Formulating Self-healable Bio-adhesive from waste proteinaceous biomaterials

A dissertation submitted in the partial fulfillment of
the requirement for the degree of
Bachelor of Science
in
Chemistry

Submitted by:
Kriti Tiwari
(SAP ID: 500084683)

Under the supervision of
Guide-Dr. Sravendra Rana
Co-Guide- Dr. Tridib Kumar Sinha

Department of Chemistry, Applied Science Cluster
School of Engineering
University of Petroleum and Energy Studies
Dehradun, Uttarakhand-248007, India

May, 2024

DECLARATION

I declare that the thesis entitled **“Formulating Self-healable Bio-adhesive from Waste Proteinaceous Biomaterials”** has been prepared by me under the supervision of **Dr. Sravendra Rana and Dr. Tridib Kumar Sinha** from **the Department of Chemistry, School of Engineering, University of Petroleum & Energy Studies, Dehradun, India.**

Kriti Tiwari.

Department of Chemistry
School of Engineering
University of Petroleum & Energy Studies
Dehradun, Uttrakhand-248007, India

CERTIFICATE

I certify that **Kriti Tiwari** has prepared his project entitled "**Formulation of self-healable bioadhesive from proteinaceous biowaste"** for the award of **B.Sc. (Hons) Chemistry,** under my/our guidance. She has carried out work at the **Department of Chemistry, School of Engineering, University of Petroleum & Energy Studies, Dehradun, India.**

Dr. Sravendra Rana
(Professor)

Department of Chemistry
School of Engineering
University of Petroleum & Energy Studies
Dehradun, Uttrakhand-248007, India

Dr. Tridib Kumar Sinha.
(Associate Professor)

Department Chemistry
School of Engineering
University of Petroleum & Energy Studies
Dehradun, Uttrakhand-248007, India

PLAGIARISM CERTIFICATE

I, **Kriti Tiwari**, herby certify that the research dissertation titled “” submitted for the partial fulfillment of a B.Sc. degree from University of Petroleum Energy & Studies, Dehradun, India is an original idea and has not been copied/taken verbatim from anyone or from any other sources.

I further certify that this dissertation has been checked for plagiarism through a plagiarism detection tool **“Turnitin”** approved by the university and “**8%** ” percentage similarities have been found as shown in the attached report.

Kriti Tiwari
Department of Physics/Chemistry/Mathematics
School of Engineering
University of Petroleum & Energy Studies
Dehradun, Uttrakhand-248007, India

Approved by:

Dr. Sravendra Rana
Department of Chemistry
School of Engineering
University of Petroleum & Energy Studies
Dehradun, Uttrakhand-248007, India

Thesis draft.docx

ORIGINALITY REPORT

8%
SIMILARITY INDEX

PRIMARY SOURCES

1	Jing Sun, Juanjuan Su, Chao Ma, Robert Göstl, Andreas Herrmann, Kai Liu, Hongjie Zhang. "Fabrication and Mechanical Properties of Engineered Protein-Based Adhesives and Fibers", Advanced Materials, 2019 Crossref	85 words	2%
2	Hind Abdellaoui, Marya Raji, Rachid Bouhfid, Abou el kacem Qaiss. "Investigation of the deformation behavior of epoxy-based composite materials", Elsevier BV, 2019 Crossref	58 words	1%
3	Sil Nevejans, Nicholas Ballard, José I. Miranda, Bernd Reck, José M. Asua. "The underlying mechanisms for self-healing of poly(disulfide)s", Phys. Chem. Chem. Phys., 2016 Crossref	58 words	1%
4	link.springer.com Internet	54 words	1%
5	omnexus.specialchem.com Internet	50 words	1%
6	Sunardi Sunardi, Dody Ariawan, Eko Surojo, Aditya Rio Prabowo, Hammar Ilham Akbar, Bo Cao, Hermes Carvalho. "Assessment of eggshell-based material as a green-composite filler: Project milestones and future potential as an engineering material", Journal of the Mechanical Behavior of Materials, 2023 Crossref	38 words	1%
7	www.coursehero.com Internet	31 words	1%
8	N. Xie, H. Wang, D. Feng. "Coating materials to increase pavement surface reflectance", Elsevier BV, 2015 Crossref	28 words	1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 1%
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 10 WORDS

Abstract

The growing emphasis on sustainability has led to increased research and development of eco-friendly materials in various industries. This project investigates into " Formulation of Self Healable Bio Adhesive Development from Waste Proteinaceous Biomaterials." By exploring the potential of utilizing waste biomaterials, such as agricultural residues, animal-based food remnants, and food processing by-products, we examine the feasibility of creating functional adhesives with a reduced environmental impact.

It starts with an overview of the sources of waste proteinaceous biomaterials and their abundance, highlighting their potential as sustainable alternatives. Extraction and modification techniques for obtaining proteins from these biomaterials are then explored, critically analyzing advancements and challenges in these processes. The formulation of self-healable adhesives takes center stage, addressing factors like self-healing, adhesion strength, flexibility, and resistance. We discuss the role of filler(ESP) and its impact on the properties of these materials, providing insights into achieving desired functionalities by formulation of composite with polymer matrix. Different formulations of composite have been formulate with different crosslinking. This project also investigates into the sustainability and environmental impact of utilizing waste proteinaceous biomaterials. Life cycle assessments and considerations of biodegradability contribute to a comprehensive analysis of the ecological footprint of these bio-based materials. The conclusion summarizes the key findings, emphasizing the significance self healable adhesives from waste proteinaceous biomaterials in achieving sustainability goals across industries.

Keywords: Bioadhesive, Self-healing property, Waste protein biomaterial,

ACKNOWLEDGEMENT

I would like to extend my deepest appreciation to the individuals and organizations who have made substantial contributions to the finalization of this thesis.

I am profoundly thankful to my supervisor, Dr. Sravendra Rana, and my co-supervisor Tridib Kumar Sinha for their guidance, assistance, and expertise during the entire research process. Their invaluable perspectives, constructive critiques, and consistent support have played a pivotal role in molding this thesis.

My gratitude is extended to the academic staff, Ph.D. scholars, and laboratory personnel for their dedication, time, and valuable contributions. Their specialized knowledge and recommendations have significantly enhanced the caliber of this research endeavor.

I am sincerely appreciative of my parents, relatives, and friends for their continuous backing, motivation, and comprehension throughout this academic odyssey. Their steadfast faith in my capabilities and their affectionate support have served as my sources of resilience.

Furthermore, I express my acknowledgment to UPES for furnishing the essential amenities, resources, and research-friendly ambiance that facilitated the completion of this thesis.

At last, I wish to convey my heartfelt gratitude to all those who have played a part in this thesis, whether explicitly acknowledged or not. Your assistance, regardless of its magnitude, has been a crucial factor in the triumphant conclusion of this research project.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

In response to the escalating global demand for sustainable and environmentally friendly products, industries are increasingly exploring innovative approaches to reduce their ecological footprint. Within this context, the development of "Self healable Bio Adhesive from Waste Proteinaceous Biomaterials" emerges as a promising avenue to address both environmental concerns and the growing need for eco-conscious materials in diverse applications.

In addition to educating the people regarding the disposal and management of waste, parallelly huge research interests are being paid to reuse the waste for developing various materials/products. In this way, we can realize the accomplishment of “Trash-to-Treasure". [**4-5**] Among various waste materials, waste biomaterials could be reused to develop a product showing biocompatibility and biodegradability. Apart from biocompatibility and biodegradability, for any product, its touch feeling/tactile performance is being given prioritized for customer preferences. [**6-10**] In this regard, many policies are being attempted to implement aiming at transforming waste materials into valuable resources through recycling, reusing, or repurposing. These policies promote sustainable waste management practices while minimizing the environmental impacts.[**11, 12**] Briefly, recycling is one of the most popular strategies that involves gathering, sorting, and processing waste materials including paper, plastic, glass, and metal to create new goods. Recycling saves energy, cuts down on landfill waste, and lessens the need to mine new resources. There are many methods to creatively utilize discarded things and convert them into treasure. Recycling is a vital technique that prevents garbage from ending up in landfills and saves resources by turning it into new goods.One of the most popular and efficient ways to turn trash into treasure is recycling. We can save resources, and energy, and minimize pollution by recycling things that might otherwise wind up in landfills. Recycling is possible for a wide range of materials, including paper, plastic, glass, metal, and electronics. Recycling (mainly), reusing, and repurposing are the important part of the “Circular Economy”.

As per the biomaterial concern, these are substances derived from biological sources that can be reused in various applications, including medical, agricultural, and industrial fields, etc. One of the most prevalent wastes produced by the food sector is eggshell waste. Eggshell waste is generated in large quantities due to the high consumption of eggs worldwide. While eggshells are typically considered waste which exerts pungent smell and polute the environment, they can actually be repurposed and used in various ways and minimize their environmental impact.**[13,14]**Indeed, eggshells have gained attention in both the academic and industrial settings for their potential use as a valuable resource. Researchers and industries have recognized the various beneficial properties of eggshells and explored their applications in different fields. Eggshells are readily available in large quantities as a byproduct of the food industry. They are often considered waste, which makes them a low-cost resource for research and industrial applications.

The Egg shell is made of an outer calcite layer (i.e., mainly calcium carbonate) holding a thin proteinaceous membrane inside. The thin layer is recognized as the eggshell membrane (ESM). In waste eggshell, the calcite layer contains hundreds of proteins that interact with the mineral phase controlling its formation and structural organization. One of this structure's distinguishing characteristics is the presence of calcite crystals in the eggshell membrane. These well-organized calcite microcrystals create a mesh-like network inside the membrane. They support the membrane's mechanical durability and toughness, protecting the egg's contents. It is thought that the eggshell membrane contains calcite crystals, which help to provide resistance to physical harm and inhibit bacterial infiltration. The calcite microcrystals' well-organized arrangement aids in preserving the membrane's integrity while enabling the flow of gases and moisture between the growing embryo and the eggshell. Since calcite makes up a large portion of the eggshell, the calcite crystals in the membrane of the eggshell are different from those in the eggshell itself. **[15,16].**

Calcium carbonate, which is present in eggshells, is good for the health of the soil and plant development. They may be made into a fine powder and used in the soil as a natural fertilizer to raise the calcium content. The ability of eggshells to regulate soil pH and give plants slow-release nutrients has also been investigated. The capacity of eggshells to remove heavy metals and other contaminants from water has been studied. Eggshells are a possible inexpensive adsorbent material for water treatment operations due to their porous nature, which allows them to adsorb the pollutants.[17,18]. The outer calcite layer is also being used as green filler to develop various polymer composites to enhance their performance in various applications. Even, the polymer-calcite composite becomes interested in coating purposes in automobile sector to enhance the touch-feeling performance.

Following the usability of outer eggshell layer, many efforts are being attempted to utilize the inner ESM layer for different advanced applications including the biomedical and health care.

Eggshells have shown good biocompatibility, i.e., they are well-tolerated by living organisms without causing adverse reactions. This property is crucial for biomedical applications, such as tissue engineering and drug delivery systems**. [19, 20]**

In addition to its chemical composition for its enhanced biocompatibility and biodegradability, because of functional groups (e.g., hydroxyl (-OH), thiol (-SH), amide (CONH2), amin (-NH_2) and carboxyl (-COOH)) that are well known to stabilize the metal nanoparticles, the ESM are being used as template for nanoparticle synthesis. **[21]** If the ESM is processed by de-crosslinking, the small proteins can be used to develop a dispersion containing ESM coated stable nanoparticles.

There are many efforts that considers the isolation of small proteins from the ESM. However, for different applications they need to be modified.

Eggshells (ESs) due to their myriad advantages, such as their low density, affordability, renewable nature, and eco-friendliness, along with exhibiting excellent thermal stability at elevated temperatures **[7].** ESs are globally abundant as a form of organic waste. Moreover, they have been identified as rich sources of calcium. The weight of ESs varies among different bird species; for instance, chicken ESs constitute approximately 11% w/w, while ducks' ESs weigh around 12.6% w/w [5]. Egg quality correlates with ES percentage, thickness, shape index, and specific gravity. Parameters for evaluating ES quality include shell weight, thickness, strength, and density **[27].**

Robust ESs are indispensable for minimizing egg damage, guarding against infections, preventing moisture loss, and serving as a calcium reservoir [28]. A reduction in membrane thickness compromises the ES's physical barrier quality and may alter its structure **[29].** The quality of ESs is significantly influenced by the age of the breeding stock. Fragile and thin ESs result in losses during production, transit, and marketing, estimated at 5–7% for the industry. Various factors affecting ES strength encompass hereditary traits, age, health, seasonal variations, production levels, dietary factors, environmental conditions [30], time of oviposition, and the housing system used **[27].** Key ES characteristics include egg weight, shape index, specific gravity, and shell ratio **[20].** ES quality is discernible through its strength, which denotes its capacity to resist external forces without fracturing or breaking. Egg-specific gravity serves as an indicator of ES strength **[20]**.

1.1 Protein-based biomaterial

Protein-based biomaterials have attracted considerable attention due to their unique structural and mechanical attributes. Silks, elastins, and collagens are frequently employed structural proteins in such biomaterials. These proteins, derived from a variety of amino acids, present distinct systems that are biocompatible, biodegradable, flexible, and non-toxic, rendering them suitable candidates for utilization in biomedicine, sensors, and tissue engineering. The intrinsic properties of proteins are primarily dictated by the amino acid sequence. The interactions among amino acids, including noncovalent and covalent interactions such as hydrogen bonding, electrostatic interaction, hydrophobic interaction, aromatic stacking, etc., facilitate the development of different secondary structures like α-helices and β-sheets. This structural variability contributes to the diverse mechanical properties of the resulting biomaterials. Through advancements in genetic engineering, significant endeavors have been made to investigate the relationship between the structure and mechanical behavior of recombinant proteins. For example, the elucidation of the primary and secondary structures of elastin has provided opportunities to finely adjust the mechanical properties of elastin-based materials. Consequently, recombinant elastin-like polypeptides (ELPs) can be customized to display a range of mechanical properties, which are highly advantageous for applications in tissue engineering and drug delivery**.[20–22]** In contrast to traditional synthetic polymers, protein-based materials offer superior flexibility and biodegradability**.[23,2**4] The remarkable level of control over their structure and mechanical behavior positions protein-based biomaterials as promising candidates for a myriad of applications.

1.2. Epoxy as Polymer matrix:-

Epoxy is a popular polymer due to its ease of processing, strong adhesiveness, and high chemical resistance. Epoxy-based composites are widely utilized in aerospace, automotive, and marine industries. Epoxy can act as either a filler or a polymer matrix in composites**.[30]** As a filler, the modification of fibers with epoxy is discussed, while as a polymer matrix, the reinforcement by natural and synthetic fibers is examined. The manufacturing processes and the performance (e.g., mechanical and thermal properties) of epoxy-based composites are investigated.**[25]**

Since its discovery in 1909 by Prileschajew, epoxy has become crucial in various industries. Epoxy, characterized by an epoxy ring with two carbon atoms bonded to a common oxygen atom, belongs to reactive prepolymers and polymers containing epoxide groups, cured with various hardeners**.[26-27]** Epoxy is a significant thermosetting polymer with wide applications due to its excellent mechanical properties, high adhesiveness, low shrinkage after curing, and good heat and chemical resistance, making it valuable as a reinforcement material, adhesive, and coating.

Epoxy is extensively used as a polymer matrix for composite applications, and its properties can be tailored by adding functional fibers. For instance, fiber-reinforced epoxy composites exhibit significantly enhanced mechanical properties. Song et al. developed carbon fiber/epoxy laminates with carbon nitride, achieving tensile strength and Young's modulus of 67 MPa and 58 GPa, respectively. Huang et al. created continuous bamboo fiber reinforced epoxy composites via resin transfer molding, which improved mechanical strain**.[26-28]** Epoxy-based composites not only boast enhanced mechanical properties but also possess unique attributes like electromagnetic interference shielding, self-healing, and heat resistance.

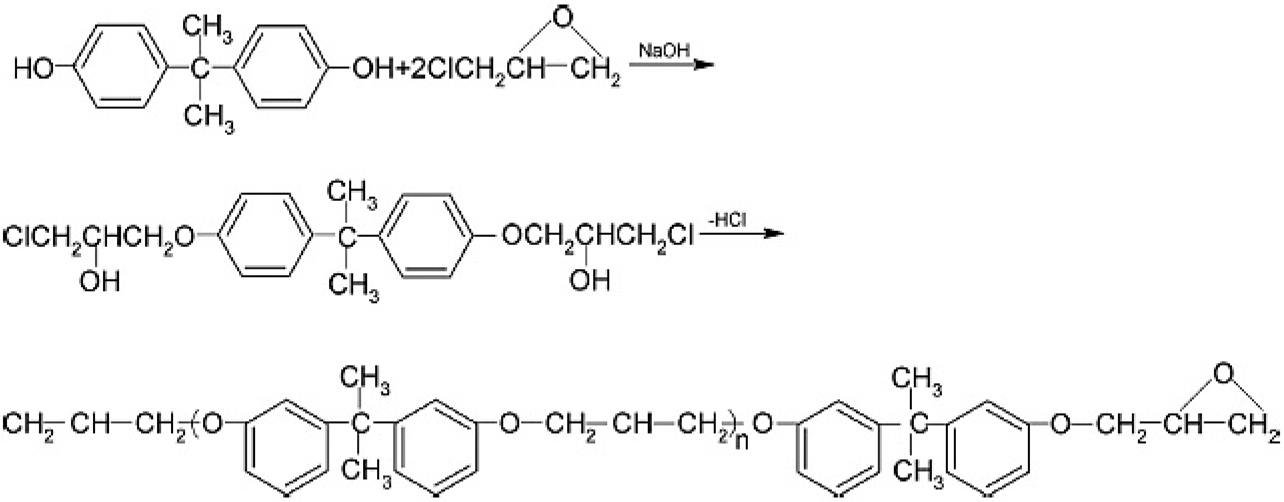
Apart from serving as a polymer matrix, epoxy can be used as a filler to reinforce polymer composites. Fillers modify polymer characteristics or reduce costs. For example, Zhao et al. used epoxy to functionalize pine fibers for reinforcing polylactic acid (PLA), improving tensile strengths and Young's moduli due to better fiber/matrix adhesion. Sujaritjun et al. achieved a 10% increase in tensile strength using epoxidized polybutadiene treated bamboo fibers to reinforce PLA. Similarly, Kyutoku et al. found that epoxy-coated cellulose fibers improved the interfacial adhesion between cellulose fibers and PLA. The high reactivity of the epoxy group in modified fibers enhances interfacial interactions, effectively reinforcing polymers.

Cured epoxy resins, however, are brittle due to high cross-linking, affecting impact strength and other properties. Thus, chemical modifications of epoxy monomers are necessary to enhance flexibility, toughness, and other properties. Efforts include introducing flexible polymers, inorganic particles, and elastomers. Despite brittleness, epoxies have poor thermal and electrical conductivity. To address these issues, conductive materials like carbon nanotubes, graphene, and carbon fiber are added, although poor interfacial interaction can reduce interfacial strength and impact toughness. Efforts to solve this include developing bio-based epoxies and epoxy-based vitrimers, driven by sustainability and recyclability concerns in composite materials[30-31]**.**

Epoxy composites represent one of the most prevalent types of polymer composites due to their extensive applications across various industries such as automotive, aerospace, marine, and oil and gas. This widespread usage is attributed to their economic viability, superior mechanical properties, high-specific characteristics, excellent adhesive properties, and notable heat resistance. Consequently, there is a growing necessity to investigate and anticipate the mechanical and tribological performance of epoxy-based composite materials.

In the advancement of reinforcement and filler materials for epoxy resin, a range of substances have been employed. Synthetic fibers like glass fiber and carbon fiber stand out as widely utilized reinforcement materials, while natural fibers such as jute fiber and bamboo fiber have gained prominence in certain applications.

Epoxy resins commonly result from the interaction between compounds containing a minimum of two active hydrogen atoms, such as polyphenolic compounds, diamines, amino phenols, heterocyclic imides and amides, aliphatic diols, and epichlorohydrin.



The oxirane group present in an epoxy monomer reacts with a variety of curing agents such as aliphatic and aromatic amines, phenols, polyamides, amidoamines, anhydrides, thiols, and acids. These chemical interactions result in the creation of stiff thermosetting materials by combining with appropriate ring-opening compounds. Nevertheless, the final cured epoxy products often exhibit brittleness as a consequence of extensive cross-linking, which may compromise their impact resistance and other key characteristics.

1.3. Polymer composite:-

Polymer composites are materials in which a polymer serves as a matrix resin, infiltrating and bonding with a reinforcing material. These composites are predominantly utilized in the automotive and aerospace industries. Typically, the epoxy composites are reinforced with ESP filler and curing agent.

Epoxy-based composite materials are extensively utilized in load-bearing applications across industries such as automotive, aerospace, construction, oil and gas, and marine, due to their affordability, excellent mechanical properties, high specific strength, strong adhesion, and robust heat and solvent resistance. Consequently, it is increasingly important to study and predict the deformation behavior of these materials under various loading conditions. The deformation of epoxy-based composites is influenced by the morphology of the epoxy matrix and the filler content. It examines the chemical composition and structural properties of epoxy polymers used as matrices in composites, and how the morphological properties of the epoxy and fillers affect deformation behavior.

1.4 Self-healing property of composite.

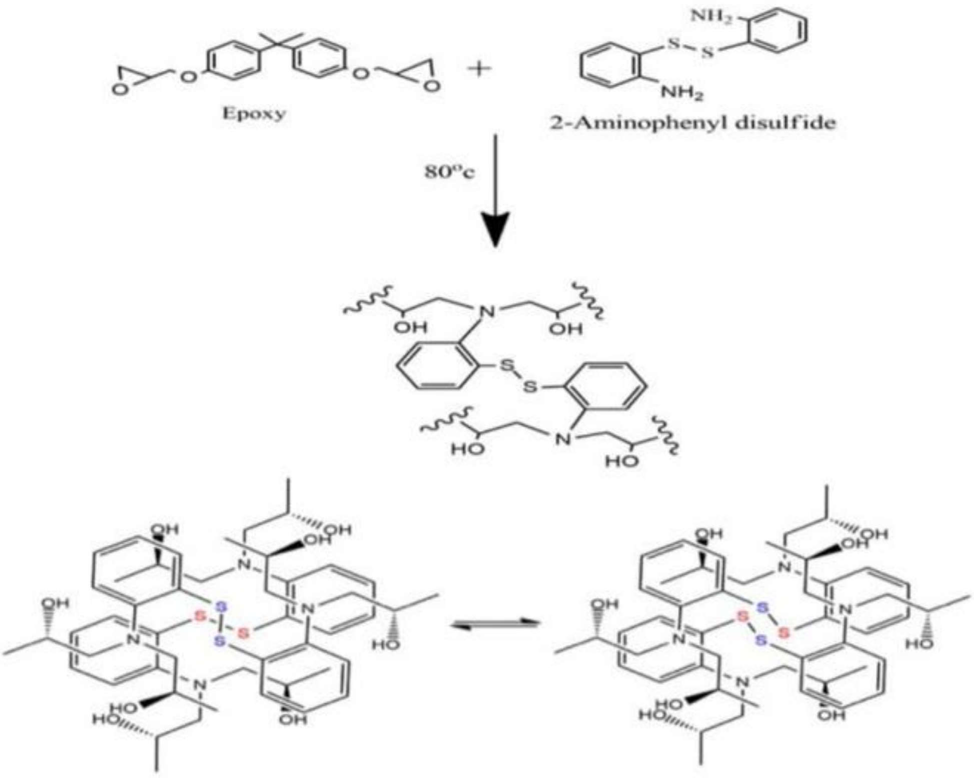
Numerous studies have explored synthetic self-healing materials, drawing inspiration from the regenerative abilities seen in biological organisms. Researchers have categorized these materials into different types based on their self-repair mechanisms. Billiet et al. distinguish between extrinsic and intrinsic self-healing materials. Extrinsic materials acquire their self-healing properties by incorporating healing agents into the material, while intrinsic materials possess inherent self-healing abilities through their own properties. Intrinsic materials exhibit self-repair properties by integrating healing agents into the material, while extrinsic materials demonstrate inherent self-healing capabilities through their own attributes**[28-29].**

Self-healing intrinsic materials commonly depend on reversible chemical or physical connections, enabling them to experience numerous healing occurrences and rendering them appropriate for prolonged utilization. For instance, reversible covalent cross-linking, such as thiol/disulfide redox dynamic exchange reactions, is employed in molecular interdiffusion to facilitate healing in polymer star gels**[31-33].** These materials can be activated for healing by various stimuli, including heat, light, redox reactions, or alterations in pH.While these stimuli-responsive methods are effective, there is a growing interest in self-healing materials that can be repaired at room temperature without external intervention. advancements include self-healing of composite elastomers, which utilize (4-aminophenyl) disulfide as a dynamic cross-linker**.[33-34]** These materials demonstrate quantitative healing efficiency at room temperature without the need for catalysts or external stimuli.

The development of materials capable of self-repair has gained significant attention in recent years due to their potential applications in enhancing the durability and longevity of various products. Inspired by nature's ability to regenerate, researchers have explored different strategies to impart self-healing properties into synthetic materials. One promising approach involves the incorporation of dynamic cross-linkers within polymer matrices, enabling the material to autonomously repair damage through reversible chemical reactions.

The synthesis of the self-healing composite materials involved the preparation of epoxy resin (Epon828) and extraction of eggshell protein from waste eggshells. 2-Aminophenyl disulfide incorporated into the epoxy resin matrix at varying concentrations. The eggshell protein was then added to the resin mixture to reinforce the composite structure.

Curing agent (2-Aminophenyl disulfide):- 2-Aminophenyl disulfide (2-AFD) serves as a curing agent that introduces dynamic disulfide bonds into epoxy vitrimers, enabling these materials to be reshaped or post-processed like thermoplastics under certain conditions. 2-AFD is an isomer of 4-aminophenyl disulfide (4-AFD), a more commonly used cross-linker for incorporating disulfide bonds into thermosetting polymers. Additionally, 2-AFD is more cost-effective than 4-AFD**. [34]**



Mechanism of 2AFD and Epon828.

1.5 Bioadhesives

Adhesives, in general, are substances with chemical or physical stickiness employed to connect surfaces. When adhering to biological and living substrates, the demand arises for bioadhesives that fulfill criteria such as biocompatibility, non-toxicity, and degradability. Nature serves as a rich source of inspiration for bioadhesives, with organisms like mussels, sandcastle worms, barnacles, caddisfly larvae, spiders, and glowworms utilizing primarily protein-based glues for various purposes **[40]**. While numerous reviews and books investigates into the use of diverse bio adhesives across various applications, the focus here centers on advancements in developing bio-inspired protein-based adhesives specifically designed for biomedical applications**[42].**

Bioadhesives are gaining significant interest due to their potential environmental benefits and biocompatibility. Traditional adhesives often contain harmful chemicals and are derived from non-renewable resources**[43].** This research aims to develop a sustainable and eco-friendly bioadhesive using soybean-based bioepoxy, a renewable and biodegradable polymer, combined with eggshell protein, a waste-derived filler, and 2AFD as the curing agent.

Bioadhesives are a category of adhesives originating from biological origins. In contrast to conventional adhesives, often composed of synthetic polymers and petroleum byproducts, bioadhesives are crafted from sustainable and biodegradable substances. This attribute renders them highly attractive across various sectors, such as medical, packaging, and construction, due to their ecological advantages and compatibility with living organisms.

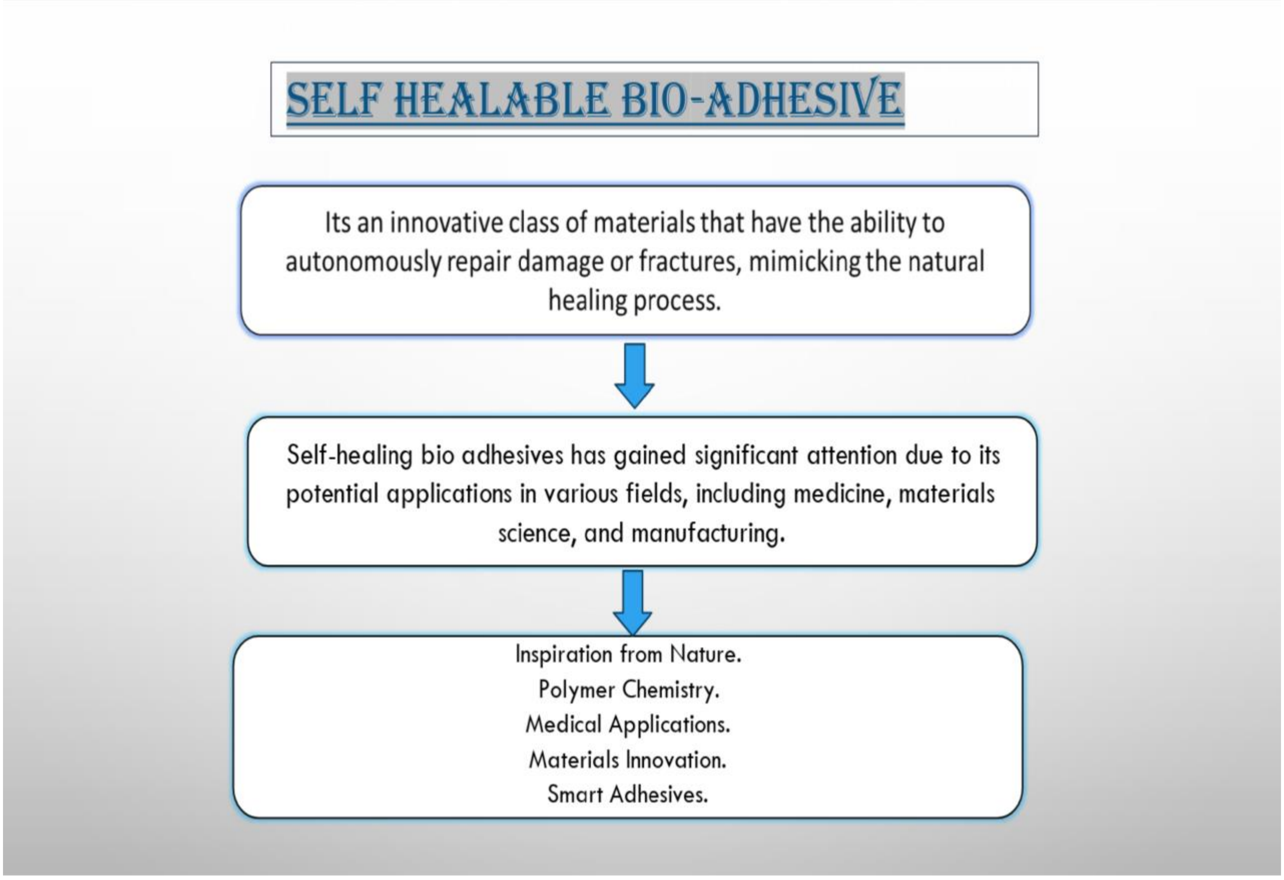
The escalating consciousness regarding the environment and the regulatory demands to diminish the utilization of finite resources have stimulated interest in bioadhesives. These organic adhesives offer multiple benefits, including decreased toxicity, heightened safety, and the potential for decomposition, aligning with the fundamentals of green chemistry and sustainable progress.

Throughout history, natural adhesives have been utilized for extended periods, with initial instances encompassing paste from starch, animal-based glues, and plant resins. Nevertheless, the evolution of contemporary bioadhesives has gained momentum with advancements in biotechnology and material science, facilitating the formulation of high-functioning adhesives from various biological sources. These sources involve proteins, polysaccharides, and lipids obtained from entities like plants, animals, and microorganisms.

The utilization of the bio-based epoxy resins emerges as a promising avenue in the advancement of bioadhesives. Ex. soybean oil is plentiful, renewable, and exhibits exceptional traits for polymer formation. Through the amalgamation of soybean-based bioepoxy with other natural components, such as proteins derived from eggshells, it becomes feasible to enhance adhesive characteristics while upholding biodegradability and sustainability**.[39-41]**

Eggshell protein, predominantly comprised of collagen, presents supplementary advantages as a filler in bioadhesives. Incorporating eggshell protein not only enhances the mechanical attributes of the adhesive but also furnishes an eco-friendly resolution for waste management, given the common occurrence of eggshells as a byproduct in the food industry**[41].**

Bioadhesives epitomize a forward-thinking approach to adhesive technology, blending environmental advantages with operational efficiency. This study delves into the formulation of a bioadhesive utilizing soybean-based bioepoxy, eggshell protein, and 2AFD, with the objective of contributing to the expanding realm of sustainable materials. The evolution and refinement of such bioadhesives hold the promise of supplanting traditional adhesives, furnishing a more environmentally sound and secure substitute for myriad applications.



SELF HEALABLE BIO-ADHESIVE

Its an innovative class of materials that have the ability to autonomously repair damage or fractures, mimicking the natural healing process.

Self-healing bio adhesives has gained significant attention due to its potential applications in various fields, including medicine, materials science, and manufacturing.

Inspiration from Nature.
Polymer Chemistry.
Medical Applications.
Materials Innovation.
Smart Adhesives.

Schematic presentation of self healable bio-adhesives

OBJECTIVE OF THE PROJECT.

- Extraction and purification of soluble protein (ESP) from waste biomaterial. (e.g. ESM, Keratin).
- Formulation of extracted protein/polymer (e.g., Epoxy resin) composite.
- Optimization of the self-healing property of the composites.
- Applying the adhesive in various potential applications.

CHAPTER 2

EXPERIMENTAL DETAILS

1. Collection of ESM and Extraction, purification, and storage of ESP

We know that ESM is a naturally bioactive biomaterial enriched with protein, collagen, and lots of minerals. To get eggshell protein (ESP) from ESM following steps have been followed:

1. Collection of waste eggshells from cafes and restaurants.
2. Cleaning the waste eggshell with a soap solution followed by acetone to remove all impurities.
3. Isolating the thinner inner eggshell membrane (ESM) via de-calcination of the outer calcite layer and peeling out the ESM in mild acidic (i.e., 1N HCl) condition.
4. Cleaning the ESM with plenty of water to remove the trace of acid, drying and collecting in an air-tight polybag.
5. Extracting the eggshell protein (ESP) via denaturation and dissolution of ESM in basic alcoholic solution followed collection of the supernatant and separating out the solid material therein.
6. Neutralizing the ESP dispersion with dilute acid (i.e., 1 N HCl)
7. Dialysis of the neutral dispersion to remove out the salt formed during neutralization.
8. Evaporating the solvent to obtain the solid dried ESP and collecting it into a air-tight container

During the dialysis, the removal of salt was confirmed by the no formation of white ppt (i.e., AgCl) in the outer aqueous part of the dialysis tube (the similar way of argentrometric chloride estimation). The whole process is schematically presented in the following. **Fig. 1**

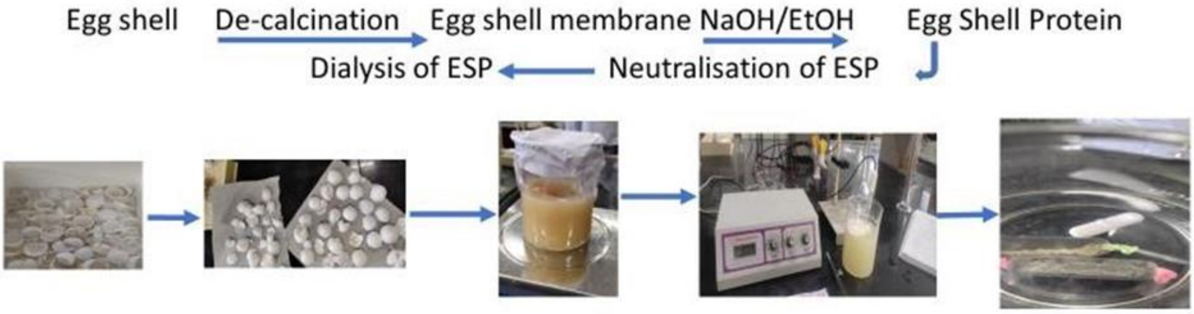


Fig.1 Schematically presentation of protein extraction

2. Formulation of composite.

Epoxy resin is used as the matrix material for adhesive composites due to several advantageous properties like adhesion strength, toughness, chemical resistant temperature resistance, dimensional stability, and versatility.

Extracted protein will be used as filler in the composite.

This aims to evaluate the effect of incorporating extracted eggshell protein into an epoxy resin (Epon 828) matrix with two different curing agents, Diethylenetriamine (DETA) and 2-aminophenyl disulfide (AFD), on the mechanical properties of the resulting composite materials.

Materials:

1. Epon 828 epoxy resin (1 gram)
2. Extracted eggshell protein (1 milligram)
3. Diethylenetriamine (DETA) curing agent (120 milligrams)
4. 2-aminophenyl disulfide (AFD) curing agent (356 milligrams)
5. Glass slides
6. Mold

Procedure:-

1. Weigh 1 gram of Epon 828 epoxy resin and place it in a voil.

2. 1 milligram of extracted eggshell protein is added to the epoxy resin.

3. Begin continuous stirring to ensure uniform dispersion of the eggshell protein within the epoxy resin matrix.

4. Once the eggshell protein is evenly distributed, add the curing agent according to the desired experimental setup:

- For Experiment 1: Added 120 milligrams of Diethylenetriamine (DETA) curing agent.
- For Experiment 2: Added 356 milligrams of 2-aminophenyl disulfide (APDS) curing agent.

5. Continue stirring the mixture until the curing agent is thoroughly mixed with the epoxy resin and eggshell protein.

6. Transfer the homogeneous mixture into a mold .

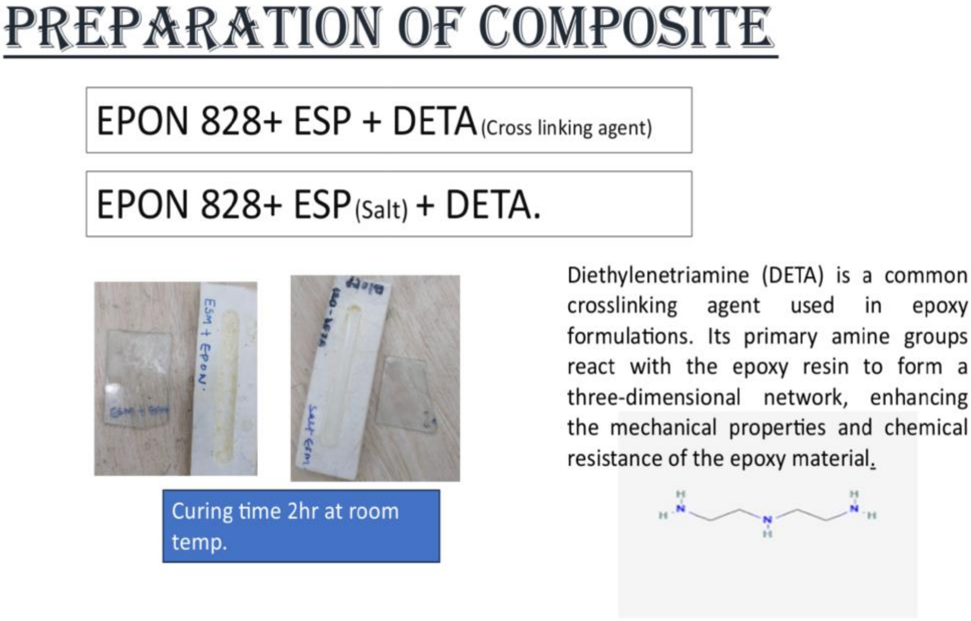
7. Coated on glass slides with a release agent to prevent adhesion.

8. Poured the epoxy mixture into the mold, ensuring even distribution and minimal air entrapment.

10. Allowed the samples to cure at room temperature for DETA curing agent and at 80c for 2hr and then for 5hr at 150c for 2AFD curing agent.

11. Monitor the curing process until epoxy resin gets fully cured and hardened.

12. After curing, carefully remove the composite samples from the mold for further testing and analysis.



PREPARATION OF COMPOSITE

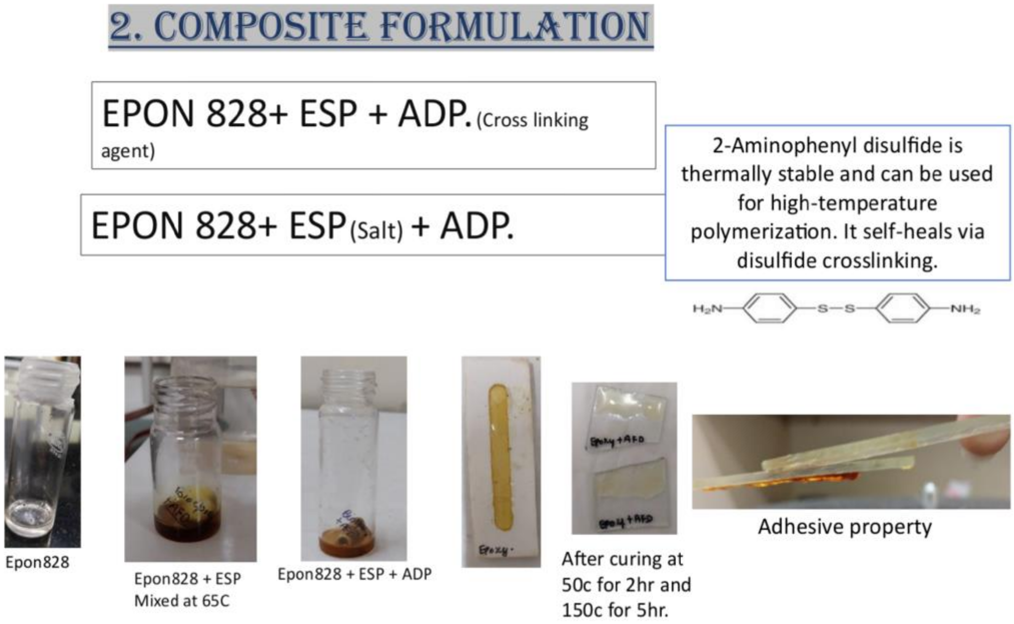
EPON 828+ ESP + DETA (Cross linking agent)

EPON 828+ ESP (Salt) + DETA.

Curing time 2hr at room temp.

Diethylenetriamine (DETA) is a common crosslinking agent used in epoxy formulations. Its primary amine groups react with the epoxy resin to form a three-dimensional network, enhancing the mechanical properties and chemical resistance of the epoxy material.

Fig.2: *Composite formulation with curing agent DETA*



2. COMPOSITE FORMULATION

EPON 828+ ESP + ADP. (Cross linking agent)

EPON 828+ ESP (Salt) + ADP.

2-Aminophenyl disulfide is thermally stable and can be used for high-temperature polymerization. It self-heals via disulfide crosslinking.

Epon828

Epon828 + ESP Mixed at 65C

Epon828 + ESP + ADP

After curing at 50c for 2hr and 150c for 5hr.

Adhesive property

Fig.3: *Composite formulation with curing agent 2AFD*

2.1 Composite from biobased epoxy

The synthesis of a bioadhesive using soybean-based bioepoxy as the polymer matrix, extracted eggshell protein as the filler, and 2-aminophenyl disulfide (2AFD) as the curing agent. Despite thorough experimentation, the curing process did not achieve the desired results due to the lack of optimization in the synthesis parameters.

Soybean-based bio epoxy was selected for its renewable origin and favorable mechanical properties.

The synthesis process involved mixing the soybean-based bio epoxy with the extracted eggshell protein and the 2AFD curing agent. Various parameters such as temperature, mixing ratios, and curing time were experimented with to optimize the curing process but we failed to synthesize it.

Despite multiple efforts to enhance the synthesis parameters, the curing procedure was unsuccessful. The bioadhesive blend did not solidify as anticipated, suggesting that the existing synthesis protocol was inadequate. Essential variables like the epoxy to filler ratio, curing agent concentration, and curing conditions necessitate additional optimization.

3. Self-healing property.

Epoxy Resin: Commercial epoxy (Epon828).

Filler: Extracted eggshell protein (specify extraction method).

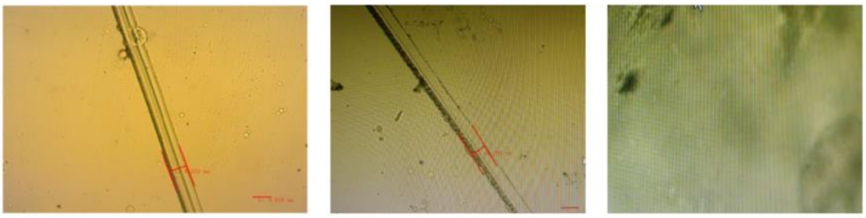
Curing Agent: 2-aminophenyl disulfide (2-AFD).

Curing Process:- Allowed the coated glass slides to cure at 80c for 2hr and at 150c for 5hr in an oven at to ensure complete cross-linking.

The self-healing testing involved using a sharp blade to create a controlled cut on the surface of the coated glass matrix. Care was taken to ensure the cut penetrated through the composite layer without damaging the underlying glass slide. The cut was then examined under an optical microscope to document the initial damage, with images captured to record the width and depth of the cut. For the self-healing process, the cut samples were placed in an oven preheated to 150°C and heated for 4 and 6 minutes to activate the self-healing mechanism facilitated by the reversible disulfide bonds introduced by 2-AFD. Following the heating, the samples were allowed to cool to room temperature. The healed regions were then re-examined under the optical microscope, with additional images captured to compare the post-healing state with the initial damage.

SELF-HEALING IN THE MATRIX.

EPON 828+ ESP + ADP. (Cross linking agent)



Before healing

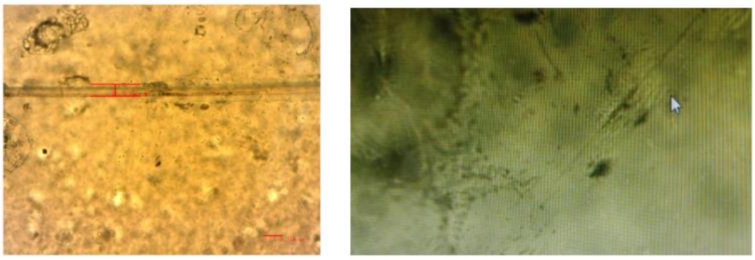
After 4min at 150C.

After 6 min at 150C

The length of the cut before healing is 0.333 um and after 4 min of heating the length is 0.240 um but after 6 min cut wasn't visible.

SELF-HEALING PROPERTY IN THE MATRIX:-

EPON 828+ ESP (Salt) + ADP.



Before healing

After healing

This matrix gets healed in 4 min. Then the length of cut before healing is 0.193 um and after 4 min at a temp of 150c no cut is visible on the matrix.

Self-healing property comparison with only epoxy and 2AFD matrix:- It took more than an hour to heal at 150c.

Fig.4: *Self-healing in polymer matrix*

Figure Caption:

Fig.1: *Schematic Representation of protein extracton.*

Fig.2: *Composite formulation with curing agent DETA*

Fig.3: *Composite formulation with curing agent 2AFD*

Fig.4: *Self-healing in polymer matrix*

CHAPTER 3

RESULTS AND DISCUSSION

1 Characterization of ESP using FTIR :

In an FTIR graph (as shown in Fig. 6), the x-axis represents the wavenumber (cm-1), which corresponds to different vibrational frequencies of the molecules, while the y-axis represents the absorbance or intensity of the infrared radiation. The FTIR spectrum of eggshell protein typically exhibits several characteristic peaks corresponding to different functional groups present in the protein structure. Here are some common peaks you may observe in the FTIR spectrum of eggshell protein: Amide I Band: This peak is typically observed around 16001700 cm-1 and is associated with the stretching vibrations of the C=O (carbonyl) groups in the protein backbone. The exact position and intensity of this peak can provide information about the protein's secondary structure.

Amide II Band: This peak appears around 1500-1600 cm-1 and is attributed to a combination of N-H bending and C-N stretching vibrations. It can also provide insights into the protein's secondary structure.

Amide A and Amide B Bands: These peaks are often observed in the lower wavenumber range (around 3100-3600 cm-1) and correspond to the stretching vibrations of N-H bonds involved in hydrogen bonding interactions within the protein structure. Additionally, other characteristic peaks related to specific functional groups like -CH, -OH, and -COO- groups may be observed depending on the composition of the eggshell protein.

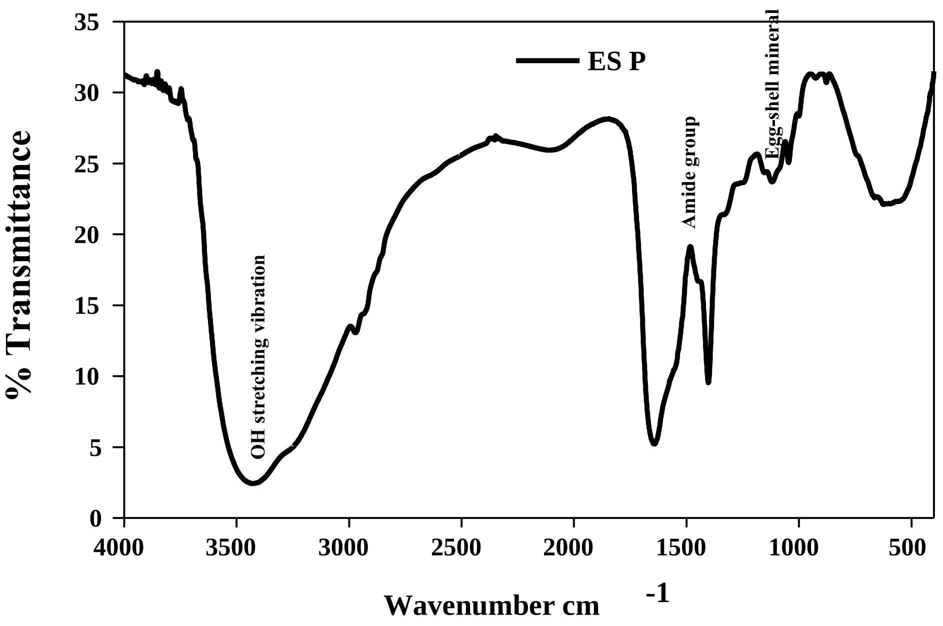


Fig. 5: *FTIR spectra of ESP*

2.Self-healing property of composite.

Self-Healing Performance of Epoxy (Epon 828) and Eggshell Protein with Salt Composite

The self-healing ability of a composite matrix comprising epoxy (Epon 828) and eggshell protein without salt was systematically investigated. Initially, the length of a cut in the composite was measured at 0.333 µm using an optical microscope. The cut sample was then subjected to a heating process in an oven preheated to 150°C. After 4 minutes of heating, the cut length was remeasured, revealing a reduction to 0.240 µm, indicating partial healing. Further heating for a total of 6 minutes resulted in the cut becoming completely invisible under the optical microscope, suggesting complete healing of the composite. These results demonstrate the effective self-healing capability of the epoxy (Epon 828) and eggshell protein composite. The significant reduction in cut length from 0.333 µm to 0.240 µm within the first 4 minutes underscores a robust initial healing phase. The complete disappearance of the cut after 6 minutes of heating confirms the dynamic self-healing mechanism, likely facilitated by the reversible disulfide bonds introduced by 2-aminophenyl disulfide.

2.1 Self-Healing Performance of Epoxy and Eggshell Protein with salt

The self-healing properties of a composite matrix composed of epoxy (Epon 828) and eggshell protein with salt were investigated. Initially, the length of a cut in the composite was measured at 0.193 µm using an optical microscope. The cut sample was then placed in an oven preheated to 150°C. After 4 minutes of heating, the cut was re-examined and found to be no longer visible under the optical microscope, indicating complete healing of the composite.

2.2 Comparative Analysis with Epoxy (Epon 828) and 2-AFD Matrix

To assess the effectiveness of the eggshell protein with salt as a healing promoter, a comparison was made with a composite matrix consisting of only epoxy (Epon 828) and 2-aminophenyl disulfide (2-AFD):

The epoxy (Epon 828) and 2-AFD matrix took more than an hour to heal at 150°C.

The composite matrix of epoxy (Epon 828) and eggshell protein with salt exhibited significantly faster self-healing properties, achieving complete healing in just 4 minutes at 150°C and without salt healing in 6 min. In contrast, the matrix composed of only epoxy (Epon 828) and 2-aminophenyl disulfide (2-AFD) required more than an hour to achieve similar healing under the same temperature conditions. This highlights the superior self-healing efficiency of the epoxy and eggshell protein with salt composite, making it a promising material for applications requiring rapid repair.

Figure Caption:

Fig.6: *FTIR spectra of ESP.*

Conclusion

In conclusion, we have successfully developed self-healing bio-adhesive composite materials by incorporating 2-aminophenyl disulfide as a dynamic cross-linker in an epoxy resin matrix reinforced with eggshell protein. The synthesized composites exhibit promising mechanical properties and autonomous repair capabilities, making them suitable for various engineering applications. Future research will focus on optimizing the composition and processing parameters and also to use the bio-based polymer matrix to enhance the performance of these materials and to make it sustainable to explore their practical applications in real-world scenarios.

References

1. Peirce, J. Jeffrey, P. Aarne Vesilind, and Ruth Weiner. Environmental pollution and control. Butterworth-Heinemann, 1998.
2. Mathur, Praveen, Sangeeta Patan, and Anand S. Shobhawat. "Need of biomedical waste management system in hospitals-An emerging issue-a review." Current World Environment 7.1 (2012): 117.
3. Harrison, Thomas R., et al. "From trash to treasure: Sourcing high-value, sustainable cellulosic materials from living bioreactor waste streams." International Journal of Biological Macromolecules (2023): 123511.
4. Russo, Ivan, et al. "From trash to treasure: The impact of consumer perception of bio-waste products in closed-loop supply chains." Journal of Cleaner Production 218 (2019): 966974.
5. Li, Huan, et al. "Melt-Compounded Keratin-TPU Self-Assembled Composite Film as Bioinspired e-Skin." Advanced Materials Interfaces 5.19 (2018): 1800635.
6. Na, Woo Seok, et al. "Eggshell membrane reinforced polypropylene biocomposite and its tactile assessment." Journal of Applied Polymer Science 137.46 (2020): 49508.
7. Pan, Hong, and Tae-Woo Lee. "Recent progress in the development of wearable pressure sensors derived from biological materials." Advanced Healthcare Materials 10.17 (2021): 2100460.
8. Sun, Qingqing, et al. "Functional biomaterials towards flexible electronics and sensors." Biosensors and Bioelectronics 119 (2018): 237-251.
9. de Sousa Victor, Rayssa, et al. "A review on Chitosan’s uses as biomaterial: tissue engineering, drug delivery systems and cancer treatment." Materials 13.21 (2020): 4995.
10. Morsada, Zinnat, et al. "Recent progress in biodegradable and bioresorbable materials: From passive implants to active electronics." Applied Materials Today 25 (2021): 101257.
11. Kabirifar, Kamyar, et al. "Construction and demolition waste management contributing factors coupled with reduce, reuse, and recycle strategies for effective waste management: A review." Journal of Cleaner Production 263 (2020): 121265.
12. Foster, Gillian. "Circular economy strategies for adaptive reuse of cultural heritage buildings to reduce environmental impacts." Resources, Conservation and Recycling 152 (2020): 104507.

13. Faridi, Hamideh, and Akbar Arabhosseini. "Application of eggshell wastes as valuable and utilizable products: A review." Research in Agricultural Engineering 64.2 (2018): 104-114.
14. Faridi, H. and Arabhosseini, A., 2018. Application of eggshell wastes as valuable and utilizable products: A review. Research in Agricultural Engineering, 64(2), pp.104-114.
15. Faridi, Hamideh, and Akbar Arabhosseini. "Application of eggshell wastes as valuable and utilizable products: A review." Research in Agricultural Engineering 64, no. 2 (2018): 104-114.
16. Jirimali, H.D., Chaudhari, B.C., Khanderay, J.C., Joshi, S.A., Singh, V., Patil, A.M. and Gite, V.V., 2018. Waste eggshell-derived calcium oxide and nanohydroxyapatite biomaterials for the preparation of LLDPE polymer nanocomposite and their thermomechanical study. Polymer-Plastics Technology and Engineering, 57(8), pp.804811.
17. Baláž M, Ficeriová J, Briančin J. Influence of milling on the adsorption ability of eggshell waste. Chemosphere. 2016 Mar 1;146:458-71.
18. Hart A. Mini-review of waste shell-derived materials’ applications. Waste Management & Research. 2020 May;38(5):514-27
19. Chaudhuri, Biswadeep, Bholanath Mondal, D. K. Modak, K. Pramanik, and B. K. Chaudhuri. "Preparation and characterization of nanocrystalline hydroxyapatite from egg shell and K2HPO4 solution." Materials Letters 97 (2013): 148-150..
20. Chaudhuri B, Mondal B, Modak DK, Pramanik K, Chaudhuri BK. Preparation and characterization of nanocrystalline hydroxyapatite from egg shell and K2HPO4 solution. Materials Letters. 2013 Apr 15;97:148-50.
21. Wang, C. G., Surat'man, N. E. B., Chang, J. J., Ong, Z. L., Li, B., Fan, X., ... & Li, Z. (2022). Polyelectrolyte Hydrogels for Tissue Engineering and Regenerative Medicine. *Chemistry–An Asian Journal*, *17*(18), e202200604.
22. Fuhrmann, S. (2013). *Synthesis of mesogenic and oligo polyols and their influence on polyurethane properties* (Doctoral dissertation, Heriot-Watt University).

23. Petriccione, M.; Mastrobuoni, F.; Pasquariello, M.S.; Zampella, L.; Nobis, E.; Capriolo, G.; Scortichini, M. Effect of Chitosan Coating on the Postharvest Quality and Antioxidant Enzyme System Response of Strawberry Fruit during Cold Storage. Foods
2015, 4, 501–523. [CrossRef].

24. U. Lafont, H. van Zeijl and S. van der Zwaag, ACS Appl.
Mater. Interfaces, 2012, 4, 6280–6288.

25 .J. Canadell, H. Goossens and B. Klumperman, Macromolecules, 2011, 44, 2536–2541.

26. B. T. Michal, C. A. Jaye, E. J. Spencer and S. J. Rowan, ACS Macro Lett., 2013, 2, 694–699.

27. Y. Amamoto, H. Otsuka, A. Takahara and K. Matyjaszewski, Adv. Mater., 2012, 24, 3975–3980.

28. G. H. Deng, F. Y. Li, H. X. Yu, F. Y. Liu, C. Y. Liu, W. X. Sun, H. F. Jiang and Y. M. Chen, ACS Macro Lett., 2012, 1, 275–279.

29. A. Rekondo, R. Martin, A. Ruiz de Luzuriaga, G. Cabanero, H. J. Grande and I. Odriozola, Mater. Horiz., 2014, 1, 237–240.

30. A. M. Belenguer, T. Frisˇˇcic´, G. M. Day and J. K. M. Sanders, Chem. Sci., 2011, 2, 696.

31. R. Caraballo, PhD thesis, KTH Royal Institute of Technology, 2010.

32. Al-Tayyar, N.A.; Youssef, A.M.; Al-Hindi, R.R. Edible coatings and antimicrobial nanoemulsions for enhancing shelf life and reducing foodborne pathogens of fruits and vegetables: A review. Sustain. Mater. Technol. 2020, 26, e00215.

33. Pandey, V.K.; Islam, R.U.; Shams, R.; Dar, A.H. A comprehensive review on the application of essential oils as bioactive compounds in Nano-emulsion based edible coatings of fruits and vegetables. Appl. Food Res. 2022, 2, 100042. [CrossRef]

34. de Oliveira Filho, J.G.; Miranda, M.; Ferreira, M.D.; Plotto, A. Nanoemulsions as Edible Coatings: A Potential Strategy for Fresh Fruits and Vegetables Preservation. Foods 2021, 10, 2438. [CrossRef]

35. Tripathi, A.D.; Sharma, R.; Agarwal, A.; Haleem, D.R. Nanoemulsions based edible coatings with potential food applications. Int.
J. Biobased Plast. 2021, 3, 112–125. [CrossRef]

36. Horison, R.; Sulaiman, F.O.; Alfredo, D.; Wardana, A. Physical characteristics of nanoemulsion from chitosan/nutmeg seed oil and evaluation of its coating against microbial growth on strawberry. Food Res. 2019, 3, 821–827. [CrossRef] [PubMed]

127. Sessa, M.; Ferrari, G.; Donsì, F. Novel Edible Coating Containing Essential Oil Nanoemulsions to Prolong the Shelf Life of Vegetable Products. Chem. Eng. Trans. 2015, 43, 55–60.

36. Kim, I.-H.; Lee, H.; Kim, J.E.; Song, K.B.; Lee, Y.S.; Chung, D.S.; Min, S.C. Plum Coatings of Lemongrass Oil-incorporating Carnauba Wax-based Nanoemulsion. J. Food Sci. 2013, 78, E1551–E1559. [CrossRef]

37. Tartaro, G.; Mateos, H.; Schirone, D.; Angelico, R.; Palazzo, G. Microemulsion Microstructure(s): A Tutorial Review. Nanomaterials 2020, 10, 1657. [CrossRef] [PubMed]

38. Paul, B.K.; Moulik, S.P. Uses and applications of microemulsions. Curr. Sci. 2001, 80, 990–1001.

40. Xue, J.; Wu, T.; Dai, Y.; Xia, Y. Electrospinning and Electrospun Nanofibers: Methods, Materials, and Applications. Chem. Rev. 2019, 119, 5298–5415. [CrossRef]

41. Shi, C.; Fang, D.; Huang, C.; Lyu, L.; Wu, W.; Li, W. Electrospun biopolymer material for antimicrobial function of fresh fruit and vegetables: Application perspective and challenges. LWT 2023, 174, 114374. [CrossRef]

42. Gagaoua, M.; Pinto, V.Z.; Göksen, G.; Alessandroni, L.; Lamri, M.; Dib, A.L.; Boukid, F. Electrospinning as a Promising Process to Preserve the Quality and Safety of Meat and Meat Products. Coatings 2022, 12, 644. [CrossRef]

43. Melendez-Rodriguez, B.; Castro-Mayorga, J.L.; Reis, M.A.M.; Sammon, C.; Cabedo, L.; Torres-Giner, S.; Lagaron, J.M. Preparation and Characterization of Electrospun Food Biopackaging Films of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Derived from Fruit Pulp Biowaste. Front. in Sustain. Food Syst. 2018, 2, 38. [CrossRef]

44. Merino, D.; Quilez-Molina, A.I.; Perotto, G.; Bassani, A.; Spigno, G.; Athanassiou, A. A second life for fruit and vegetable waste: A review on bioplastic films and coatings for potential food protection applications. Green Chem. 2022, 24, 4703–4727. [CrossRef]

45. Tavares, L.; Souza, H.K.S.; Gonçalves, M.P.; Rocha, C.M.R. Physicochemical and microstructural properties of composite edible film obtained by complex coacervation between chitosan and whey protein isolate. Food Hydrocoll. 2021, 113, 106471. [CrossRef]

46. Ramos, M.; Mellinas, C.; Solaberrieta, I.; Garrigós, M.C.; Jiménez, A. Emulsions Incorporated in Polysaccharide-Based Active Coatings for Fresh and Minimally Processed Vegetables. Foods 2021, 10, 665. [CrossRef]

47. Jose, A.; Pareek, S.; Radhakrishnan, E.K. Advances in Edible Fruit Coating Materials. In Advances in Agri-Food Biotechnology; Sharma, T.R., Deshmukh, R., Sonah, H., Eds.; Springer Singapore: Singapore, 2020; pp. 391–408.