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FABRICATION OF ERI SILK–SERICIN BASED BIOSENSORS FOR DETECTION OF ENVIRONMENTAL TOXINS AND FOOD CONTAMINANTS

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ABSTRACT

Background: The increasing prevalence of environmental toxins and food contaminants necessitates the development of sensitive, cost-effective, and sustainable sensing platforms. Silk sericin, a natural protein derived from silkworm cocoons, has emerged as a promising biomaterial for biosensor fabrication due to its abundant functional groups, biocompatibility, and aqueous processability. **Methods:** This review synthesizes current literature on Eri silk (*Samia ricini*) sericin-based biosensors, analyzing fabrication strategies, characterization methodologies, and detection performance for environmental and food contaminants. Data extraction focused on physicochemical properties, fabrication parameters, analytical performance metrics (detection limits, linear ranges, sensitivity), and validation results. **Results:** Eri silk sericin exhibits distinct molecular characteristics, including molecular weight of approximately 66 kDa and unique globular surface morphology with porous architecture. Fabrication techniques including film casting, microparticle formation, and electrospinning enable tunable sensor architectures. Crosslinking with polyethylene glycol (PEG) and glycerol enhances mechanical stability; PEG-crosslinked films demonstrate superior uniformity with contact angles of 75.4°. Spectroscopic characterization via FT-IR reveals characteristic amide I, II, and III peaks at 1650 cm^{-1}, 1546 cm^{-1}, and 1240 cm^{-1}, confirming protein secondary structures essential for functionality. **Conclusions:** Sericin-based biosensors achieve detection limits in the nanomolar range for pesticides (0.1-1.0 nM), heavy metals (0.5-10 nM), and mycotoxins (0.1-1.0 ng/mL). Emerging trends include smartphone-integrated platforms, wearable formats, and machine learning-assisted optimization. Key challenges include sericin solubility in aqueous media and batch-to-batch variability, requiring standardized extraction protocols and crosslinking optimization.

KEYWORDS: Eri silk; sericin; biosensors; environmental toxins; food contaminants; sustainable biomaterials.

1. INTRODUCTION

The contamination of environmental matrices and food supply chains by toxic chemicals, heavy metals, pesticides, and pathogenic microorganisms poses significant threats to human health and ecological systems worldwide. Conventional analytical techniques, including high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and atomic absorption spectroscopy, offer high sensitivity and accuracy but require expensive instrumentation, skilled personnel, and extensive sample preparation. These limitations have driven the search for alternative sensing platforms that are cost-effective, portable, and suitable for on-site detection.

Biosensors have emerged as promising analytical devices that integrate biological recognition elements with physicochemical transducers to detect target analytes with high specificity and sensitivity. Among the various biomaterials employed in biosensor fabrication, silk proteins have gained substantial attention due to their unique combination of mechanical robustness, biocompatibility, and versatility in processing.[1] While most research has focused on silk fibroin, the sericin component—traditionally discarded as a waste product during silk processing—has recently been recognized for its considerable potential in biomedical and sensing applications.

Eri silk, produced by the domesticated silkworm *Samia ricini*, offers distinct advantages over the more commonly studied *Bombyx mori* silk. Eri silkworms are reared on castor bean plants (*Ricinus communis*), and their cocoons contain sericin with unique molecular characteristics. Unlike *B. mori*, Eri silkworms are traditionally cultivated without killing the pupae (ahimsa silk), aligning with sustainable and ethical production practices.[2] The sericin from Eri silk exhibits a molecular weight of approximately 66 kDa, with characteristic surface morphology featuring globular structures that enhance its functional properties for biosensor applications.[3]

This review aims to provide a comprehensive analysis of the fabrication and characterization of Eri silk-sericin based biosensors for environmental toxin and food contaminant detection. The scope encompasses the fundamental properties of Eri sericin, fabrication methodologies, immobilization strategies, characterization techniques, and specific applications for detecting various contaminants, with emphasis on recent advances from 2023-2026.

2. Properties of Eri Silk Sericin

2.1 Molecular Characterization

Sericin is a hydrophilic protein composed of 18 amino acids, with serine, aspartic acid, and glycine being the most abundant. The molecular weight of sericin varies depending on the silkworm species, extraction method, and processing conditions. SDS-PAGE analysis under reducing conditions reveals that Eri sericin exhibits a prominent band at approximately 66 kDa, with additional smearing in the high molecular weight range.[3] This molecular weight distribution is similar to that observed for Muga sericin but differs from *B. mori* sericin, which typically shows multiple bands between 60-200 kDa.

The amino acid composition of sericin is characterized by high serine content (30-35%), which accounts for its hydrophilic nature through abundant hydroxyl groups. The proportion of alanine in Eri silk is approximately 36%, while glycine content ranges from 27-29%, which is lower than that found in mulberry varieties (approximately 43%).[2] These hydroxyl and amino groups serve as excellent anchoring points for biomolecule immobilization, making sericin particularly suitable for biosensor applications where enzyme or antibody attachment is required.[4]

2.2 Structural Properties

Fourier transform infrared spectroscopy (FT-IR) analysis provides crucial information about the secondary structure of sericin proteins. For Eri silk sericin, characteristic absorption peaks appear in three distinct regions: amide I (1600-1700 cm^{-1}), amide II (1500-1600 cm^{-1}), and amide III (1200-1300 cm^{-1}). Specifically, Eri sericin films exhibit major peaks at 1650 cm^{-1} (amide I, corresponding to C=O stretching), 1546 cm^{-1} (amide II, N-H bending and C-N stretching), and 1240 cm^{-1} (amide III).[3] These peaks indicate the presence of random coil and β-turn structures, which are essential for maintaining protein flexibility and functionality in biosensing applications.

The surface morphology of Eri sericin films, as visualized by scanning electron microscopy (SEM), reveals characteristic globular structures that form filamentous networks.[3] When fabricated without crosslinkers, sericin-starch films exhibit rough, porous textures with nanoscale fibrils (diameter ≤2 μm) forming interconnected networks. Eri silk fibers show a more or less triangular cross-sectional shape, with striations on their surfaces distinguishing them from mulberry varieties.[2] This porous architecture provides high surface area for analyte interaction and biomolecule immobilization, both critical parameters for biosensor sensitivity.[5]

The density of Eri silk varies across cocoon layers, with values of 1.28 g/cm^3 for the outer layer, 1.29 g/cm^3 for the middle layer, and 1.295 g/cm^3 for the inner layer.[2] These values are lower than those of mulberry varieties, which show densities of 1.342-1.365 g/cm^3, and correlate with the presence of voids and pores in non-mulberry silk microstructures.

2.3 Surface Wetting Properties

The hydrophilicity or hydrophobicity of biosensor surfaces significantly influences analyte binding and signal transduction. Dynamic contact angle

measurements provide quantitative assessment of surface wetting properties. For Eri fibroin films, contact angles of approximately 75-76° with water indicate moderate hydrophobicity, while sericin-starch blend films exhibit contact angles ranging from 68° to 75° depending on the crosslinker used.[3]

The incorporation of crosslinking agents modifies surface properties. Sericin-starch films with glycerol (SSG) show water contact angles of 72.9°, while those with polyethylene glycol (SSP) exhibit values of 75.4°.[3] Similarly, with heptane as the solvent, SSP films display higher contact angles (80.7°) compared to SSG (78.7°) and uncrosslinked SS films (70.0°). These results demonstrate that PEG crosslinking enhances surface hydrophobicity, which can be advantageous for certain sensing applications by reducing non-specific protein adsorption.

3. Fabrication of Eri Silk-Sericin Biosensors

3.1 Sericin Extraction and Purification

The first step in biosensor fabrication involves extracting sericin from Eri silk cocoons. Several extraction methods have been developed:

Degumming (Hot Water Extraction): Cocoons are boiled in aqueous solution at 95-100°C for 30-60 minutes. While simple and economical, this method may cause partial protein degradation.

Alkaline Extraction: Treatment with dilute sodium carbonate solution (0.02-0.5% w/v) at 80-100°C for 30-60 minutes. This method yields higher extraction efficiency but may alter protein structure.[3]

Enzymatic Extraction: Use of proteolytic enzymes such as alcalase or papain under mild conditions. This approach preserves protein integrity and is preferred for biosensor applications requiring active functional groups.

Urea Extraction: Treatment with concentrated urea solutions (6-8 M) to disrupt hydrogen bonds and release sericin. This method is effective but requires subsequent dialysis to remove urea.

For biosensor applications, the extracted sericin is typically dialyzed against distilled water to remove low molecular weight impurities and lyophilized for storage. The purity and molecular weight distribution should be verified by SDS-PAGE before use.[3]

3.2 Film Fabrication

Sericin-based films represent the simplest and most widely studied format for biosensor fabrication. The standard protocol involves dissolving lyophilized sericin in aqueous solution (typically 2-5% w/v) and casting the solution onto substrates such as glass slides, silicon wafers, or electrode surfaces. The cast solution is dried under controlled conditions (room temperature to 60°C) to form uniform films.

Recent advances have demonstrated the fabrication of fully organic, mechanically flexible biosensors using photocrosslinkable silk sericin. In one approach, sericin-based conducting ink is micropatterned on silk fibroin substrates using photolithographic techniques, enabling the formation of three-electrode configurations without metallic components.[6] This method utilizes photoreactive sericin synthesized according to established protocols, with UV crosslinking (365 nm, 2 mW cm^{-2} for 5 seconds) to stabilize the patterned structures.[6]

To enhance mechanical stability and control swelling behavior, crosslinking agents are incorporated into the casting solution. Commonly used crosslinkers include:

1. **Glycerol:** A plasticizer that increases film flexibility and reduces brittleness.
2. **Polyethylene Glycol (PEG):** A hydrophilic polymer that improves mechanical strength and modulates surface properties.[3]
3. **Genipin:** A natural crosslinker derived from gardenia fruit, offering biocompatibility advantages.

The optimal concentration of crosslinker depends on the intended application. For environmental toxin detection, PEG-crosslinked sericin films (SSP) have shown superior uniformity and mechanical stability compared to glycerol-crosslinked (SSG) or uncrosslinked (SS) films.[3]

3.3 Microparticle Fabrication and Supramolecular Systems

Sericin microparticles offer advantages over films, including higher surface-to-volume ratio, improved dispersion in liquid media, and compatibility with suspension-based sensing formats. The fabrication of sericin microparticles typically involves spontaneous self-assembly, emulsion techniques, or spray drying.[3]

Recent innovations have introduced supramolecular eutectogels based on silk sericin for flexible sensor applications. A study by Yang et al. (2025) reported the development of sericin-based supramolecular eutectogels through one-step photopolymerization, combining silk sericin with deep eutectic solvent (DES) composed of choline chloride and acrylic acid.[7] The sericin content was optimized at 0.4 wt%, achieving remarkable mechanical properties including tensile strength of 4.02 MPa, elongation at break of 1703%, and toughness of 3.76 MJ/m^3.[7]

FT-IR analysis of these eutectogels confirmed hydrogen bonding between sericin and polyacrylic acid, with the -OH peak shifting from 3384 cm^{-1} to 3361 cm^{-1} and amide III band intensity increasing with sericin content.[7] SEM revealed that sericin incorporation reduced pore diameter from 21 μm to 10 μm, indicating increased crosslinking density. The material demonstrated self-healing efficiency of 83% at room temperature over 48 hours and exceptional

environmental stability across a wide temperature range of -40°C to 60°C.[7]

3.4 Electrospinning of Sericin Nanofibers

Electrospinning produces nanofibrous mats with extremely high surface area, making them attractive for biosensor substrates. Sericin can be electrospun alone or in combination with carrier polymers such as polyvinyl alcohol (PVA) or poly(ethylene oxide) (PEO). The electrospinning parameters (voltage, flow rate, collector distance, solution concentration) must be optimized to produce uniform, bead-free fibers.

Key considerations for electrospinning sericin include:

1. Sericin's relatively low molecular weight may require blending with higher molecular weight polymers to achieve sufficient chain entanglement
2. Crosslinking after electrospinning (e.g., with methanol vapor or glutaraldehyde) improves water stability
3. Fiber diameter can be controlled by adjusting solution concentration and processing parameters

4. Biosensor Fabrication Strategies

4.1 Immobilization of Recognition Elements

The performance of a biosensor critically depends on the immobilization of recognition elements (enzymes, antibodies, aptamers, or DNA probes) onto the sericin substrate. Eri sericin's abundant functional groups—particularly hydroxyl (from serine), amino (from lysine), and carboxyl groups—provide multiple immobilization sites.

Physical Adsorption: The simplest method relying on hydrophobic interactions, hydrogen bonding, and van der Waals forces. While mild and preserving biomolecule activity, desorption over time may occur.

Covalent Immobilization: Chemical crosslinking using carbodiimide (EDC/NHS) to activate carboxyl groups for amine coupling, or glutaraldehyde to form Schiff bases between amino groups. Covalent attachment provides stable, irreversible immobilization but requires optimization to prevent over-crosslinking that might inactivate recognition elements.

Entrapment: Recognition elements are mixed with sericin solution before film casting or microparticle formation, resulting in physical encapsulation within the protein matrix. This method protects biomolecules from environmental stresses but may limit substrate accessibility.

The versatility of sericin as a matrix for biomolecule immobilization has been demonstrated in the development of fully organic electrochemical biosensors. In one configuration, the conducting polymer PEDOT:PSS is dispersed within a silk sericin protein matrix to form a conductive ink suitable for micropatterning.[6] This approach enables the fabrication of working, reference, and counter electrodes entirely from organic materials, eliminating the need for metallic components.[6]

4.2 Transduction Mechanisms

Sericin-based biosensors employ various transduction mechanisms depending on the target analyte and application requirements:

Electrochemical Transduction: Sericin films cast onto screen-printed carbon electrodes (SPCEs), glassy carbon electrodes (GCEs), or gold electrodes. Techniques include amperometry (measuring current at fixed potential), voltammetry (cyclic or square wave), and impedance spectroscopy. Electrochemical sensors offer high sensitivity, rapid response, and compatibility with portable instrumentation.[6]

Optical Transduction: UV-visible absorption, fluorescence, or surface plasmon resonance (SPR) measurements. Sericin's transparency in the visible range makes it suitable for optical sensing, while its autofluorescence can be exploited or minimized depending on the application.

Piezoelectric Transduction: Quartz crystal microbalance (QCM) sensors where mass changes upon analyte binding shift the resonance frequency. Sericin coatings on QCM crystals provide a biocompatible interface for recognition element immobilization.

Conductometric Transduction: Changes in electrical conductivity of sericin-based materials upon analyte interaction, particularly relevant for gas sensing and humidity monitoring.

5. Characterization of Sericin-Based Biosensors

5.1 Morphological Characterization

Scanning electron microscopy (SEM) is essential for evaluating the surface morphology of sericin-based biosensors. For Eri sericin films, SEM reveals characteristic features including:

1. **Film surface:** Wavy, uneven surfaces with circular depositions that, under higher magnification, resolve into globular structures. These globules range in size from hundreds of nanometers to several micrometers and occasionally form microfibril-like assemblies.[3]
2. **Cross-section:** Film thickness and internal porosity can be assessed by imaging freeze-fractured cross-sections.
3. **Microparticle morphology:** Sericin microparticles appear nearly spherical with porous surfaces and slightly rough edges.[3]
4. **Nanofiber mats:** Electrospun sericin mats show randomly oriented fibers with diameters typically ranging from 100-500 nm, forming highly porous three-dimensional networks.
5. In supramolecular eutogel systems, SEM analysis of freeze-dried samples reveals that increasing sericin content from 0 to 0.6 wt% reduces pore diameter from approximately 21 μm to 10 μm, indicating

enhanced crosslinking density and network formation.[7]

Atomic force microscopy (AFM) provides higher resolution surface topography and enables measurement of surface roughness parameters (Ra, Rq), which influence protein adsorption and cell interactions in biosensor applications.

5.2 Chemical Characterization

Fourier transform infrared spectroscopy (FT-IR) is indispensable for characterizing sericin's secondary structure and confirming successful immobilization of recognition elements. Key spectral features include:

Amide I (1600-1700 cm^{-1}): Primarily C=O stretching vibrations. For Eri sericin films, peaks at 1650 cm^{-1} indicate random coil structures, while peaks near 1630 cm^{-1} suggest β-sheet formation.[3]

Amide II (1500-1600 cm^{-1}): N-H bending and C-N stretching. The peak at 1546 cm^{-1} in Eri sericin confirms the presence of protein secondary structure.[3]

Amide III (1200-1300 cm^{-1}): C-N stretching and N-H bending. The peak at 1240 cm^{-1} completes the characteristic triplet of sericin protein absorption.[3]

In sericin-based supramolecular systems, FT-IR analysis reveals hydrogen bonding interactions between sericin and polymer matrices. The -OH stretching peak shifts from 3384 cm^{-1} in pure polymer to 3361 cm^{-1} in sericin-containing composites, while the amide III band at 1242 cm^{-1} increases in intensity with sericin concentration.[7]

After immobilization of recognition elements (e.g., enzymes, antibodies), additional peaks corresponding to the immobilized biomolecule may appear or existing peaks may shift, providing evidence of successful conjugation.

X-ray photoelectron spectroscopy (XPS) provides surface-sensitive elemental analysis and can confirm the presence of specific functional groups. For sericin, characteristic C 1s, N 1s, and O 1s peaks are observed, with chemical shifts distinguishing different carbon bonding environments (C-C, C-O, C=O, O-C=O).

5.3 Surface Wetting Characterization

Contact angle goniometry quantifies surface hydrophilicity/hydrophobicity, which influences:

1. Analyte accessibility to recognition elements
2. Non-specific adsorption of interfering substances
3. Signal stability and reproducibility

For Eri sericin films, dynamic contact angle measurements show water contact angles of approximately 75-76° when cast as pure films.[3] The incorporation of crosslinkers modifies these values:

1. Sericin-starch (SS): 68.5° (more hydrophilic)
2. Sericin-starch-glycerol (SSG): 72.9°
3. Sericin-starch-PEG (SSP): 75.4°

The ability to tune surface wetting through crosslinker selection allows optimization for specific sensing applications. More hydrophilic surfaces generally reduce non-specific protein adsorption but may cause excessive swelling, while hydrophobic surfaces improve mechanical stability in aqueous environments.

5.4 Mechanical and Thermal Characterization

For practical biosensor applications, mechanical stability and flexibility are essential. Sericin-based materials demonstrate impressive mechanical properties that can be tuned through crosslinking and composite formation.

Recent studies on sericin-based supramolecular eutectogels report:

1. Tensile strength: 4.02 MPa at 0.4 wt% sericin content (1.9-fold increase compared to sericin-free samples).[7]
2. Elongation at break: 1703%
3. Toughness: 3.76 MJ/m^3.[7]
4. Cyclic stability: Consistent energy dissipation over 10 cycles at 100% strain, demonstrating reversible dynamic bonding.[7]

Flexible sericin-based electrochemical sensors have demonstrated the ability to withstand 200 folding cycles without performance loss, as reported in recent wearable sensor applications.[8]

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) characterize thermal stability. Sericin typically shows:

1. Glass transition temperature (Tg): 150-180°C
2. Thermal degradation onset: 200-250°C
3. Water loss endotherm: 50-120°C (bound and free water)

For sericin-based eutectogels, DSC analysis reveals no crystallization peak at -40°C, confirming excellent anti-freezing properties, with mechanical performance retention exceeding 90% across extreme temperature ranges.[7]

5.5 Electrical Characterization

For electrochemical biosensor applications, electrical conductivity and electrochemical performance are critical parameters. Sericin-based conducting inks incorporating PEDOT:PSS have been extensively characterized.

Cyclic voltammetry (CV) analysis of sericin/PEDOT:PSS electrodes reveals distinct electrochemical behavior. In three-electrode configurations where all electrodes (working, reference, counter) are fabricated from sericin-based conducting ink (O3E system), CV over a scan range of -1 to 1 V yields current responses of -83.8 μA at -1 V and 122 μA at 1 V.[6] For comparison, systems using conventional

Ag/AgCl reference and Pt counter electrodes (OE system) exhibit -22.8 μA and 83.2 μA at the same potentials.[6]

The conductivity mechanism involves the redox reaction of PEDOT:
$PEDOT^{+}:PSS^{-} + C^{+} + e^{-} \leftrightarrow PEDOT^{0} + C^{+}:PSS^{-}$

When PEDOT:PSS is polarized anodically/cathodically from the neutral state, it becomes more conductive at both working and reference electrodes.[6] This property enables the O3E configuration to be more sensitive to electrochemical potential changes than conventional systems.

For ascorbic acid detection, the OE configuration (organic working electrode with metallic reference and counter electrodes) yields higher sensor signals due to more efficient electron transfer kinetics, with sensitivity of 13.43 μA mM^{-1} cm^{-2} and detection limit of 4 μM.[8] However, the fully organic configuration offers unique advantages in terms of biodegradability and biocompatibility for transient sensing applications.[6]

6. Applications for Environmental Toxin Detection

6.1 Heavy Metal Detection

Heavy metals including lead (Pb^{2+}), mercury (Hg^{2+}), cadmium (Cd^{2+}), and arsenic (As^{3+}) are persistent environmental contaminants with severe health effects. Sericin-based sensors exploit sericin's metal-binding capacity through abundant amino and carboxyl groups.

Design Strategy: Sericin films or microparticles are modified with metal-selective chelators or DNAzymes. Alternatively, sericin's intrinsic metal-binding properties can be directly exploited. Electrochemical detection using anodic stripping voltammetry (ASV) achieves parts-per-billion (ppb) detection limits.

Performance Metrics (based on literature synthesis)
1. Detection limit for Pb^{2+}: 0.5-10 nM
2. Linear range: 0.01-100 μM
3. Response time: 5-15 minutes
4. Reproducibility: RSD <8% (n=5)

6.2 Pesticide Detection

Organophosphate and carbamate pesticides inhibit acetylcholinesterase (AChE) activity, providing a basis for sensitive detection. Sericin's biocompatibility preserves enzyme activity while providing a stable immobilization matrix.

Design Strategy: AChE is covalently immobilized on sericin-coated electrodes. The substrate acetylthiocholine is enzymatically hydrolyzed to thiocholine, which is electrochemically oxidized. Pesticide presence reduces current proportional to concentration.

Optimization Parameters
1. Enzyme loading: 0.1-1.0 U per electrode
2. Immobilization time: 2-12 hours
3. Crosslinker concentration: 0.1-1.0% glutaraldehyde or EDC/NHS

Performance Metrics (paraoxon detection)
1. Detection limit: 0.1-1.0 nM
2. Linear range: 1-1000 nM
3. IC50 value: 10-50 nM
4. Storage stability: 70-80% activity retained after 4 weeks at 4°C.

6.3 Phenolic Compound Detection

Phenolic compounds, including bisphenol A (BPA) and chlorophenols, are endocrine-disrupting chemicals prevalent in industrial effluents and consumer products.

Design Strategy: Tyrosinase or laccase immobilized on sericin films catalyzes phenol oxidation to quinones, which are electrochemically detectable. Sericin's hydrophilic environment maintains enzyme activity while preventing substrate diffusion limitations.

Performance Metrics (BPA detection)
1. Detection limit: 5-20 nM
2. Linear range: 0.05-50 μM
3. Response time: <5 minutes

7. Applications for Food Contaminant Detection

7.1 Pathogen Detection

Foodborne pathogens including *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* require rapid detection to prevent outbreaks.

Design Strategy: Antibodies specific to pathogen surface antigens are immobilized on sericin-coated surfaces or microparticles. Detection formats include:
1. Sandwich ELISA using sericin microwells
2. Electrochemical immunosensors with HRP or ALP labels
3. Lateral flow strips incorporating sericin for signal enhancement

Performance Metrics (E. coli detection)
1. Detection limit: 10^{2}-10^{3} CFU/mL
2. Assay time: 30-60 minutes
3. Specificity: No cross-reactivity with other Enterobacteriaceae

7.2 Mycotoxin Detection

Mycotoxins (aflatoxins, ochratoxin A, fumonisins) contaminate agricultural products and pose carcinogenic and hepatotoxic risks.

Design Strategy: Aptamer-based biosensors (aptasensors) offer advantages over antibodies, including higher stability and lower cost. Sericin's functional groups enable aptamer immobilization through streptavidin-biotin linkage or direct covalent attachment.

Performance Metrics (Aflatoxin B1 detection)
1. Detection limit: 0.1-1.0 ng/mL
2. Linear range: 0.5-100 ng/mL
3. Recovery in food matrices: 85-115%
4. Reproducibility: RSD <10%

7.3 Food Additive and Preservative Detection

Excessive use of food additives (sulfites, nitrites, benzoates) requires monitoring to ensure compliance with safety regulations.

Design Strategy: Direct electrochemical oxidation or reduction of the target analyte at sericin-modified electrodes. Sericin enhances electron transfer and reduces electrode fouling. For sulfite detection, sulfite oxidase immobilization provides enzymatic specificity.

Performance Metrics (sulfite detection)
1. Detection limit: 0.5-2.0 μM
2. Linear range: 5-500 μM
3. Interference: Minimal from common food matrix components

8. Statistical Analysis and Performance Benchmarking

8.1 Comparative Performance Metrics

Table 1: Analytical performance of sericin-based biosensors for environmental and food contaminants.

Analyte	Sensor Type	Detection Limit	Linear Range	Sensitivity
Glucose	Enzyme-free flexible sensor	4 μM	25-400 μM	13.43 μA mM^{-1} cm^{-2}
Ascorbic Acid	Organic electrochemical	2.5 μM	10-500 μM	-
Paraoxon	AChE/Sericin electrode	0.5 nM	1-1000 nM	-
Aflatoxin B1	Aptasensor	0.5 ng/mL	1-100 ng/mL	-
Pb^{2+}	ASV/Sericin	2 nM	0.01-50 μM	-

8.2 Mechanical and Physical Property Statistics

Table 2: Physicomechanical properties of sericin-based materials.

Property	Sericin Film	Sericin-Starch	Sericin-PEG	Supramolecular Eutectogel
Tensile Strength (MPa)	5-15	8-20	12-25	4.02
Elongation at Break (%)	2-5	5-10	3-8	1703
Contact Angle (°)	75-76	68.5 [3]	75.4 [3]	-
Swelling Ratio (%)	>200	150-200	100-150	-
Conductivity (S/cm)	-	-	0.1-10	-

8.3 Environmental Stability Statistics

Sericin-based supramolecular eutectogels demonstrate remarkable environmental stability:
1. Temperature operating range: -40°C to 60°C
2. Mechanical performance retention in extreme temperatures: >90%
3. Self-healing efficiency: 83% at room temperature (48 hours), 87% at 60°C
4. Signal stability after 5 healing cycles: No significant degradation

Flexible sericin-based sensors maintain performance after 200 folding cycles.[8] Biodegradation studies indicate functional stability for several days in protease-containing solutions prior to complete degradation.[6]

8.4 Strain Sensing Performance

For wearable sensor applications, sericin-based eutectogels exhibit gauge factor (GF) values:
1. GF = 4.23 in strain range 0-400%
2. GF = 8.42 in strain range 650-925%
3. Response time: 60 ms
4. Recovery time: 40 ms

These performance metrics compare favorably with conventional hydrogel-based strain sensors, while offering superior environmental stability and biodegradability.[7]

9. Current Challenges and Limitations

9.1 Sericin Solubility and Stability

Sericin's high solubility in aqueous media presents a fundamental challenge for biosensor applications that require operation in water or biological fluids. Without adequate crosslinking, sericin films may dissolve or swell excessively, leading to loss of immobilized recognition elements and sensor failure.

Mitigation Strategies
1. Chemical crosslinking with glutaraldehyde, genipin, or PEG
2. Physical crosslinking through methanol or ethanol treatment
3. Blending with more stable polymers (starch, PVA, chitosan)
4. Supramolecular network formation with DES components

However, extensive crosslinking may reduce sericin's functional group availability and alter its biocompatibility, requiring careful optimization for each application.

9.2 Batch-to-Batch Variability

Sericin properties vary depending on:
1. Silkworm rearing conditions (diet, temperature, humidity)
2. Extraction method (temperature, time, chemical reagents)

3. Storage conditions (temperature, humidity, light exposure)

Standardized extraction protocols and thorough characterization of each batch (molecular weight, purity, FT-IR spectrum) are essential for reproducible sensor fabrication.

9.3 Sensitivity and Detection Limits

While sericin-based biosensors achieve detection limits in the nanomolar to picomolar range, they may not match the sensitivity of conventional analytical techniques (LC-MS/MS, ICP-MS) for certain applications. As demonstrated in comparative electrochemical studies, fully organic sericin-based configurations (O3E) exhibit lower conductivity than systems using metallic reference electrodes.[6] Improving sensitivity requires:

1. Optimization of immobilization density and orientation
2. Signal amplification strategies (nanoparticles, enzymatic amplification)
3. Hybrid configurations combining organic working electrodes with conventional reference systems where biodegradability requirements permit.

9.4 Long-Term Storage Stability

The biological recognition elements (enzymes, antibodies) in sericin-based biosensors gradually lose activity during storage. While sericin's protein matrix provides some protective effect, significant activity loss occurs over weeks to months.

Storage Optimization

1. Lyophilization (freeze-drying) of sericin-based sensors
2. Storage at -20°C or -80°C with desiccant
3. Addition of stabilizing agents (trehalose, sucrose, BSA)

9.5 Scalability and Manufacturing

Transitioning from laboratory-scale fabrication to commercial manufacturing presents challenges including:

1. Maintaining uniform film thickness and quality across large areas
2. Reproducible micropatterning of sericin-based conductive inks
3. Quality control metrics for batch release
4. Cost competitiveness with synthetic polymer alternatives

Recent advances in photolithographic patterning of photocrosslinkable sericin offer promise for scalable microfabrication, with room-temperature processing using water-based solvents.

10. Future Perspectives

10.1 Sustainable and Green Manufacturing

Eri silk sericin represents an underutilized resource that is typically discarded as waste during silk processing. Developing value-added applications such as biosensors aligns with circular economy principles and sustainable manufacturing. Future research should focus on:

1. Low-energy extraction methods (room temperature, enzymatic)
2. Solvent-free processing techniques
3. Biodegradable sensor components to reduce electronic waste

10.2 Wearable and Continuous Monitoring Systems

Recent developments in flexible and stretchable sericin-based materials enable wearable sensing applications. The supramolecular eutectogel platform demonstrates:

1. Adhesion to various substrates including metal (Fe), plastic (PTFE), and skin (0.07-1.53 MPa adhesion strength)
2. Capability to monitor finger bending (0-90°), swallowing motions, and voice signals (e.g., "hydrogel" pronunciation)
3. Stable signal output under dynamic deformation

Future developments should focus on integrating these materials with wireless data transmission for real-time health and environmental monitoring.

10.3 Transient and Bioresorbable Sensors

The biodegradability of silk proteins offers unique advantages for transient sensing applications where device removal is impractical. Fully organic sericin-based electrochemical systems have been shown to function for several days in protease-containing solutions before complete degradation.[6] Such systems can provide useful tools for:

1. In vivo biomonitoring of analytes for controlled periods
2. Environmental sensors that degrade after deployment
3. Implantable systems that eliminate the need for retrieval surgery

10.4 Smartphone-Integrated Sensors

The ubiquity of smartphones with high-resolution cameras and computing power enables portable, user-friendly biosensing. Smartphone-integrated sericin-based sensors could include:

1. Colorimetric paper strips with sericin coatings for image analysis
2. Fluorescence detection using phone camera and external LED excitation
3. Electrochemical readout via phone audio jack or Bluetooth-connected potentiostats

10.5 Machine Learning for Sensor Optimization

Machine learning algorithms can accelerate the optimization of sericin-based biosensor fabrication parameters (crosslinker concentration, immobilization conditions, transduction settings) that would otherwise require extensive empirical testing. Applications include:

1. Predicting optimal crosslinking conditions for specific analytes

2. Interpreting complex electrochemical or impedance spectra
3. Calibrating sensor responses for matrix effects in real samples

10.6 Multiplexed Detection Platforms

Current sericin-based sensors typically detect single analytes. Integrating multiple recognition elements on a single sericin substrate would enable simultaneous detection of several contaminants, improving efficiency and reducing costs. Strategies include:

1. Microarray patterning of different antibodies or aptamers
2. Multi-channel electrode arrays with distinct recognition elements
3. Colorimetric arrays with pattern recognition (electronic tongue)

11. CONCLUSION

Eri silk sericin has emerged as a versatile and sustainable biomaterial for biosensor fabrication, offering distinct advantages over synthetic polymers and even other silk proteins. Its molecular characteristics—including a molecular weight of approximately 66 kDa, abundant functional groups for biomolecule immobilization, and unique globular surface morphology—make it particularly well-suited for detecting environmental toxins and food contaminants.[3]

Recent advances have demonstrated the versatility of sericin-based materials across multiple sensing platforms. Conducting polymer composites with PEDOT:PSS enable fully organic electrochemical sensors that eliminate metallic components while maintaining competitive detection performance for metabolites such as ascorbic acid (detection limit 2.5 μM).[6] Supramolecular eutectogels incorporating sericin achieve remarkable mechanical properties (tensile strength 4.02 MPa, elongation 1703%) and environmental stability across -40°C to 60°C, with self-healing efficiency of 83%.[7] Flexible enzyme-free glucose sensors based on sericin-AuNP composites demonstrate detection limits of 4 μM and withstand 200 folding cycles without performance degradation.[8]

Fabrication strategies ranging from simple film casting to electrospun nanofibers, microparticles, and supramolecular networks provide flexibility in sensor design. Crosslinking with agents such as PEG and glycerol enables tuning of mechanical properties, surface wetting, and stability.[3] Comprehensive characterization using SEM, FT-IR, contact angle goniometry, and electrochemical methods ensures quality control and optimization of sensor performance.

Current applications demonstrate detection of heavy metals, pesticides, phenolic compounds, pathogens, and mycotoxins with detection limits in the nanomolar range. While challenges remain—particularly regarding sericin solubility in aqueous media and batch-to-batch variability—recent developments in supramolecular chemistry, photocrosslinkable sericin derivatives, and nanocomposite formulations offer promising solutions.

The convergence of sericin-based materials with wearable device platforms, smartphone readout systems, and machine learning optimization positions this technology for translation from laboratory research to practical applications. As environmental monitoring and food safety requirements become increasingly stringent, the need for low-cost, portable, and sustainable sensing solutions will continue to grow. Eri silk-sericin based biosensors, combining the unique properties of a traditional natural material with modern biosensing technologies, are well-positioned to address this need.

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