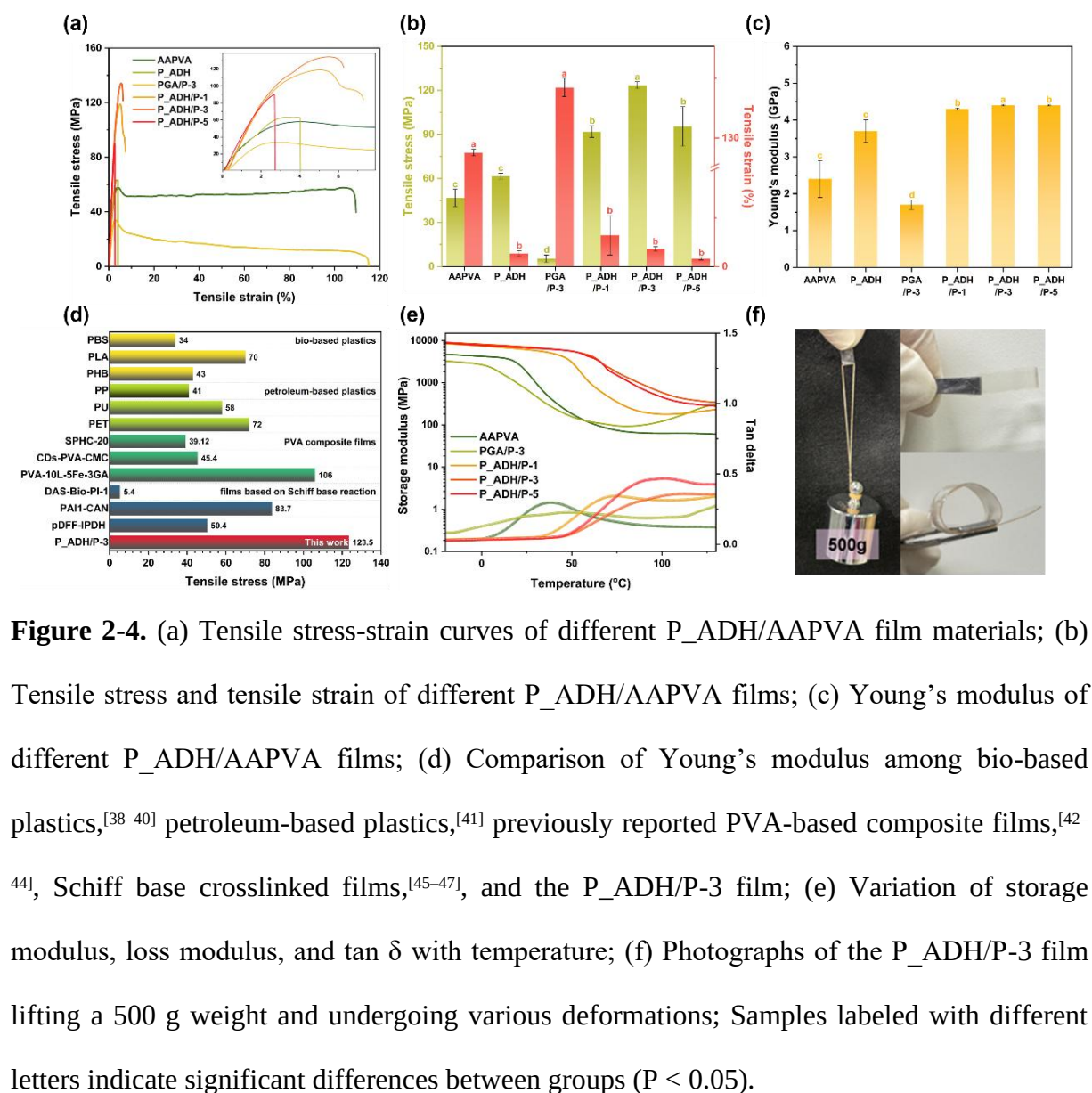
properties by disrupting the formation of an efficient crosslinked structure. The notable reduction in the elongation at break was attributed to restricted chain mobility following the formation of hydrazone linkages. **Figure 2-4d** presents a comparative analysis of the tensile strength of the P_ADH/P-3 film in relation to bio-based plastics, petroleum-based plastics, previously reported PVA-based composite films, and Schiff base-crosslinked films. Common bioplastics such as PBS, PLA, and PHB exhibit tensile strengths of approximately 34 , 70 , and 43 MPa, respectively, which are markedly lower than those of the P_ADH/AAPVA films. These results demonstrate that the P_ADH/P-3 film exhibits mechanical performance on par with or superior to that of conventional plastics, highlighting its potential as a sustainable alternative.

Table 2-2. Thickness(μm), Tensile strength (MPa), Elongation at break (%) and Young's modulus of AAPVA, P_ADH, PGA/P-3 and P_ADH/AAPVA films.

Films	Film thickness (mm)	Tensile stress at break (MPa)	Elongation at break (%)	Young's modulus (GPa)
AAPVA	0.083	46.79 ± 5.82	114 ± 3.7	2.4 ± 0.50
P_ADH	0.113	61.43 ± 2.16	4.71 ± 1.0	3.7 ± 0.31
PGA/P – 3	0.069	5.422 ± 2.44	185 ± 9.9	1.7 ± 0.13
P_ADH/P – 1	0.075	91.81 ± 3.91	11.3 ± 7.1	4.3 ± 0.03
P_ADH/P – 3	0.076	123.5 ± 2.32	6.32 ± 0.8	4.4 ± 0.02
P_ADH/P – 5	0.078	95.37 ± 13.4	2.75 ± 0.4	4.4 ± 0.02

The dynamic mechanical and thermal properties of the films were further investigated using Dynamic Mechanical Analysis (DMA). Variations in storage modulus (E') and loss factor ($\tan \delta$) with temperature are depicted in **Figure 2-4e**. The storage modulus of the P_ADH/AAPVA films was significantly higher than those of pure AAPVA and physically blended PGA/P-3 films, indicating enhanced mechanical rigidity. As the temperature increased, the storage modulus gradually decreased, suggesting the activation of the dynamic exchange of

acylhydrazone bonds, which facilitated polymer chain mobility within the P_ADH/AAPVA network. The glass transition temperatures (T_g) of P_ADH/P-1, P_ADH/P-3, and P_ADH/P-5 films were determined to be 68.3°C, 106.2°C, and 96.4°C, respectively, correlating with the acylhydrazone crosslinking content. Furthermore, the P_ADH/P-3 film (approximately 100 μm thick and 1 cm wide) was able to lift a 500 g weight (**Figure 2-4f**), demonstrating its excellent mechanical strength, flexibility, and ductility. These findings underscore the superior mechanical performance of P_ADH/AAPVA films, making them promising candidates for high-performance, sustainable material applications.



2.3.3. Optical Properties of P_ADH/AAPVA Films

The UV-visible transmittance spectra of various films in the 200–800 nm wavelength range, along with their transparency values at 600 nm, are presented in **Figure 2-5a, 2-5b** and **Table 2-3**. The results indicate that the pure AAPVA and P_ADH films exhibited high transparency but limited UV-blocking capability. In contrast, the P_ADH/P-3 film demonstrates an ~85.8% transmittance at 600 nm with an optical transparency of 0.76 ± 0.16 , while blocking UV light in the 200–320 nm range, thereby exhibiting excellent UV-shielding performance. This improvement was attributed to the dense cross-linked structure and the presence of C=N bonds within the polymer network, which effectively absorbed UV radiation and minimized undesired reflection and scattering, resulting in a uniform refractive index and improved optical clarity.^[48] Although the AAPVA/P-1 and AAPVA/P-5 films also maintained high transparency (~85.4% and 87.9%, respectively), their UV-shielding performances were comparatively lower. The surface and cross-sectional SEM images (**Figure 2-6**) the P_ADH/AAPVA films exhibited smooth surfaces and densely packed microstructures, which contributed to their excellent optical appearance. To further illustrate the high transparency of the films, an AAPVA/P-3 film was placed over half of the image and remained clearly visible throughout the film (**Figure 2-5c**). Overall, the combination of superior optical transparency

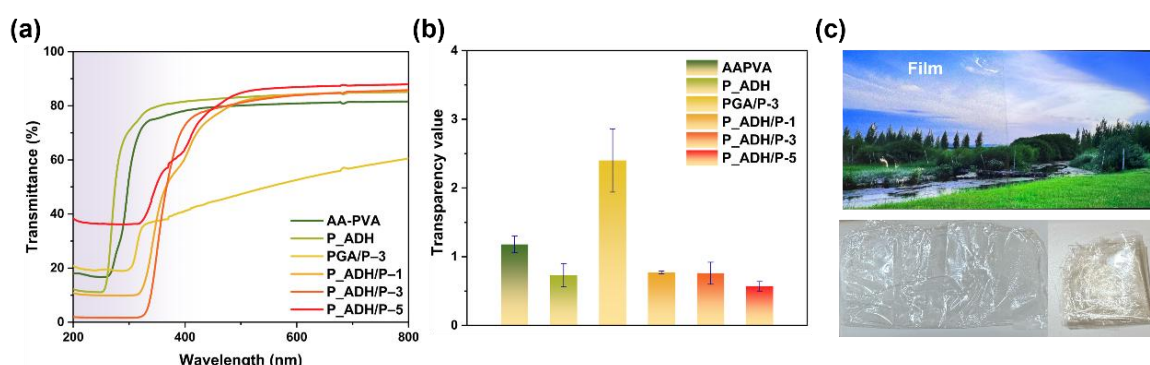


Figure 2-5. (a) UV-visible transmittance spectra of different P_ADH/AAPVA films; (b) Transparency value of different P_ADH/AAPVA films at a wavelength of 600 nm; (c) Photographs of transparent P_ADH/P-3 film and large-scale P_ADH/P-3 film.

and outstanding UV-blocking capability highlights the significant potential of the P_ADH/AAPVA films for applications in food packaging, biomedical devices, and other advanced material fields.

Table 2-3. Transparency value (at 600 nm) of AAPVA, P_ADH, PGA/P-3 and P_ADH/AAPVA films.

Films	Transparency value (at 600 nm)
AA-PVA	1.18 ± 0.12
P_ADH	0.73 ± 0.17
PGA/P – 3	2.40 ± 0.46
P_ADH/P – 1	0.77 ± 0.02
P_ADH/P– 3	0.76 ± 0.16
P_ADH/P – 5	0.57 ± 0.07

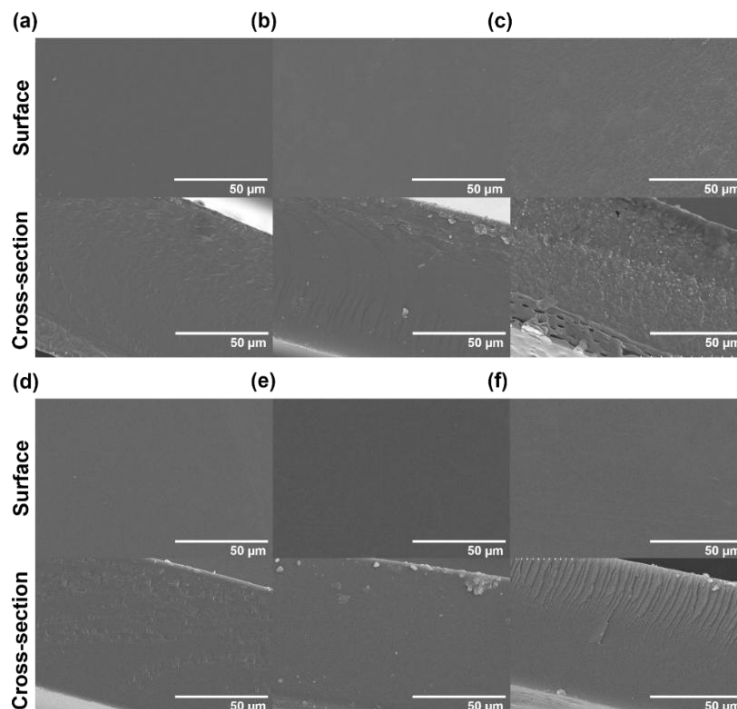


Figure 2-6. Scanning electron microscopy of surface and cross-section of (a) AAPVA, (b) P_ADH, (c) PGA/P-3, (d) P_ADH/P-1, (e) P_ADH/P-3, (f) P_ADH/P-5 films.

2.3.1. Stability and Recyclability of P_ADH/AAPVA Films

In addition to their exceptional mechanical properties and high transparency, water stability and chemical resistance are critical for the practical application of plastic alternatives. Owing to the abundance of hydrophilic functional groups in both AAPVA and P_ADH, these materials inherently exhibit high water affinity and are readily soluble in water, limiting their practical applications. As shown in **Figure 2-7a** and **2-7b**, AAPVA exhibits a higher water contact angle than the other films because of the presence of acetoacetyl groups; however, it also displays an extremely high-water absorption rate of 3222%. In contrast, the P_ADH/AAPVA films benefited from the densely crosslinked surface formed via Schiff base reactions, which significantly improved their water resistance. After 90 s, the water contact angles of the P_ADH/P-1, P_ADH/P-3, and P_ADH/P-5 films were 44.4°, 46.9°, and 40.9°, respectively, with water absorption rates of 988%, 877%, and 1685%. Notably, the water absorption rate of the P_ADH/P-3 film was reduced by 72.8% compared to that of AAPVA, and its structural integrity was retained even after three months of immersion, demonstrating

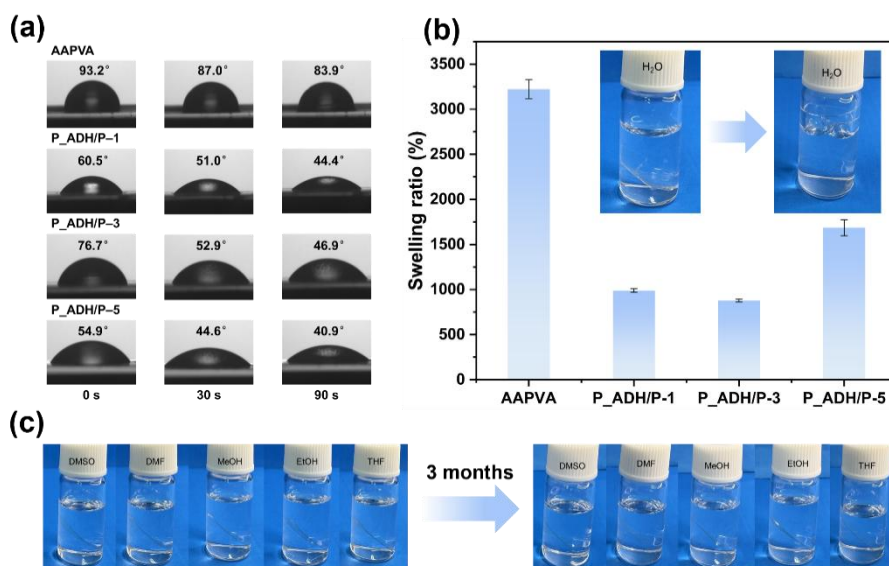


Figure 2-7. (a) Water contact angles of P_ADH/AAPVA films; (b) Swelling ratio of P_ADH/AAPVA films; (c) Chemical stability of P_ADH/AAPVA films after immersion in organic solvents for three months.

that the dense network structure and chemical modification improved water resistance. The P_ADH/P-3 film also demonstrated excellent chemical resistance, retaining its original color, shape, and mechanical integrity even after three months of immersion in various commercially available organic solvents without visible deformation or degradation (**Figure 2-7c**). The TGA and differential thermal gravimetry (DTG) curves (**Figure 2-8**) revealed three distinct stages of mass loss occurring at 30–180°C, 220–380°C, and 380–500°C, corresponding to the evaporation of absorbed and bound water, thermal degradation of P_ADH and AAPVA molecular chains, and decomposition of the dynamic hydrazone crosslinked network, respectively. These results confirmed that the P_ADH/AAPVA films possessed excellent thermal stability, ensuring their safety and reliability at elevated temperatures.

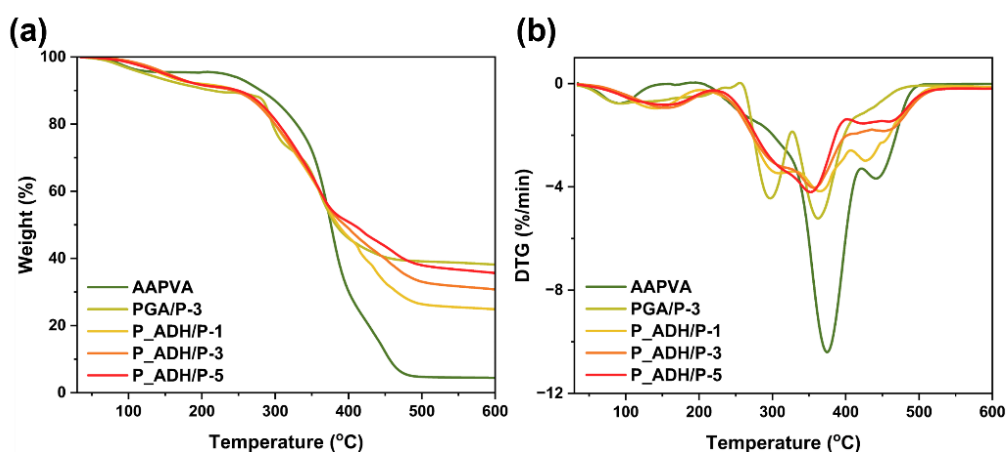


Figure 2-8. TGA analysis showing the a) weight loss and b) DTG.

Unlike conventional non-recyclable thermoset materials, the P-ADH/AAPVA film series demonstrates excellent chemical recyclability owing to the dynamic and reversible nature of the acylhydrazone bonds.^[49] To verify this property, a representative P-ADH/P-3 film was immersed in a 1M HCl/H₂O solution and stirred at 50 °C for 24 h. As shown in **Figure 2-9a**, the film completely degraded under these conditions. This degradation was attributed to the acid-catalyzed hydrolysis of the acylhydrazone bonds within the dynamic crosslinked network, leading to the breakdown of the film structure. The degradation solution was purified and

freeze-dried to obtain a white solid powder. The recycled product was structurally characterized using ^1H NMR and FTIR spectroscopy (**Figure 2-9b** and **2-9c**). The ^1H NMR spectrum confirmed the presence of α (3.97 ppm), β (1.76–1.88 ppm), and γ (2.20 ppm) protons, indicating that the structural features of γ -PGA in the recycled P_ADH were retained. In addition, a signal at 1.46 ppm, corresponding to the methylene protons in ADH, was still observed, suggesting that the P_ADH structure remained largely intact. The FTIR spectrum of the newly formed film from recycled P_ADH showed the reappearance of the characteristic C=N stretching band at 1640–1643 cm^{-1} , while the peak associated with the acetoacetyl group disappeared, confirming successful reconstruction of the acylhydrazone crosslinked network.

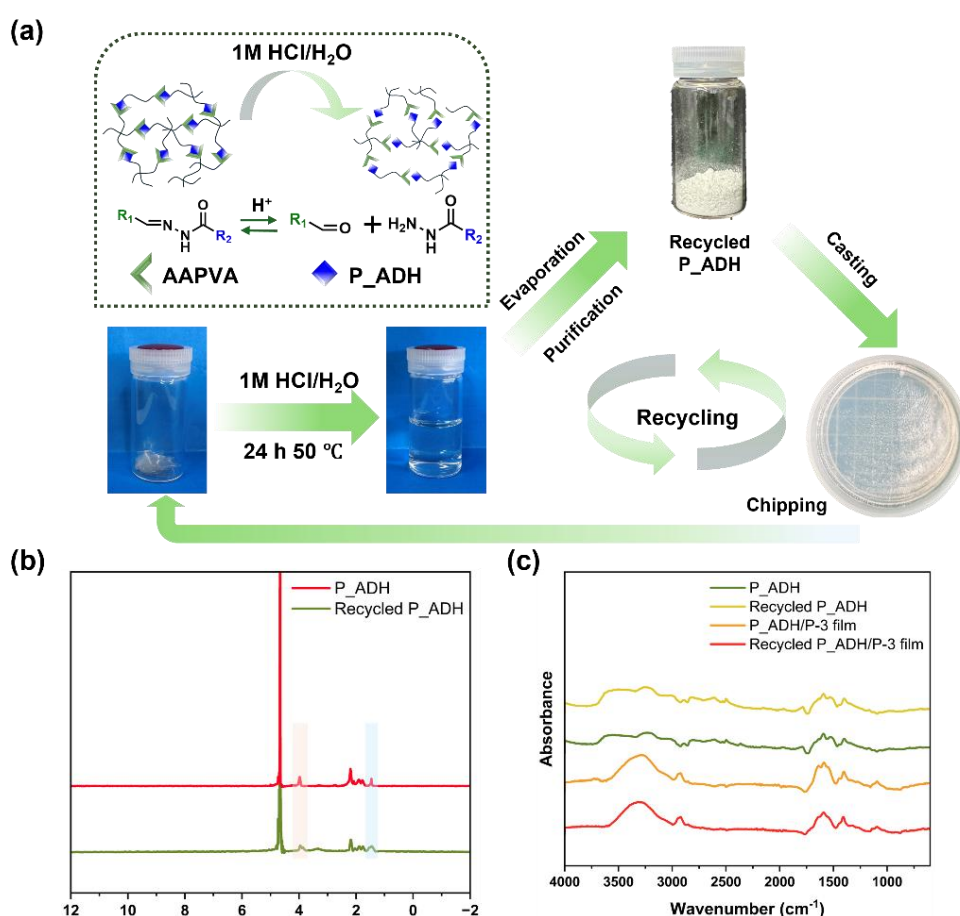


Figure 2-9. (a) Chemical degradation and recycle of P_ADH/P-3 films in 1 M HCl/H₂O solution; (b) ^1H NMR spectra of P_ADH and recycled P_ADH; (c) FT-IR spectra of P_ADH, recycled P_ADH, P_ADH/P-3 film and recycled P_ADH/P-3 film.

Notably, the regenerated film retained approximately 30% of its original tensile strength, which is comparable to those of some commercial plastic films (**Figure 2-10**), demonstrating the practical feasibility of this recyclable system. Overall, the P-ADH/AAPVA films exhibited outstanding chemical recyclability owing to the reversible nature of the dynamic acylhydrazone bonds, offering an effective strategy for the development of degradable and recyclable high-performance bio-based thermoset materials.

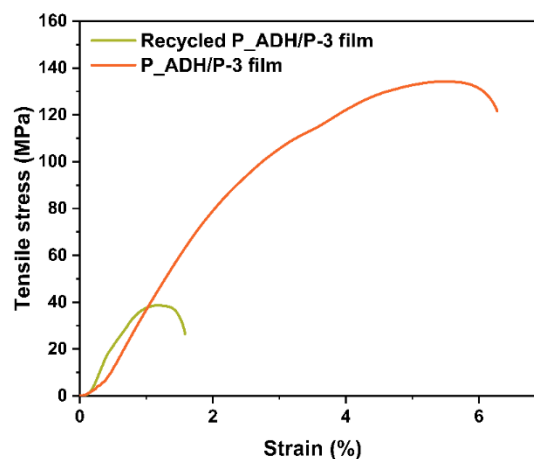


Figure 2-10. Tensile stress–strain curves of P_ADH/P-3 and recycled P_ADH/P-3 films.

2.4. Conclusions

In this study, γ -PGA was functionalized with ADH to produce P-ADH, which was subsequently crosslinked with AAPVA via a Schiff base reaction to form films containing dynamic acylhydrazone linkages. Among the various formulations, the P-ADH/P-3 film exhibited the highest tensile strength (123.5 ± 2.32 MPa) and Young's modulus (4.4 ± 0.022 GPa), along with a T_g of 106.2 °C, indicating mechanical robustness comparable to that of conventional high-performance plastics. In addition to their excellent mechanical performance, the P-ADH/AAPVA films exhibited outstanding optical transparency. Notably, the P-ADH/P-3 film exhibited UV-blocking capabilities, demonstrating its effectiveness as a UV-shielding material. These films also displayed excellent water resistance and chemical stability while maintaining their structural integrity when exposed to common organic solvents. Owing to the dynamic acylhydrazone bonds within the crosslinked network, the P-ADH/P-3 film was fully degraded in 1 M HCl/H₂O solution, confirming its chemical recyclability. The recovered P-

ADH was reused to fabricate a new film, illustrating the circularity of the material system. Collectively, these attributes underscore the strong potential of the P-ADH/AAPVA films for smart packaging and other renewable materials, thereby advancing the development of sustainable polymer alternatives.

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Chapter 3

Chitosan/Acetoacetylated Poly(vinyl alcohol) Nanofibers Incorporating Starch-Derived Carbon Quantum Dots with pH-Responsive Color Change for Wound Dressings

3.1. Introduction

With the growing challenges posed by population aging, diabetes, and infection-related complications, skin injuries resulting from diabetic foot ulcers, burns, and other conditions frequently progress into chronic wounds.^[1,2] These wounds are characterized by slow healing and high susceptibility to infection, significantly compromising patients' quality of life and imposing a substantial socioeconomic and healthcare burden.^[3,4] Although frequent debridement and dressing changes are clinically beneficial for infection control, they may also disrupt newly formed tissue, delay healing, and increase the risk of secondary infections.^[5,6] Therefore, the development of smart wound dressings capable of non-invasively indicating wound status has become a key research focus, as such dressings could enable real-time monitoring and reduce unnecessary interventions, ultimately improving patient comfort and care efficiency.^[7,8]

Different stages of wound healing are associated with characteristic changes in specific biomarkers, among which the pH of the wound microenvironment is widely regarded as a key physiological parameter reflecting the healing status of the tissue.^[9,10] Generally, healthy skin maintains a slightly acidic pH ranging from 4.0 to 6.0. In contrast, chronic wounds often exhibit a mildly alkaline environment ($\text{pH} > 7.0$), with dynamic fluctuations throughout the inflammatory, infectious, and reparative phases.^[11,12] Therefore, real-time monitoring of wound

pH can thus provide reliable insights into the healing stage, infection risk, and treatment response, offering clinicians critical information for timely and personalized interventions.^[13]

At present, a variety of pH-responsive materials have been widely explored for application in intelligent wound dressings, including colorimetric dyes (e.g., phenol red and anthocyanins),^[14,15] fluorescence-emitting probes,^[16,17] and conductive polymers exhibiting pH-dependent electrical changes (e.g., polyaniline and PEDOT: PSS).^[18,19] Among them, carbon quantum dots (CQDs), a new class of carbon-based fluorescent nanomaterials, have attracted increasing attention for visual pH monitoring owing to their excellent optical stability, good biocompatibility, and easily functionalizable surface chemistry.^[20,21] Polysaccharides have emerged as ideal carbon sources for the green synthesis of biocompatible CQDs due to their abundant availability, structural diversity, and favorable carbonization characteristics.^[22] Starch is a naturally occurring polysaccharide composed of glucose units linked by α -1,4 and α -1,6 glycosidic bonds.^[23] Its high hydroxyl content, regular structure, and large surface area make it a promising precursor for preparing CQDs with tunable optical properties and high pH sensitivity.^[24,25] However, pristine starch suffers from limited solubility and stability, which are unfavorable for forming a uniform and stable carbonization environment.^[26,27] In contrast, hydroxypropyl starch (HPS), obtained by substituting hydroxyl groups with hydroxypropyl moieties, exhibits enhanced water solubility and structural stability due to weakened internal hydrogen bonding, increased hydrophilicity, and the formation of an expanded three-dimensional network structure, thus providing a more suitable carbon source.^[28] Therefore, synthesizing fluorescent, pH-responsive CQDs from HPS aligns with sustainable material development principles and offers a new route for constructing non-toxic, efficient, and responsive platforms for intelligent wound monitoring.

Previous studies have explored the incorporation of CQDs into hydrogel systems for visual pH monitoring of wounds. For example, Yang et al.^[29] developed a tannic

acid/quaternized carboxymethyl chitosan/oxidized sodium alginate@carbon quantum dots hydrogel for monitoring diabetic wounds, while Zheng et al.^[30] co-doped phenol red and CQDs into hydrogels to achieve multimodal pH-responsive sensing. Despite the excellent moisture retention and biocompatibility of hydrogel-based wound dressings, their inherently weak mechanical properties limit their applicability in dynamic wound environments.^[31,32] In contrast, electrospun nanofiber dressings offer superior mechanical flexibility, conformability, breathability, and high surface area, making them more suitable for addressing the dual requirements of functionality and structural integrity in chronic wound management.^[33] Electrospinning, due to its compatibility with diverse polymer systems, precise structural control, and low fabrication cost, has become an ideal technique for constructing nanofibrous wound dressings.^[34] Conventional electrospinning typically employs organic solvents, which pose potential environmental and biosafety concerns; therefore, water-based electrospinning (green electrospinning) has garnered increasing attention.^[35–37] However, such nanofibers often suffer from insufficient water resistance and mechanical strength under hydrated conditions, making crosslinking an effective strategy to enhance their structural stability and functionality.^[38] Various crosslinking approaches for electrospun nanofibers have been reported, including in situ crosslinking, solution immersion, vapor-phase, and spray-based methods, encompassing both physical and chemical strategies.^[39]

Numerous studies have reported electrospun systems based on natural polymers such as collagen, oxidized cellulose, cellulose, and chitosan, which not only capitalize on the advantages of renewable resources but also exhibit excellent biocompatibility and biodegradability.^[40,41] Chitosan, the second most abundant natural linear aminopolysaccharide after cellulose, has been widely used in biomedical applications such as tissue repair and wound dressings due to its excellent biocompatibility, non-toxicity, and enzymatic degradability, and can be electrospun at high fractions using environmentally friendly solvent systems such as

aqueous concentrated acetic acid.^[42,43] Building on this foundation, we introduced acetoacetylated polyvinyl alcohol (AAPVA), a non-toxic, environmentally friendly polymer with excellent mechanical properties.^[44] Beyond improving processability and biosafety, the acetoacetyl groups in AAPVA impart multifunctional reactivity, enabling the formation of stable covalent crosslinked networks with functional polymers such as chitosan, thereby further enhancing the structural integrity and mechanical strength of the nanofibers under hydrated conditions.^[45,46] Such a water-based design strategy holds great potential for developing intelligent wound dressings that combine mechanical robustness with functional responsiveness, particularly in terms of wet-state stability and biological safety.

Based on the above background, the objective of this study was to develop a novel pH-responsive electrospun nanofibrous dressing composed of CQDs, CS, and AAPVA. We hypothesized that the abundant polar functional groups on starch-derived CQDs could endow the composite nanofibers with excellent pH sensitivity, while the biocompatible CS would form a stable covalent crosslinked network with AAPVA through Schiff base reactions, thereby enhancing the structural integrity and stability of the nanofibers under moist conditions. Based on this design concept, composite nanofibers were fabricated using an aqueous green electrospinning method, and their physicochemical properties and biocompatibility were systematically evaluated. Finally, a visual pH detection model was established using RGB image analysis to further verify their potential for intelligent management and remote monitoring of chronic wounds.

3.2. Experimental Section

3.2.1. Materials

The hydroxypropyl starch (propylene chlorohydrin compounds: less than 1.0 ppm, a degree of hydroxypropyl substitution of 20.0 %, corresponding to a molar substitution of

approximately 0.7 per anhydroglucose unit) was obtained from Matsutani Chemical Industry Co., Ltd. (Itami, Japan). The high molecular weight chitosan (CAS RN: 9012-76-4, 310–375 kDa, 73% deacetylated) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Ethylenediamine (EDA) was purchased from Fujifilm Wako Pure Chemical Co. (Osaka, Japan). Acetoacetylated polyvinyl alcohol (AAPVA, degree of hydrolysis: 99%, $M_w \approx 44,000 \text{ g mol}^{-1}$, acetoacetyl substitution degree: 5%) was kindly supplied by The Nippon Synthetic Chemical Industry Co., Ltd. (Tokyo, Japan).

3.2.2. Synthesis of CQDs

CQDs were synthesized via a one-step hydrothermal method. Specifically, 3 g of starch was gelatinized in 16 mL of deionized water at 90 °C under magnetic stirring for 1 h until complete gelatinization occurred. After complete dissolution, the mixed solution was transferred into a Teflon-lined autoclave, adding 4 mL of ethylenediamine with thorough mixing. The reaction was conducted at 200 °C for 6 hours, during which the polysaccharide chains underwent hydrolysis and ring-opening reactions to generate reactive aldehyde intermediates. These intermediates further participated in condensation and polymerization reactions, accompanied by partial carbonization. Upon completion, a brownish-yellow colloidal solution was obtained. The mixture was centrifuged at 12,000 rpm to remove large particulate impurities, and the supernatant was further filtered through a 0.22 μm microporous membrane. The resulting filtrate was subjected to dialysis against 1000 mL of deionized water for 3 days using a dialysis bag (molecular weight cutoff is 1kDa) to remove small molecular impurities. The solution was freeze-dried at $-40 \text{ }^{\circ}\text{C}$ under a vacuum pressure of 10 MPa to yield a light-yellow solid powder as the final CQD product. The powder was then stored under controlled conditions at 25 °C and 40 % relative humidity until further use.

3.2.3. Synthesis of CQDs/CS/AAPVA nanofibers

Preliminary experiments were conducted to optimize the electrospinning precursor composition. AAPVA solutions at 5%, 10%, and 15% (w/v) were first evaluated under electrospinning conditions. Subsequently, CQDs were incorporated into 10% (w/v) AAPVA solutions at concentrations of 0%, 0.5% and 1% (w/v). For CS incorporation, 0%, 0.5% and 1% (w/v) CS solutions prepared in 2% (v/v) acetic acid were blended with the CQDs/AAPVA solution, and different mixing ratios (90:10, 80:20, and 70:30, v/v) were investigated. Based on the optimized parameters, a homogeneous precursor solution containing 10% (w/v) AAPVA and 1% (w/v) CQDs was prepared in deionized water at 90 °C under magnetic stirring for 6 hours. Separately, a 1% (w/v) CS solution was prepared in 2% (v/v) acetic acid ultrasonicated at room temperature for 1 h and mixed with the CQDs/AAPVA solution at a volume ratio of 80:20 under magnetic stirring at room temperature for 10 min to obtain the electrospinning blend. Electrospinning was carried out at 25 °C and 45% relative humidity using a syringe with a 0.8 mm diameter needle, a flow rate of 0.5 mL/h, an applied voltage of 20 kV, and a tip-to-collector distance of 10 cm. Four types of nanofibrous scaffolds were fabricated according to this procedure: (I) AAPVA, (II) CQDs/AAPVA, (III) CS/AAPVA, and (IV) CQDs/CS/AAPVA.

3.2.4. Characterization

The morphology of the CQDs was characterized using transmission electron microscopy (TEM, JEM-2100, JEOL Ltd., Tokyo, Japan) by dropping the dispersion onto a high-resolution carbon film and then drying under vacuum. The height of the CQDs was measured using atomic force microscopy (AFM, SPI3800N/SPA-400, Seiko Instruments Inc., Tokyo, Japan) after depositing the dispersion onto a freshly cleaved mica surface and allowing it to air-dry. The hydrodynamic diameter was determined using a particle size analyzer (ELSZ-

2000 Series, Otsuka Electronics Co. Ltd., Osaka, Japan). All the above measurements were carried out using a 0.2 wt% CQD aqueous dispersion. The chemical composition and structural features of the CQDs and electrospun nanofiber membranes were confirmed by X-ray diffraction (XRD, SmartLab, Rigaku Co. Ltd., Tokyo, Japan) and X-ray photoelectron spectroscopy (XPS, KRATOS ULTRA2, Shimadzu Co. Ltd., Kyoto, Japan). Fourier transform infrared (FT-IR) spectra in the 500–4000 cm^{-1} range were recorded using a Nicolet iS5 spectrometer (Thermo Fisher Scientific, Waltham, USA). Ultraviolet-visible (UV-vis) absorption spectra of the CQDs were obtained using a UV-vis spectrophotometer (U-2810, Hitachi, Ltd., Tokyo, Japan). A microplate reader (SH-9000Lab, Yamato Scientific Co. Ltd., Tokyo, Japan) was used to analyze the photoluminescence (PL) spectra, 3D fluorescence contour map and Commission Internationale de L'Eclairage (CIE) coordinate of the CQDs. The nanofibers were mounted on aluminum stubs and sputter-coated with a 20 nm gold–palladium layer before morphological and diameter observation using a scanning electron microscope (Quattro S, Thermo Fisher Scientific, Waltham, USA). The water contact angle of the nanofibers was measured at 25 °C using a contact angle goniometer (Drop Master DM300, Kyowa Interface Science Co., Ltd., Saitama, Japan) by applying a 0.5 μL droplet of deionized water onto the nanofibers. Mechanical properties were evaluated using a universal testing machine (EZ Graph, Shimadzu Co., Kyoto, Japan) equipped with a 10 N load cell at a strain rate of 20 mm/min, under conditions of 25 °C and 40% relative humidity, with sample dimensions of 5 $\times$ 20 mm. Thermogravimetric analysis (TGA) was conducted to assess thermal stability using a thermogravimetric analyzer (STA200RV, Hitachi High-Tech Science Co., Tokyo, Japan) by heating the samples from 30 °C to 500 °C at a rate of 10 °C/min under a nitrogen atmosphere to evaluate their thermal stability.

3.2.5. Swelling ratio

Dried samples were weighed and immersed in 10 mL of phosphate-buffered saline (PBS, pH 7.4) at 37 °C to evaluate the swelling ratio (SR). At predetermined time intervals, the samples were carefully removed, excess surface liquid was gently blotted using filter paper, and the swollen samples were weighed. The swelling ratio was calculated using the **Eq. (3-1)**.^[47]

$$SR = \frac{W_{ss} - W_{ds}}{W_{ds}} \times 100 \quad (3-1)$$

where W_{ds} and W_{ss} represent the sample weights before and after swelling, respectively. Each experiment was conducted in triplicate.

3.2.6. In vitro degradation

Nanofibrous membranes with equal initial weights were immersed in 10 mL of PBS solution (pH 7.4) at 37 °C. After 7 days, the samples were removed, rinsed with distilled water, and dried. The weight retention (WR) of the membranes was calculated using the **Eq. (3-2)**.^[48]

$$WR = \frac{W_m - W_n}{W_n} \times 100 \quad (3-2)$$

where W_n and W_m represent the sample weights before and after degradation, respectively. Each experiment was conducted in triplicate.

3.2.7. Morphological stability

To assess the morphological stability during *in vitro* degradation, samples were immersed in 10 mL of PBS buffer (pH 7.4) at 37 °C and retrieved after 1, 3, and 5 days. After rinsing with distilled water, samples were immediately frozen in liquid nitrogen and subsequently freeze-dried at −40 °C under 10 MPa. The surface morphology of the freeze-dried samples was examined using scanning electron microscopy (SEM, SU5000, Hitachi High-Tech

Science Co., Japan). Before observation, the samples underwent gold sputtering on the MSP-1S magnetron sputter and were imaged using a Schottky field-emission electron gun.

3.2.8. pH-responsive testing of CQDs/CS/AAPVA nanofibers

The color changes of CQDs/CS/AAPVA nanofibers in buffer solutions with different pH values were investigated. Rectangular nanofiber samples (1×2 cm) were prepared and immersed in buffer solutions with pH values of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 at 37 °C for 30 seconds. After exposure, the color-changed nanofibers were photographed using a smartphone camera. The images were then processed using the free image analysis software to extract the corresponding RGB values. To minimize discrepancies caused by variations in ambient lighting between the captured image and the actual color of the sample, each measurement was repeated three times.

3.2.9. Biocompatibility: In vitro MEM elution cytotoxicity

According to the recommendations of ISO-10993 standards, cell viability was evaluated via an indirect assay.^[49] The extraction media were prepared by incubating pieces of the electrospun scaffolds ($6 \text{ cm}^2/\text{ml}$) in cell culture medium at 37°C for three days. L929 fibroblast cells were seeded at a concentration of 3,000 cells/well into 96-well plates and cultured with DMEM (Gibco, United States), 10% Fetal calf serum (FSC, Gibco, United States), 1% Penicillin-Streptomycin/Amphotericin B Mix (PanBiotech, Germany) for 24 hours. Subsequently, the culture medium was removed and replaced with extraction media, and the cells were further incubated for another 24 hours. Normal medium was used as the negative control, while medium containing 10% DMSO (Sigma-Aldrich, United States) was the positive control. Five wells were tested for each material ($n = 5$). Cell metabolic activity after exposure to different extract media was assessed using the CellTiter-Blue® Cell Viability Assay

(Promega, Germany). To evaluate cell morphology, fluorescence imaging (Nikon, Japan) was performed using Phalloidin-iFluor™ 488 (Cayman Chemicals, United States) and DAPI (Carl ROTH, Germany) for nuclear staining, allowing visualization of cell morphology and viability.

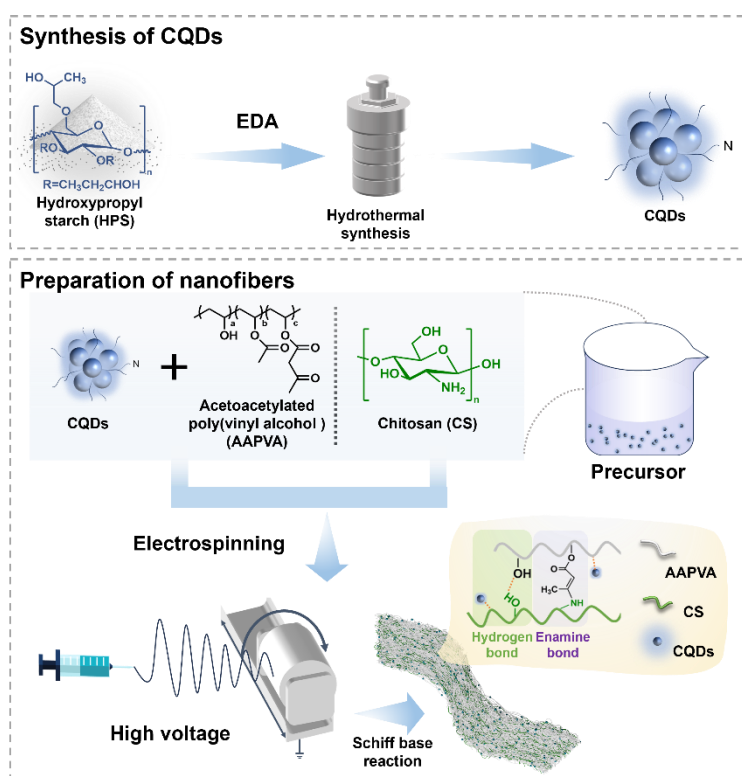
3.2.10. Statistical analysis

All experimental data from three or more independent experiments are expressed as mean $\pm$ standard deviation. One-way ANOVA was used to estimate all data. (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

3.3. Results and Discussion

3.3.1. Synthesis and characterization of CQDs

The synthesis process of CQDs using HPS as the carbon precursor and ethylenediamine as the nitrogen source via a one-pot hydrothermal method is illustrated in **Figure 3-1**. TEM and



Scheme 3-1. Schematic of the preparation procedure of CQDs and electrospun nanofibers.

AFM images revealed that the resulting CQDs were quasi-spherical nanoparticles with an average diameter of 2.39 ± 0.64 nm (**Figure 3-2a** and **3-2b**). The presence of these functional groups ensures excellent dispersibility of the CQDs in aqueous media. The average hydrodynamic diameter of the CQD aqueous suspension was 4.3 nm, slightly larger than the TEM-measured size (**Figure 3-2c**). XRD pattern of the synthesized CQDs (**Figure 3-3a**) displayed a broad diffraction peak centered around $2\theta \approx 20^\circ$, which is larger than the (002) interlayer spacing of graphite, indicating a highly disordered (amorphous) carbon structure. This is likely attributed to the abundance of oxygen-containing surface groups.^[50]

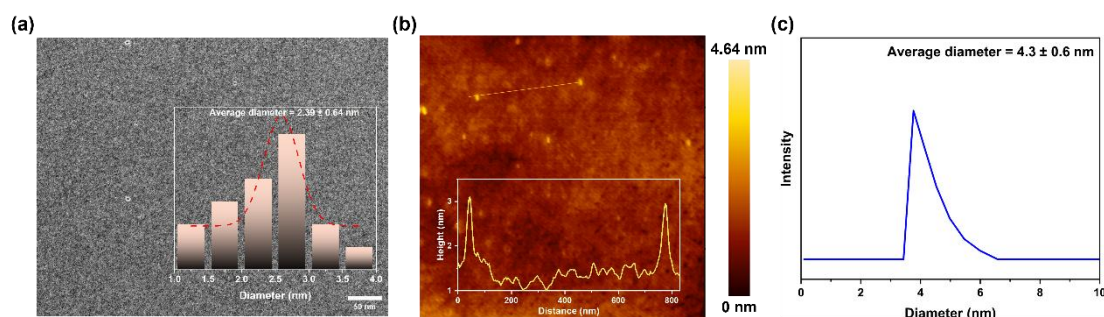


Figure 3-2. (a) TEM images of CQDs, a-Inset: Particle size distribution of CQDs; (b) AFM images of CQDs, b-Inset: Height of CQDs; (b) particle size distribution of CQDs obtained by DLS analysis.

The chemical structure of the CQDs was characterized by FT-IR and XPS. As shown in **Figure 3-3b**, new absorption bands emerged at ~ 1650 cm^{-1} and 1560 cm^{-1} in the CQD spectrum, corresponding to C=O and C=C stretching vibrations, respectively.^[51,52] A peak at 1405 cm^{-1} was assigned to C–N stretching, confirming the introduction of nitrogen-related groups.^[53] The full survey spectrum (**Figure 3-3c**) shows characteristic peaks at binding energies of 284.0, 399.0, and 532.0 eV, corresponding to C 1s, N 1s, and O 1s, respectively. This successful nitrogen doping is expected to enrich the surface states of the CQD framework, thereby enhancing their photoluminescent properties. As shown in the high-resolution C 1s spectrum (**Figure 3-3d**), four peaks were observed at 284.60, 285.94, 286.37, and 287.39 eV,

corresponding to C–C/C=C, C–O, C–N, and C=O, respectively. The N 1s spectrum (**Figure 3-3e**) revealed three nitrogen configurations: pyridinic N (398.90 eV), pyrrolic N (399.76 eV), and graphitic N (401.63 eV). In the O 1s spectrum (**Figure 3-3f**), two peaks at 532.38 and 533.81 eV were attributed to C=O and C–O, respectively. These results collectively confirm the functional groups identified by FTIR and demonstrate the successful incorporation of nitrogen atoms into the CQD structure.^[54,55]

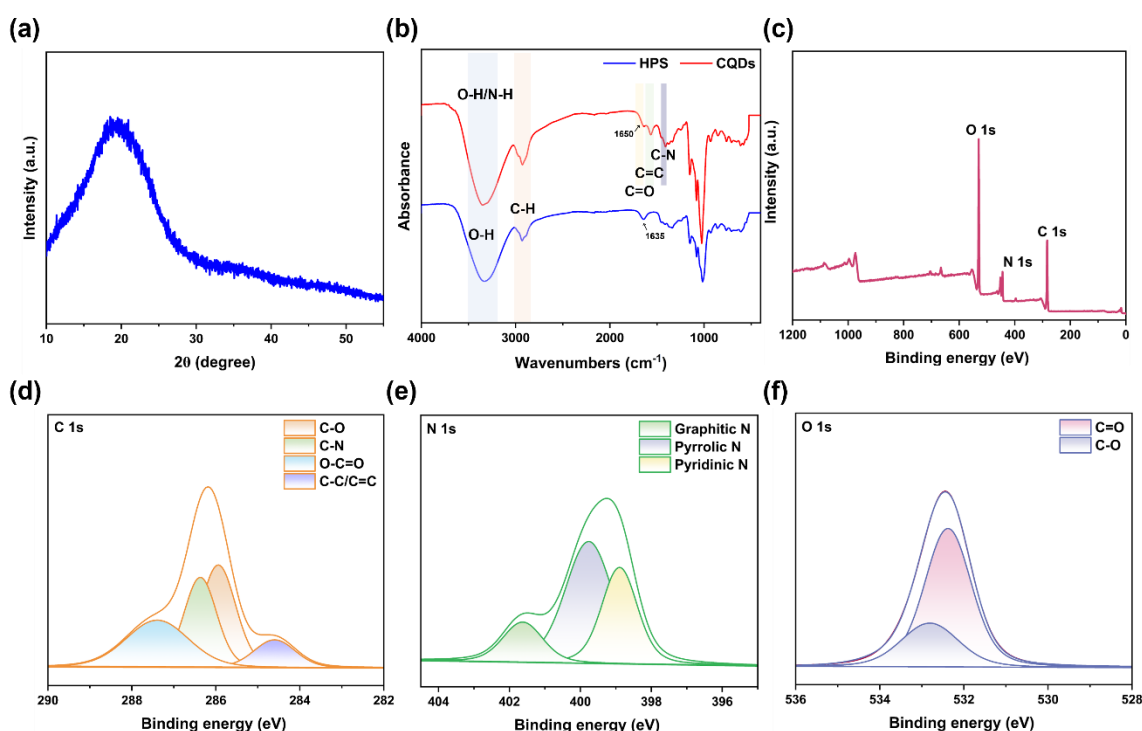


Figure 3-3. (a) XRD patterns of CQDs; (b) FTIR spectra of HPS and CQDs; (c) full-scan XPS spectra, (d) C 1s, (e) N 1s, and (f) O 1s XPS spectra of the prepared CQDs.

3.3.2. Fluorescence properties of CQDs

The photophysical properties of the CQDs are presented in **Figure 3-4**. As shown in **Figure 3-4a**, the CQD aqueous solution appeared transparent under daylight (DL), while a distinct cyan–blue fluorescence was observed under 365 nm ultraviolet (UV) light irradiation. The UV–vis absorption spectrum (**Figure 3-4b**) displayed three characteristic peaks. A broad absorption band centered at 230 nm is attributed to the π – π^* transitions of C=C bonds within

the sp^2 -hybridized carbon core. A shoulder peak around 283 nm corresponds to $n-\pi^*$ transitions associated with oxygen-containing surface functional groups on sp^3 -hybridized carbon. Additionally, a weak shoulder at approximately 370 nm is ascribed to heteroatom-doped surface states or energy traps arising from the $n-\pi^*$ transitions of surface functional groups.^[56] These absorption features indicate the coexistence of conjugated carbon domains and oxygenated functional groups on the CQD surface.^[57] The optical band gap (E_g) of the CQDs was estimated from the Tauc plot (**Figure 3-4c**) using the **Eq. (3-3)**.^[58]

$$(\alpha h\nu)^2 = A(h\nu - E_g) \quad (3-3)$$

Where α is the normalized absorption coefficient, $h\nu$ is the photon energy, and E_g represents the optical band gap. By extrapolating the linear portion of the Tauc curve, the direct band gap of the CQDs was determined to be approximately 2.94 eV. This band gap value is consistent

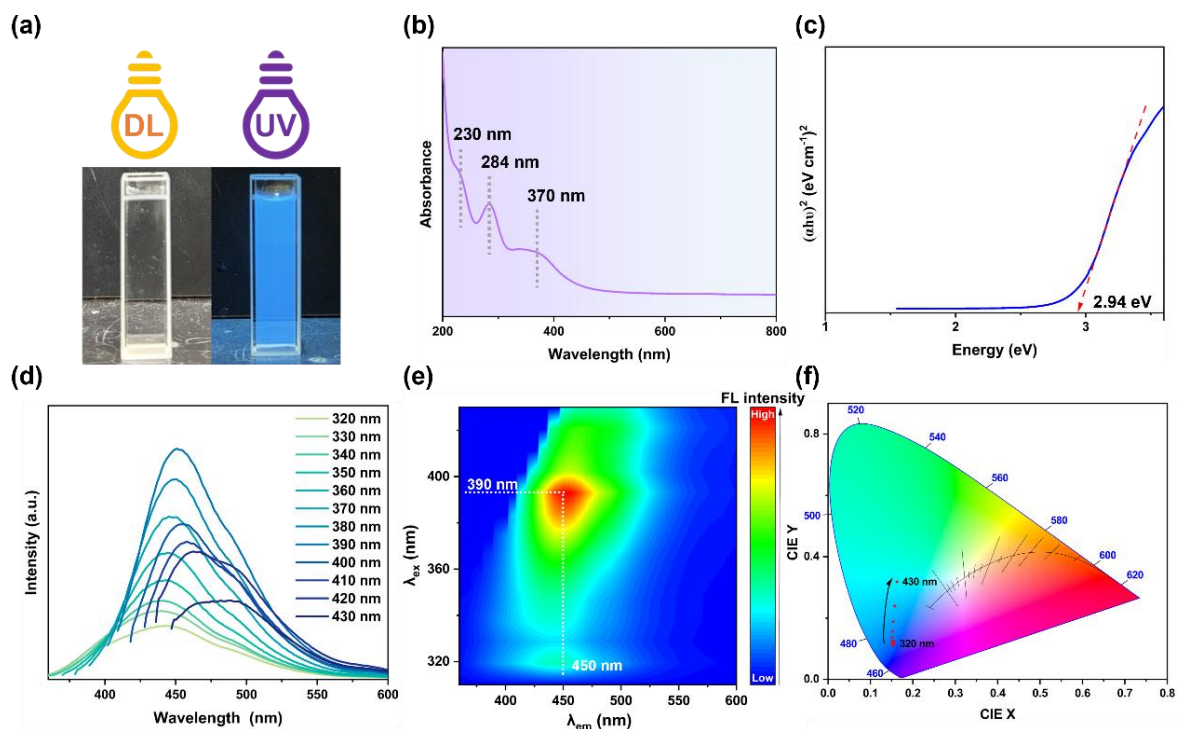


Figure 3-4. (a) digital photographs of CQDs solutions under visible and UV light excitation; (b) UV–vis absorption spectra of CQDs; (c) Tauc plots of CQDs; (d) Excitation-dependent PL spectra of CQDs; (e) Three-dimensional fluorescence mapping of CQDs; (f) CIE coordinate of CQDs.

with the cyan–blue fluorescence emission observed under 365 nm excitation, indicating that the electrons in the CQDs can be readily excited to the conduction band, resulting in strong photoluminescence even under low-energy excitation.^[56] The band gap of CQDs is highly dependent on their core carbon structure, surface states, and heteroatom doping.

To further investigate the photoluminescence (PL) properties, fluorescence emission spectra under different excitation wavelengths were recorded (**Figure 3-4d**). The emission peak was most intense at 450 nm when excited at 390 nm. As the excitation wavelength increased from 320 to 430 nm, a progressive red shift of the emission peak was observed, indicating a typical excitation-dependent behavior, which suggests the involvement of multiple emissive energy states or surface defect states in the PL process. Based on this, a 3D fluorescence contour map (**Figure 3-4e**) was obtained, which confirmed the optimal excitation and emission wavelengths of 390 nm and 450 nm, respectively. Additionally, the color evolution of the emitted light under different excitation wavelengths was analyzed using the CIE coordinate (**Figure 3-4f**). The emission color gradually shifted from blue to cyan blue as the excitation wavelength increased from 320 to 430 nm, confirming the excitation-dependent fluorescence behavior. These results demonstrate that the synthesized CQDs possess moderate band gap energy, pronounced excitation-dependent fluorescence, and excellent photostability, providing a robust functional basis for their application in pH-responsive, visually trackable wound dressings for real-time monitoring of the healing process.

To evaluate the photostability of the CQDs, their fluorescence was monitored under continuous UV irradiation in both aqueous and PBS solutions (**Figure 3-5a and 3-5b**). Although a gradual decrease in intensity was observed over time, the CQDs retained more than 85% of their initial fluorescence after 3 h of irradiation, demonstrating sufficient stability for practical use. Furthermore, the photoluminescence response of freshly prepared samples and those stored for six months under ambient conditions showed no obvious difference under 365

nm excitation, confirming the long-term stability of the synthesized CQDs (**Figure 3-5c**). These results demonstrate that the synthesized CQDs possess moderate band gap energy, pronounced excitation-dependent fluorescence, and excellent photostability, providing a robust functional basis for their application in pH-responsive, visually trackable wound dressings for real-time monitoring of the healing process.

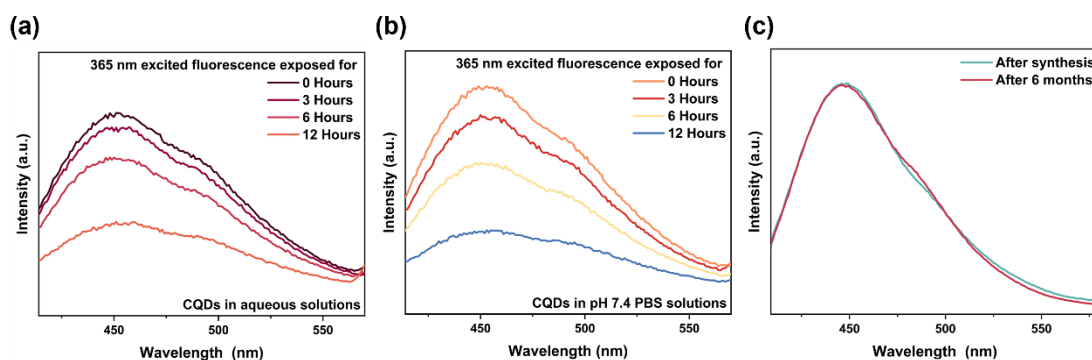


Figure 3-5. Fluorescence spectra of CQDs after UV irradiation for 0, 3, 6, and 12 h in (a) aqueous solution and (b) pH 7.4 buffer solution; (c) fluorescence spectra of freshly synthesized CQDs and those stored for six months under 365 nm excitation.

3.3.3. Fabrication and characterization of nanofibers

To address the issue of secondary tissue damage caused by repeated infections and frequent dressing changes, and to facilitate wound healing alongside real-time monitoring and

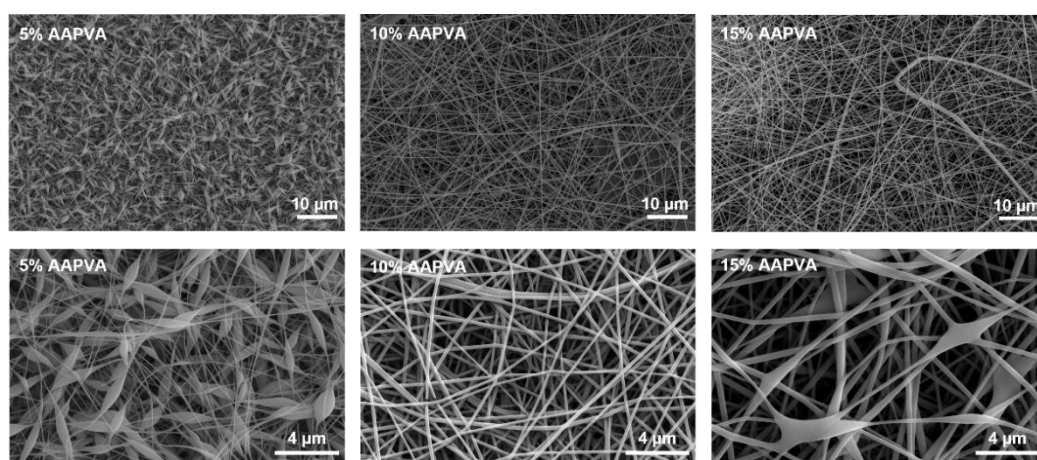


Figure 3-6. SEM Images of nanofibers containing different AAPVA concentrations (5%, 10%, 15%).

auxiliary treatment, a smart-responsive CQDs/CS/AAPVA composite nanofiber dressing was fabricated via electrospinning. In preliminary optimization, 10% (w/v) CQDs observed under UV light.

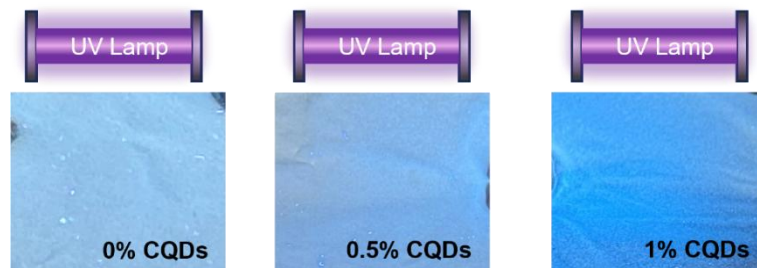


Figure 3-7. Nanofibers containing 0%, 0.5%, and 1% CQDs observed under UV light.

AAPVA was identified as the most suitable concentration, as lower or higher concentrations resulted in bead formation or unstable jetting (**Figure 3-6**). For CQDs loading, 1% (w/v) provided uniform and strong fluorescence under UV illumination, whereas 0.5% (w/v) produced weak and uneven signals (**Figure 3-7**). With respect to the CS fraction, the 80:20 (CQDs/AAPVA:CS) mixing ratio offered a balance between viscosity, conductivity, and spinnability, leading to uniform fibers with minimal defects (**Figure 3-8**). Attempts to electrospin CS/CQDs alone under comparable conditions failed to yield continuous fibers, likely due to insufficient entanglement and high conductivity. These optimized conditions were therefore adopted for subsequent scaffold fabrication.

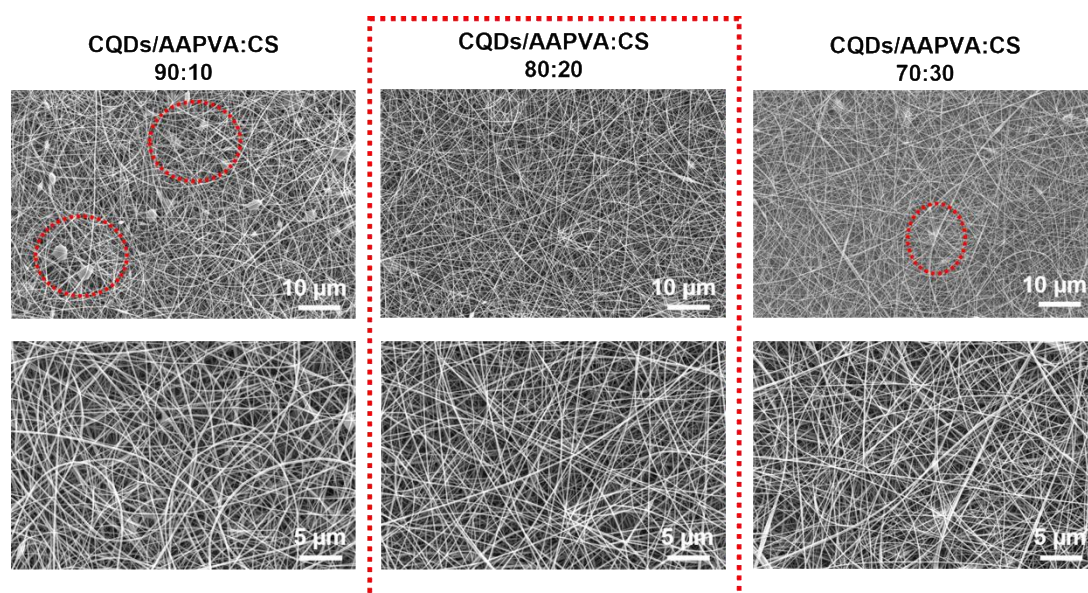


Figure 3-7. SEM morphology of CQDs/CS/AAPVA nanofibers with different mixing ratios.

The surface morphology and fiber diameter distribution were analyzed by SEM (**Figure 3-8a** and **3-8b**). The results revealed a progressive reduction in fiber diameter across the four groups: AAPVA (261.95 ± 30.78 nm), CQDs/AAPVA (207.46 ± 33.24 nm), CS/AAPVA (167.46 ± 34.58 nm), and CQDs/CS/AAPVA (141.49 ± 18.95 nm). Incorporating polar functional groups from both CQDs and chitosan significantly increased the conductivity of the electrospinning solution, thereby enhancing the electrostatic stretching effect under high voltage conditions.^[59] This improvement facilitated the formation of thinner nanofibers and a denser, more uniform fiber network in the CQDs/CS/AAPVA group (**Table 3-1**). The wettability of the nanofiber dressings was evaluated by static water contact angle (WCA) measurements (**Figure 3-8c**). A water droplet was placed on the fiber surface, and the contact angle was recorded at 0 s and 60 s. Due to the abundance of hydrophilic hydroxyl groups on the fiber surface, the water contact angle rapidly decreased within a short period, demonstrating pronounced hydrophilicity.^[60] This behavior is advantageous for the rapid absorption of wound exudates and supports the establishment of a moist environment conducive to wound healing.

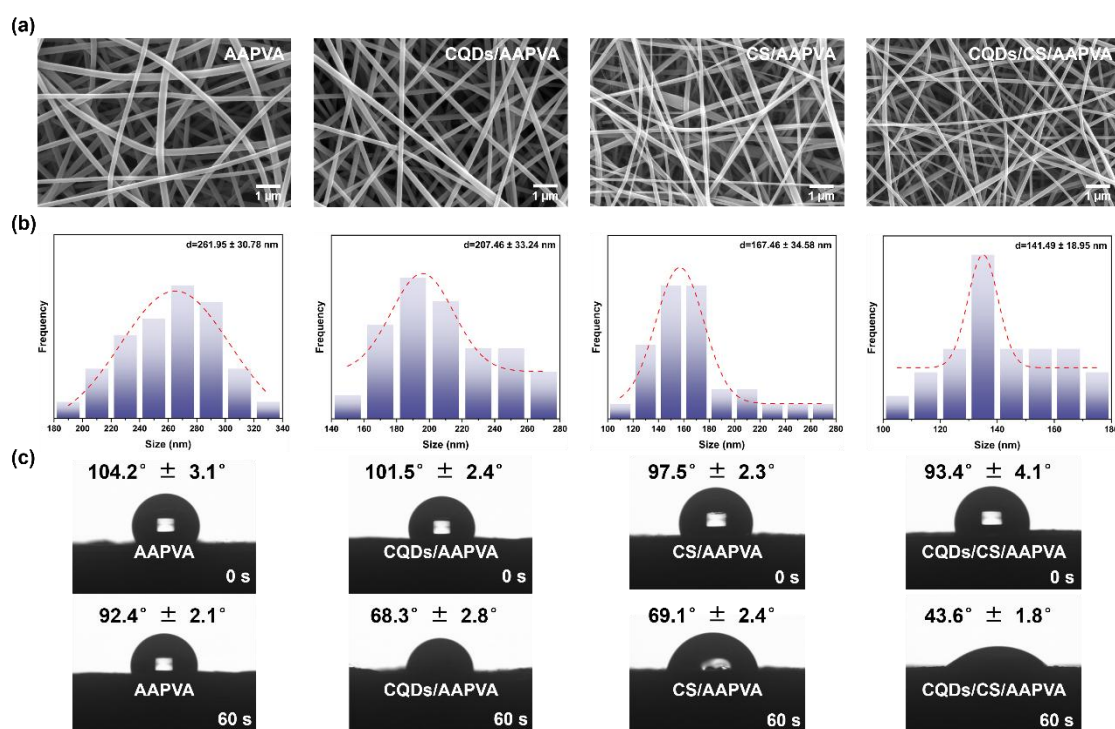


Figure 3-8. (a) SEM micrographs, (b) diameter distribution, and (c) water contact angles (0 and 60 s) of AAPVA, CODs/AAPVA, CS/AAPVA, and CODs/CS/AAPVA nanofibers.

Table 3-1 The diameters of AAPVA, CQDs/AAPVA, CS/AAPVA, and CQDs/CS/AAPVA nanofibers.

Nanofibers	Diameters (nm)
AAPVA	261.95 ± 30.78
CQDs/AAPVA	207.46 ± 33.24
CS/AAPVA	167.46 ± 34.58
CQDs/CS/AAPVA	141.49 ± 18.95

The chemical structure of the nanofibers was further explored by FT-IR (**Figure 3-9a**). In the spectra of CS/AAPVA and CQDs/CS/AAPVA composite nanofibers, a clear shift was observed in the characteristic stretching vibration band of the acetoacetyl C=O group ($1700\text{--}1730\text{ cm}^{-1}$), along with the emergence of new absorption peaks at ~ 1650 and $\sim 1605\text{ cm}^{-1}$,

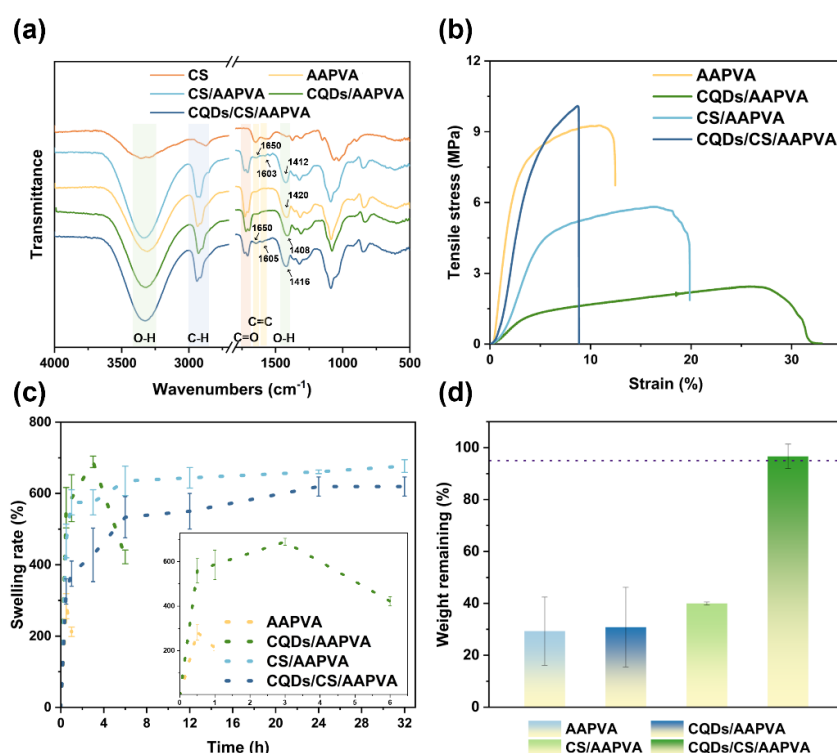


Figure 3-9. (a) FT-IR of CS powder, AAPVA, CS/AAPVA, CQDs/AAPVA and CQDs/CS/AAPVA nanofibers; (b) the stress-strain curve, (c) the swelling rate, and (d) *in vitro* degradation properties in PBS solution (pH = 7.4) within 5 days of AAPVA, CS/AAPVA, CQDs/AAPVA and CQDs/CS/AAPVA nanofibers.

representing the formation of enamine bonds.^[61]

These results indicate the occurrence of Schiff base reactions between the amino groups of CS and the acetoacetyl groups of AAPVA.

Moreover, the interaction between CQDs and AAPVA can be assigned to hydrogen bonding, which helps immobilize CQDs on the AAPVA chains, evidenced by a shift of the –OH bending

vibration peak from 1420 cm^{-1} to around 1408 cm^{-1} . In addition, the observed blue shift of the –OH vibration band further indicates the presence of hydrogen-bonding interactions among AAPVA, CQDs, and CS, suggesting that both chemical and physical interactions contribute to the stability and structural modulation of the composite nanofibers. The crystalline structures of CS, AAPVA, CQDs/AAPVA, CS/AAPVA, and CQDs/CS/AAPVA nanofibers were characterized by XRD (**Figure 3-10**). All samples exhibited broad diffraction humps centered around $2\theta \approx 20^\circ$, with no sharp crystalline peaks observed, indicating predominantly amorphous structures. This amorphous nature mainly originates from the AAPVA matrix, and importantly, the incorporation of CQDs and CS into the electrospinning solution prior to fiber formation did not disrupt the amorphous nature or the uniform morphology of the resulting nanofibers. Amorphous materials typically possess good flexibility and film-forming ability, which are advantageous for producing uniform, dense, and functionally integrated nanofiber dressings, particularly suitable for flexible and conformable wound care applications.

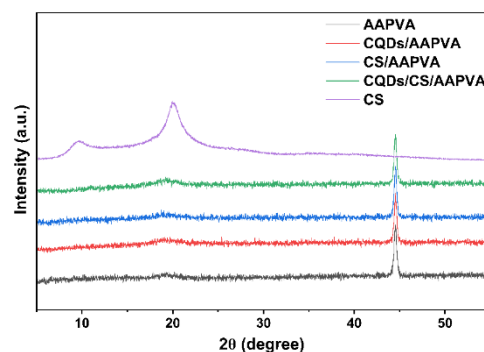


Figure 3-10. XRD patterns of CS powder, AAPVA, CS/AAPVA, CQDs/AAPVA and CQDs/CS/AAPVA nanofibers.

Representative stress-strain curves for nanofiber dressings are shown in **Figure 3-9b** and **Table 3-2**. The incorporation of chitosan significantly improved the mechanical performance by forming chemical crosslinks through Schiff base reactions with AAPVA. This chemical interaction enhanced inter-chain connectivity, enabling the material to maintain

sufficient strength while exhibiting improved ductility. Furthermore, although incorporating CQDs slightly reduced the ultimate tensile strength, it notably enhanced the flexibility and toughness of the nanofibers. This enhancement is likely attributed to forming a flexible hydrogen-bonded network between CQDs and polymer chains. Comparative analysis of the mechanical properties of CS/AAPVA and CQDs/AAPVA nanofibers revealed a synergistic effect between CQDs and CS, further improving the tensile performance. The resulting CQDs/CS/AAPVA composite nanofibers exhibited a maximum tensile strength of approximately 10 MPa, along with adequate flexibility, indicating excellent overall mechanical performance. Such flexibility and strength are particularly beneficial for enabling close conformity of the dressing to wounds on various anatomical surfaces, thus improving clinical applicability.

Table 3-2. Transparency value (at 600 nm) of AAPVA, P_ADH, PGA/P-3 and P_ADH/AAPVA films.

Nanofibers	Tensile stress at break (MPa)	Elongation at break (%)
AAPVA	8.2 ± 0.2	10.7 ± 1.6
CQDs/AAPVA	2.7 ± 0.6	26.9 ± 0.7
CS/AAPVA	5.2 ± 1.0	25.7 ± 6.2
CQDs/CS/AAPVA	10.0 ± 1.2	9.6 ± 0.8

Figure 3-9c illustrates the swelling behavior of the nanofiber materials in pH 7.4 PBS buffer. AAPVA and CQDs/AAPVA fibers exhibited structural collapse within 2 h and 6 h, preventing further quantitative measurement of swelling. In contrast, CS/AAPVA and CQDs/CS/AAPVA fibers displayed a multi-stage swelling profile with apparent plateaus at ~2, 4, 6, and 24 h, reaching $676.9 \pm 17.8\%$ and $619.4 \pm 26.8\%$ at 24 h while maintaining fibrous integrity. FTIR spectra of CS/AAPVA and CQDs/CS/AAPVA after 24 h revealed a blue shift of the O–H band, indicating new hydrogen bond formation, which together with the more stable enamine linkages contributed to progressive network densification (**Figure 3-11**).

To further evaluate *in vitro* degradation behavior, the mass loss of nanofibers after 5 days of immersion in PBS (pH = 7.4) was measured, as shown in **Figure 3-9d**. Only the CQDs/CS/AAPVA sample exhibited a mass loss below 5%,

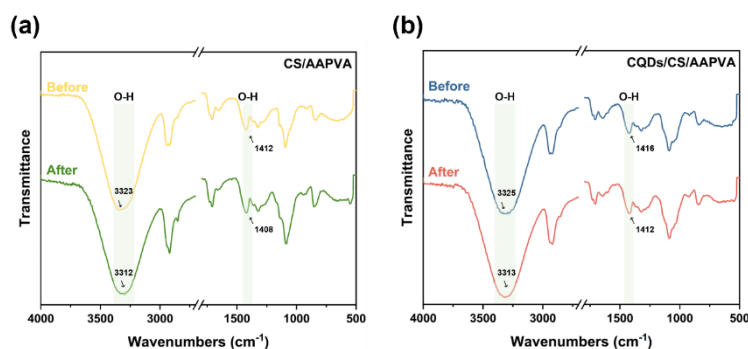


Figure 3-11. FTIR spectra of (a) CS/AAPVA nanofiber and (b) CQDs/CS/AAPVA nanofiber before and after wetted by water.

suggesting that the synergistic interaction between CQDs and CS with AAPVA generated a denser and more stable nanofiber network. In contrast, the other samples underwent pronounced disintegration, leaving only large fragments as residual mass. This stability was further confirmed by SEM analysis (**Figure 3-12**), which revealed distinct morphological evolution at 1, 3, and 5 days. Specifically, AAPVA and CQDs/AAPVA gradually lost their fibrous

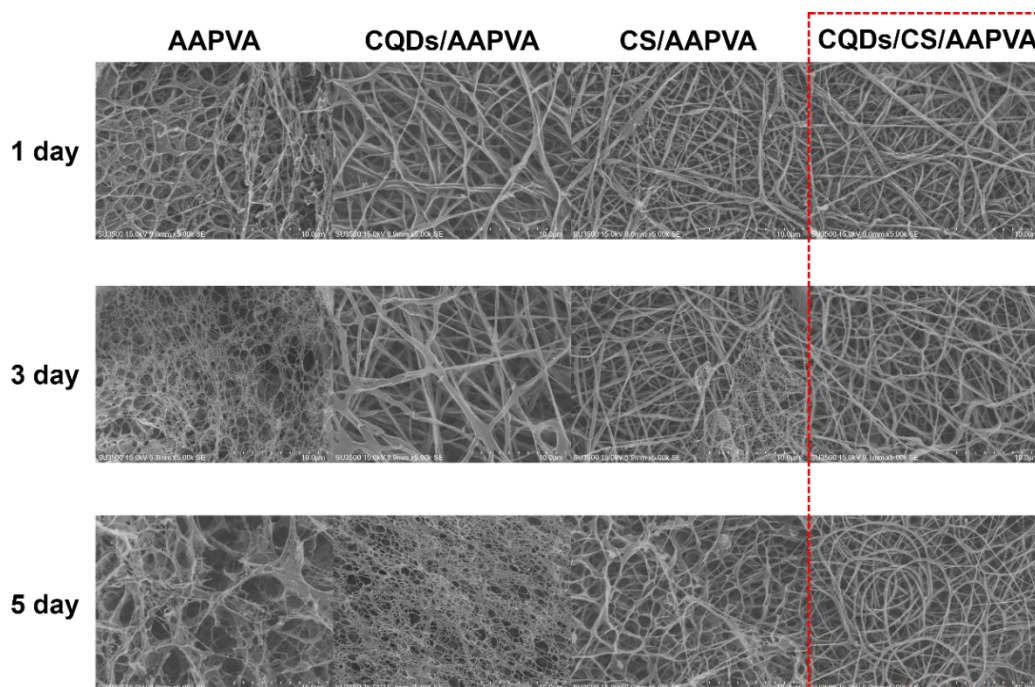


Figure 3-12. SEM morphology of AAPVA, CQDs/AAPVA, CS/AAPVA, and CQDs/CS/AAPVA nanofibers in PBS (pH=7.4) buffer for 1-, 3-, and 5-day *in vitro* degradation tests.

morphology due to swelling, network expansion, and collapse, while CS/AAPVA and especially CQDs/CS/AAPVA largely preserved their fibrous structure even after 24 h, consistent with swelling results. The superior stability of CQDs/CS/AAPVA is attributed to both the chemical crosslinking between CS and AAPVA and the additional physical interactions introduced by CQDs, which synergistically enhance crosslinking density and suppress fiber merging. Additionally, the thermal stability of the nanofibers was assessed using thermogravimetric analysis (TGA) (**Figure 3-13**). All nanofiber samples exhibited initial decomposition temperatures above 200 °C, indicating satisfactory thermal resistance suitable for biomedical applications. The major degradation peaks were observed at ~365 °C for AAPVA, ~345 °C for CQDs/AAPVA, ~358 °C for CS/AAPVA, and ~352 °C for CQDs/CS/AAPVA. The lower degradation temperature of CQDs/AAPVA indicates that the interaction between CQDs and AAPVA is mainly physical, providing interfacial contact but limited thermal reinforcement. In contrast, the incorporation of CS introduced partial crosslinking with AAPVA, which slightly stabilized the structure and shifted the degradation peak upward. Overall, the addition of CQDs or CS caused only minor variations, suggesting that the overall thermal stability of the composite nanofibers remained largely unchanged.

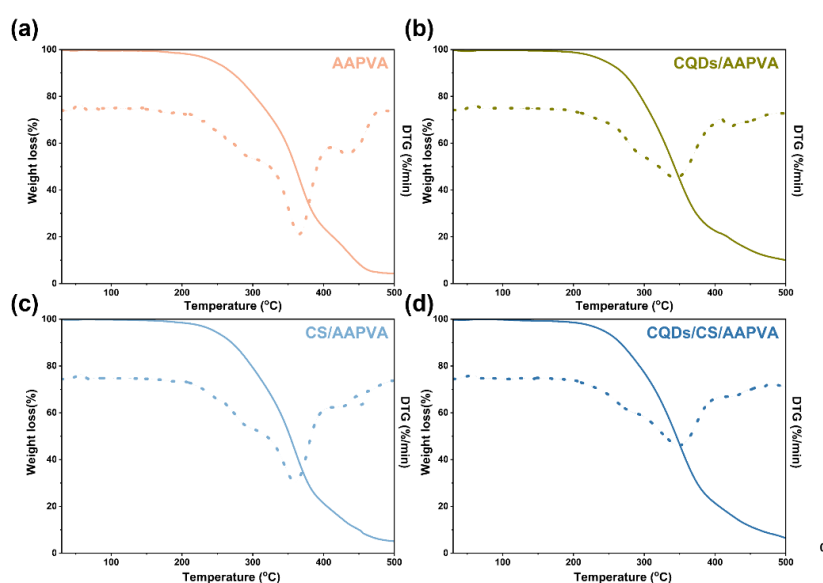


Figure 3-13. TGA and DTG thermogram of (a) AAPVA, (b) CQDs/AAPVA, (c) CS/AAPVA, and (d) CQDs/CS/AAPVA nanofibers.

3.3.4. pH responsiveness of CQDs/CS/AAPVA nanofibers

The pH-responsive fluorescence behavior of CQDs is of great importance for their application in wound monitoring. To evaluate this property, the optical response of CQDs in aqueous solutions with pH values ranging from 5 to 8 was investigated (**Figure 3-14a**). In dispersion, the fluorescence intensity of CQDs significantly decreased with increasing pH from 5 to 8 (**Figure 3-14b and 3-14c**), which can be attributed to the deprotonation of surface functional groups. To translate this intrinsic behavior into a practical dressing system, CQDs were further embedded into the nanofiber matrix.

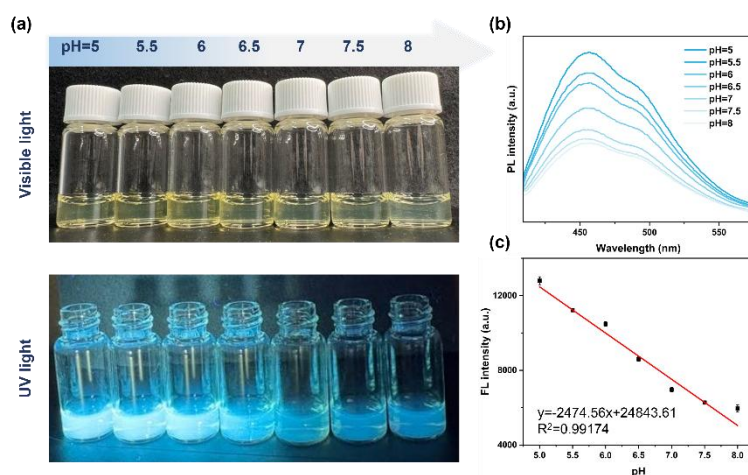


Figure 3-14. (a) Digital photographs of CQDs in aqueous solution at pH = 5-8 under visible light and UV light; (b) fluorescence spectra of CQDs in different pH aqueous solutions; (c) A linear relationship between excitation wavelength and pH values of CQDs aqueous solution.

A pH-responsive CQDs/CS/AAPVA nanofiber dressing was developed, in which CQDs were uniformly distributed throughout the matrix, ensuring stable fluorescence and making the system suitable for chronic wounds with moderate and persistent exudation (**Figure 3-15a**). This platform enables dual-mode pH visualization under both visible and UV light (**Figure 3-15b**). Visually, the dressing transitioned from translucent to opaque white with increasing pH, providing a clear visual cue of pH changes. Under UV light, the fluorescence color shifted from pale blue to cyan, enhancing the visual contrast of the fluorescent response. In contrast to the

dispersion behavior, embedding CQDs within the CS/AAPVA nanofiber matrix led to an overall increase in fluorescence intensity under UV light with rising pH. Such a reversed trend is not due to the intrinsic response of CQDs themselves but results from pH-induced structural rearrangements in the nanofiber matrix, which enhance light scattering, decrease transparency, and thereby amplify the apparent fluorescence signal.^[62,63] Considering that most human-perceived colors can be quantified using the RGB color model, an image-based pH sensing system was proposed using smartphone-assisted acquisition and RGB extraction to represent wound pH digitally. **Figure 3-15c** and **3-15d** show strong linear correlations between pH values and corresponding RGB intensities under visible and UV light. Accordingly, pH estimation equations were constructed under visible (**Eq. (3-4)**) and UV illumination (**Eq. (3-5)**). This approach provides a convenient and low-cost platform for portable, remote, and real-time monitoring of wound pH, offering promising prospects for intelligent wound management.

$$pH = 4.99 + \frac{R}{0.096(R+G+B)} \quad (3-4)$$

$$pH = 0.42 + \frac{R}{0.026(R+G+B)} \quad (3-5)$$

To further contextualize the practical relevance of the developed nanofiber dressing, a comparison was made with existing smart and passive wound dressings, including PVA-based foams, gels, and nanofibers, as well as several commercially available dressings (**Figure 3-15e**). The results demonstrate that while reported foams and gels exhibit high swelling ratios, they generally suffer from poor mechanical strength, which limits their structural integrity under moist wound conditions. In contrast, nanofiber systems provide better mechanical stability but usually compromise on fluid uptake. The present CQDs/CS/AAPVA nanofiber dressing effectively combines these advantages, exhibiting both a significantly higher swelling ratio than typical nanofiber dressings and a markedly improved tensile strength compared to hydrogels and foams. Although its swelling ratio may not surpass that of some hydrogel- or foam-based systems, the overall balance between fluid absorption and mechanical robustness highlights the

material's comprehensive performance. Compared with commercial dressings, it provides a more balanced performance between fluid management and mechanical robustness, underscoring its potential for chronic wound care.

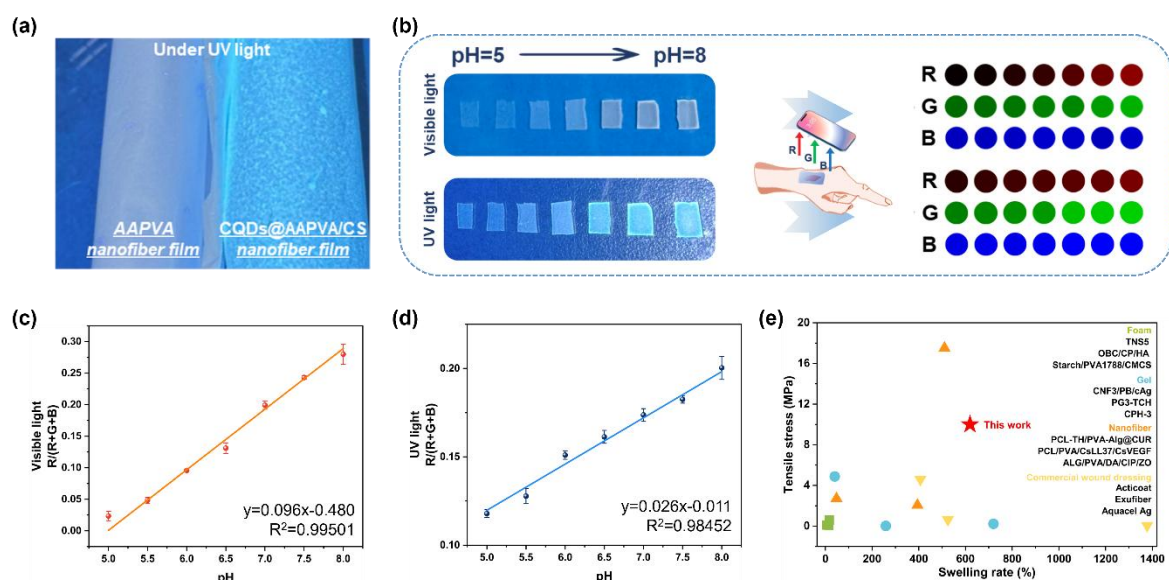


Figure 3-15. (a) Digital photographs of AAPVA and CQDs/CS/AAPVA nanofibers under UV light; (b) Photographs and RGB images of CQDs/CS/AAPVA nanofibers with pH values ranging from 5-8 under UV and visible light; RGB values and fitted curves of CQDs/CS/AAPVA nanofibers as a function of pH under (c) visible light and (d) UV light. Data are presented as mean $\pm$ SD ($n = 3$); (e) Comparison of the developed CQDs/CS/AAPVA nanofiber dressing with existing smart and passive wound dressings, including PVA-based foams,^[64–66] gels,^[67–69] and nanofibers,^[70–72] as well as several commercially available dressings.^[73]

3.3.5. Cell viability of nanofibers

Cytotoxicity evaluation is critical for assessing the suitability of wound dressings in clinical wound management. This study assessed the cytocompatibility of AAPVA, CQDs/AAPVA, CS/AAPVA, and CQDs/CS/AAPVA nanofibers using ISO 10993 standards. As shown in the fluorescence microscopy images (**Figure 3-16a**), L929 fibroblasts exhibited

morphological features characteristic of active, mature cells, comparable to those cultured in fresh medium, indicating good cytocompatibility. Furthermore, quantitative analysis of cellular metabolic activity (**Figure 3-16b**) demonstrated that the relative viability of fibroblasts cultured with extracts from AAPVA, CQDs/AAPVA, CS/AAPVA, and CQDs/CS/AAPVA nanofibers was $108.4 \pm 9.7\%$, $109.2 \pm 6.2\%$, $100.4 \pm 8.0\%$, and $109.1 \pm 6.8\%$, respectively. Cell viability in all experimental groups exceeded 100% relative to the negative control, confirming the excellent cytocompatibility of the developed composite dressings. This enhancement can be attributed to the synergistic biological effects of the three polymeric components. AAPVA contributes to a moist and stable microenvironment due to its hydrophilic nature and reactive acetoacetyl groups, CS promotes cell adhesion/ and regeneration through its cationic bioactivity, and CQDs are known to stimulate mitochondrial activity and modulate intracellular signaling pathways via their abundant surface functional groups.^[74–76]

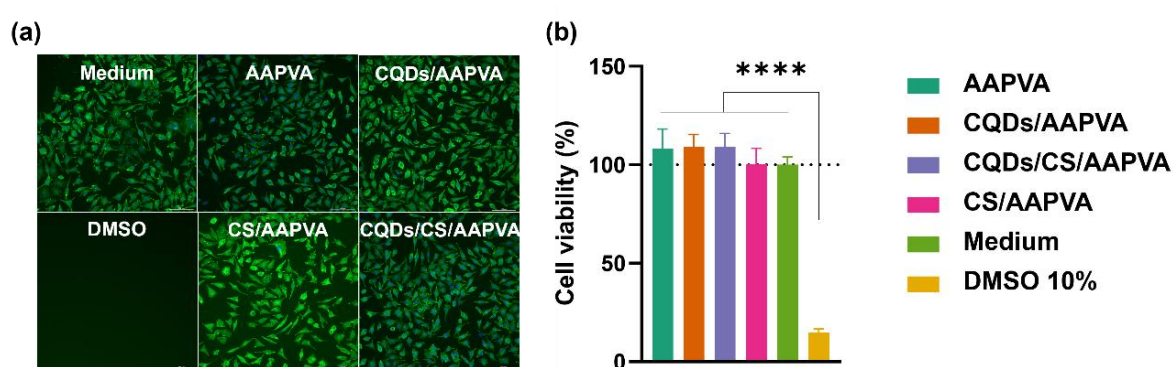


Figure 3-16. *In vitro* cytocompatibility assessment of nanofibers. (a) Fluorescence microscopy images and (b) cell viability of L929 cells cultivated with AAPVA, CS/AAPVA,

3.4. Conclusions

Nitrogen-doped CQDs were successfully synthesized using starch as a biomass-derived carbon source through a green hydrothermal method. These CQDs were incorporated into an aqueous electrospinning system comprising AAPVA and CS, resulting in a composite nanofibrous dressing with excellent biocompatibility and environmental safety. The fabricated

CQDs/CS/AAPVA nanofibers demonstrated strong mechanical properties (tensile strength of 10.0 MPa), high swelling capacity ($\sim 619.4 \pm 26.8 \%$), and outstanding structural integrity, retaining clear fibrous morphology after 5 days in PBS (pH 7.4) with a mass loss of less than 5%. Notably, the dressing exhibited highly visible and reversible optical responses to pH changes within the range of 5–8 under both daylight and UV light. By integrating RGB image analysis via mobile devices, these pH-responsive color and fluorescence changes enabled accurate, non-invasive quantification of wound pH. The developed CQDs/CS/AAPVA nanofibers present a facile, cost-effective, and sustainable strategy for developing innovative wound dressings capable of real-time monitoring and intelligent management of chronic wounds.

3.5. References

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Concluding Remarks

This study developed three types of high-performance bio-based composite systems using starch (plant-derived), poly(γ -glutamic acid) (γ -PGA, microbial-derived), and chitosan (animal-derived) as biomass matrices. Polyvinyl alcohol was employed as a universal reinforcing component, and both hydrogen bonding and chemical crosslinking mechanisms were introduced to achieve synergistic enhancements in mechanical properties, water stability, and functional performance of the resulting composites.

In **Chapter 1**, the reinforcing effect of PVA with different DS and DP on PVA/starch composite films was investigated. Two types of modified starch—hydroxypropyl starch (HPS) and octenyl succinate starch (OctS)—were compared to determine the optimal material composition. Notably, films fabricated using high-DS and high-DP PVA blended with OctS at a 7:3 ratio exhibited excellent optical transparency, mechanical strength, thermal stability, and water resistance, highlighting their potential for sustainable packaging applications.

In **Chapter 2**, a novel strategy for fabricating recyclable poly(γ -glutamic acid) (γ -PGA)-based films via dynamic covalent Schiff base crosslinking is presented. γ -PGA was first grafted with adipic dihydrazide (P-ADH) and then crosslinked with acetoacetylated polyvinyl alcohol (AAPVA) under mild, catalyst-free conditions to form acylhydrazone crosslinked networks, resulting in high-performance, recyclable thermoset films. The resulting films exhibited excellent mechanical strength, high optical transparency, UV shielding ability, and solvent resistance. Notably, the dynamic crosslinked networks enabled acid-triggered bond cleavage, allowing recovery of macromolecular components and their reprocessing into new films, thereby demonstrating chemical recyclability and reusability.

In **Chapter 3**, a composite nanofiber wound dressing was developed using AAPVA as the polymer matrix, starch-derived carbon quantum dots (CQDs) as the pH-responsive component

via hydrogen bonding, and chitosan (CS) as the crosslinking agent through Schiff base chemistry. The resulting CQDs/CS/AAPVA nanofibers displayed pronounced optical responsiveness within the pH range of 5–8, exhibiting visible color and fluorescence changes under both visible and UV light. Additionally, quantitative pH sensing was achieved through RGB image analysis, demonstrating promising potential for wound monitoring. Due to the synergistic interaction between CQDs and CS, the composite nanofibers also exhibited excellent mechanical strength, surface wettability, structural stability, and biocompatibility, highlighting their application potential in intelligent chronic wound management and remote monitoring.

In summary, this study successfully constructed and optimized a series of high-performance, sustainable bio-based materials by integrating biomass-derived polymers with PVA and its functional derivatives. The outcomes demonstrate broad practical potential in fields such as packaging and smart wound care, offering valuable insights for the development of green functional materials and the high-value utilization of diverse biomass resources.

List of Publications

1. Enhanced Physical Properties and Water Resistance of Films Using Modified Starch Blend with Poly(vinyl alcohol)

Ying Yao, Yu-I Hsu*, and Hiroshi Uyama*

Industrial Crops and Products, **2024**, 222, 119870.

DOI: 10.1016/j.indcrop.2024.119870

2. Schiff Base Crosslinked Poly(γ -glutamic acid) Composite Films with High Mechanical Strength and Remoldability

Ying Yao, Yu-I Hsu*, and Hiroshi Uyama*

Polymer Degradation and Stability, **2025**, 240, 111450.

DOI: 10.1016/j.polymdegradstab.2025.111450

3. Chitosan/Acetoacetylated Poly(vinyl alcohol) Nanofibers Incorporating Starch-Derived Carbon Quantum Dots with pH-Responsive Color Change for Wound Dressings

Ying Yao, Yu-I Hsu*, Selina Sonntag, Alicia Fernández-Colino, Stefan Jockenhövel, and Hiroshi Uyama*

Carbohydrate Polymers, **2025**, 124477.

DOI: 10.1016/j.carbpol.2025.124477

Acknowledgements

This research was conducted in the Department of Applied Chemistry, Graduate School of Engineering, The University of Osaka, from 2020 to 2025. During this period, I not only advanced academically but also grew personally. I was fortunate to work with many outstanding individuals whose support made this dissertation possible.

First and foremost, I would like to express my deepest gratitude to my supervisor, Prof. Hiroshi Uyama, for allowing me to join his laboratory. His continuous encouragement and numerous opportunities greatly shaped my development as a researcher and have left a lasting impact on my academic journey. I would also like to thank the other members of my dissertation committee, Prof. Satoshi Minakata and Prof. Masaya Nogi, for their insightful comments and for dedicating their valuable time to reviewing my work.

My sincere thanks also go to Assoc. Prof. Yu-I Hsu, whose consistent support, from my early days in the lab to the present, has been invaluable. Her advice, shared experience, and encouragement have guided my research in meaningful ways. I also appreciate the helpful suggestions from Assist. Prof. Akihide Sugawara, which significantly enhanced the quality of my experiments.

I would also like to express my sincere gratitude to Prof. Stefan Jockenhövel, Dr. Alicia Fernandez Colino, and Ms. Selina Sonntag from RWTH Aachen University for their valuable suggestions and kind support regarding the third part of my research.

I really appreciate Ms. Yoko Uenishi and Ms. Tomoko Shimizu for their kind help and care during these five years. I would also like to express my sincere gratitude to all past and present members of Uyama Laboratory: Dr. Yan Wang, Dr. Luwei Zhang, Dr. Meng Wei, Dr. Mark Adam Malaluan Ferry, Dr. Yuxiang Jia, Dr. Nontarin Roopsung, Dr. Madhurangika Panchabashini Horathal Pedige, Dr. Yu Cao, Dr. Manjie He, Dr. Emil Hajili, Dr. Peng Du, Dr. Juan Wang, Dr. Kazuki Shibasaki, Dr. Atsuki Takagi, Dr. May Myat Noe, Dr. Guan Wang, Ms.

Hasinah Binti Mohamed Rafiq, Ms. Judit Rebeka Molnar. Mr. Ruiqi Zhang, Mr. Yihan Gao, Ms. Izzah Binti Haji Abdul Hamid Durrati, Ms. Sooyeon Noh, Ms. Qianying Li, Mr. Yazhou Su, Mr. Jiahui Dong, Mr. Kai Cheng, Mr. Yihe Chen, Ms. An Thuy Le Huynh, Ms. Rika Onishi, Mr. Hiroshi Hasegawa, Mr. Shotaro Yano, Mr. Kippe Yamamura, Mr. Alejandro Adrian Ayala Escamilla, Mr. Ziyu Meng, Ms. Shafri Shafinee Yarnina Hj Md, Ms. Akane Odagaki, Ms. Rina Kugimiya, Mr. Shunya Kubo, Mr. Sota Nakagawa, Ms. Maoko Hayashi, Ms. Daniela Yacine Sebastião Bravo Da Costa, Ms. Peixuan Shao, Mr. Kohnosuke Yoshida, Ms. Chua Siaw Thong, Mr. Chenxu Zhang, Mr. Naoaki Ishihara, Mr. Yuta Okuda, Ms. Kanako Sakai, Mr. Yuta Kitagaki, Mr. Daiki Morimoto, Mr. Yoshiki Imazato, Mr. Naoki Ueda, Ms. Supritha Muppuri, Mr. Yuto Kirihata, Mr. Tomoya Ushiroda, Ms. Akiko Mano, Ms. Erina Katsuragawa, Ms. Chikako Abe, Ms. Kyoko Tanimura, Ms. Kyoko Fukuma, etc.

I would like to express my heartfelt gratitude to the QLEAR Fellowship for financially supporting my doctoral studies. I am especially thankful to Prof. Shigehiko Hasegawa and Prof. Akira Oiwa for their invaluable support throughout my research and for their kind assistance that greatly eased my life as an international student. My sincere thanks also go to Ms. Yoko Yoshida and other staff members for their ongoing help and encouragement throughout this journey.

I am deeply grateful to my friends for their unwavering support throughout this journey. I would like to express my sincere thanks to Ms. Minghe Li and Ms. Xiaojie Tian. Your companionship, care and encouragement have helped me grow and become a clearer and better version of myself. Finally, I would like to express my heartfelt appreciation to my parents and family members. Their unconditional love and steadfast support have always been the foundation that enables me to pursue what I love and follow the path I choose with confidence.

YAO YING
September 2025