

Investigating the Characteristics of Freeze-Dried Mucin Extract from Snail *Achatina Fulica* and Silicone Adhesive Films for the Development of Wound Healing Patches

Muhammad Aminuddin Zulkifle¹, Aliff Hisyam A Razak^{1*}

¹ Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh Education Hub, KM 1, Jalan Panchor, 86400 Muar, Johor, MALAYSIA

*Corresponding Author: aliff@uthm.edu.my

DOI: <https://doi.org/10.30880/peat.2025.06.01.062>

Article Info

Received: 18 January 2025

Accepted: 06 February 2025

Available online: 30 April 2025

Keywords

Achatina Fulica, freeze-dried snail mucin, silicone, phr, patch

Abstract

This study investigates the characterization of freeze-dried snail mucin extract from *Achatina Fulica* with silicone adhesive film for its potential use in wound healing patches. The research aims to address the problem of current wound healing patches that face limitations such as moisture buildup and adhesive-induced skin irritation, which can compromise patient comfort, delay healing, and reduce overall efficiency by develop a wound healing patch containing freeze-dried snail mucin from *Achatina Fulica* to overcome the issues. By employing freeze-drying techniques within ethical guidelines, the study seeks to optimize mucin extraction by carefully removing moisture under low temperatures in range of -50°C until 25°C to preserve its bioactive properties. An optimization method was used to identify key factors such as mixing time, curing time and loading of freeze-dried snail mucin that influence adhesiveness and permeability of the patch. The optimized conditions were found to be 10 minutes, 50 minutes, and 5 phr. The silicone encapsulated the snail mucin lacked antimicrobial properties due to contamination, making it ineffective against *E. coli* and *S. epidermidis*. The findings highlight the potential of freeze-dried mucin extract and silicone adhesive films as innovative components for wound healing patches, emphasizing both their effectiveness in enhancing skin repair.

1. Introduction

Wound healing patches currently face limitations that impact their effectiveness, particularly issues like moisture buildup and skin irritation. Adhesive components, essential for holding the patch in place and ensuring proper wound closure, are often the primary cause of irritation, affecting patient comfort and recovery outcomes (1). These challenges can lead to slower healing and reduced efficiency in the recovery process. Addressing these concerns is crucial to improve the functionality and patient experience of wound healing patches. Therefore, it is important to use a suitable patch that can protect the skin.

This study uses a technology involving a freeze-drying method to remove the moisture content, ensuring to preserves the integrity of the bioactive compounds (2). Research shows that snail mucin contains bioactive substances beneficial for skin health. The study examines the biological properties of the silicone patch contain mucin in detail. Biologically, the mucin's ability for antibacterial activity to confirm it can stop harmful germs on the skin will be evaluated.

The study aims to investigate the biological properties of freeze-dried snail mucin extract from *Achatina Fulica* with silicone adhesive film in cosmeceutical product. Key questions include assessing particle size of mucin while for the patch, it includes detect the antibacterial properties. Additionally, this research explores the ethical extraction of mucin from *Achatina Fulica* snails and investigates its combination with silicone adhesive films to create effective, ethically-sound wound healing patches. The study aims to understand the potential benefits of freeze-dried mucin in enhancing the adhesive properties and overall performance of the patches.

2. Methodology

2.1 Preparation of freeze-dried snail mucin from *Achatina Fulica*

The freeze-drying process is used to prepare snail mucin from *Achatina Fulica*, with commercial mucin sourced from the COSRX brand. The mucin solution is carefully poured onto a stainless-steel tray and frozen at -40°C for at least 24 hours until it solidifies completely. The frozen trays are then transferred to a freeze dryer, where the primary drying process occurs via sublimation at approximately -50°C and 0.1 mbar vacuum pressure, leaving behind a porous dried solid (3). A secondary drying step at $20\text{-}25^{\circ}\text{C}$ under vacuum removes residual moisture, and the resulting freeze-dried mucin is immediately sealed in a moisture-proof container and stored in a cool, dry environment until further analysis for wound healing patch production.

2.2 Preparation of the patch outer layer

The outer layer was designed to combine with hydrophilic silicone, ensuring desired properties like high elasticity and hydrophobicity. Hydrophobic silicone, consisting of Part A and Part B, was used, mixed at a 1:1 ratio with 85% heptane in a beaker (10 g each of Part A and Part B). The mixture was stirred with a glass rod, poured onto a clean glass surface, and formed into a $20\ \mu\text{m}$ thick film (4). The film was cured at 100°C for 10 minutes to remove moisture, and the outer layers were cast with the adhesive silicone layer.

2.3 Preparation of silicone adhesive patch with freeze-dried snail mucin (inner layer)

The hydrophilic silicone used for adhesive film production is a two-part liquid kit (Part A and Part B) mixed in a 10:1 weight ratio with varying amounts of freeze-dried snail mucin. 20 g of silicone from Part A is combined with 2 g of silicone from Part B. Automated mixing techniques are used, and a speed mixer set at 1750 rpm ensures uniform blending without issues.

For a casting part, an applicator with a thickness of $20\ \mu\text{m}$ is used for casting. The glass surface is cleaned with 70% ethanol before and after casting to remove impurities and prevent contamination. Proper clipping of the glass surface ensures a smooth silicone adhesive film formation. The cast silicone undergoes curing in a drying oven. Curing transforms the liquid silicone into a solid elastomer through heat-induced cross-linking. Key parameters affecting curing are temperature and time, which influence adhesion and permeability. The silicone containing snail mucin is cured at 50°C for 50 to 80 minutes.

After curing, the samples are cut and stored in protective packaging. Anti-slip silicone acts as a temporary outer layer, while a thin wrapper prevents contamination. Samples are then prepared for optimization tests, including peel adhesion and water absorption tests.

2.4 Peel adhesion force to determine the optimised silicone adhesive patch with freeze-dried snail mucin

The peel adhesion test measures the force needed to remove an adhesive from a surface (5). In this study, a $15\ \text{cm} \times 2\ \text{cm}$ silicone sample with freeze-dried snail mucin was tested using a Hot Tack Tester. The sample was pulled at a 180° angle at a speed of $100\ \text{mm}/\text{min}$ to measure the force required to separate the silicone from the outer layer. The results were shown as a graph of load (N) versus time (seconds).

2.5 Water absorption measurements to determine the optimised silicone adhesive patch with freeze-dried snail mucin

The water absorption test measures how much water a material can absorb, important for evaluating durability and moisture resistance (6). Silicone films containing snail mucin were cut into $4\ \text{cm} \times 2\ \text{cm}$ samples, weighed, and immersed in 150 mL of water at $30\text{-}33^{\circ}\text{C}$ for 8 hours. Afterward, the samples were dried and reweighed to measure weight changes due to water absorption.

2.6 Antimicrobial activity using disk diffusion

The agar medium was prepared by mixing 28 g of agar powder with 1000 mL of distilled water. For this test, 0.2381 g of agar was mixed with 150 mL of water. The solution was stirred until smooth, then sterilized in an

autoclave for 15 minutes, with an extra hour for temperature adjustments. After cooling, the solution was poured into agar plates in a biosafety cabinet, sterilized with 70% ethanol (7), and stored in a chiller overnight to solidify.

On day two, three plates were incubated for 5 hours to remove water vapor, making the medium ready for bacterial culture. *E. coli* and *Staphylococcus epidermidis* were cultured on these plates using the 3-quadrant streak method, focusing on isolating individual colonies. The plates were incubated overnight at 37°C.

On day three, individual colonies of *E. coli* and *Staphylococcus epidermidis* were transferred to fresh plates. Two plates (A and B) were prepared for the sub-cultured bacteria, with samples A and B placed on the respective plates. The plates were incubated at 37°C for 4 days (8).

3. Result and Discussion

3.1 Peel adhesion force as response 1

The silicone adhesive with snail mucin is designed to provide strong adhesion without causing skin irritation or discomfort. Adhesion forces for the patches are expected to range from 0.9 N to 2.7 N (9). Adhesion testing for 15 hydrophilic silicone samples was conducted using a Hot Tack Tester. Table 1 and Figure 1 summarize the adhesion forces across samples, highlighting the influence of snail mucin loading (1-5 phr), mixing time, and curing time.

Table 1: Result of the peel adhesion force of silicone patches with freeze-dried snail mucin from *Achatina Fulica*

Run	Mixing Time (min)	Curing Time (min)	Loading of Freeze-Dried Snail Mucin (phr)	Peel Adhesion Force (N)
1	20	80	3	6.92
2	20	65	5	2.12
3	20	65	1	2.25
4	15	65	3	4.13
5	15	80	1	6.28
6	15	50	5	2.30
7	15	65	3	1.20
8	15	80	5	1.86
9	10	50	3	4.58
10	15	50	1	1.60
11	10	80	3	1.17
12	10	65	5	1.12
13	20	50	3	5.42
14	15	65	3	1.20
15	10	65	1	1.54

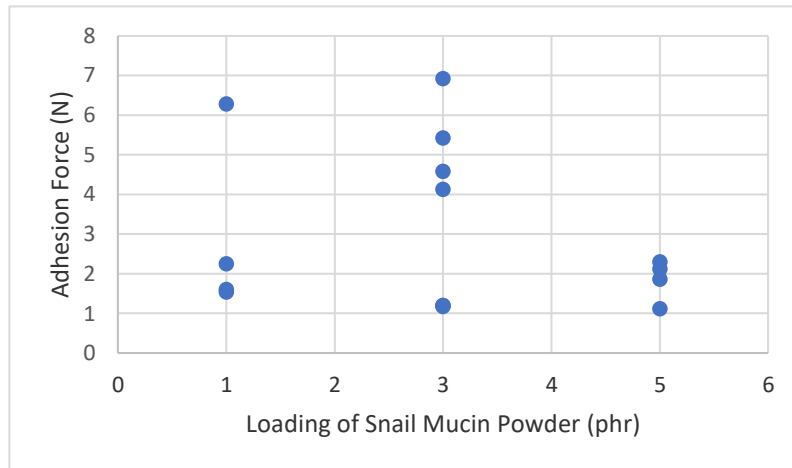


Fig. 1: Adhesion force (N) against loading snail mucin powder from *Achatina Fulica*

At 1 phr, the adhesion force varies widely from 1.54 N to 6.28 N, likely due to uneven dispersion or incomplete integration of the mucin powder in the silicone matrix, leading to inconsistent results. At 3 phr, adhesion forces range from 1.17 N to 6.92 N, with some samples showing high adhesion but others performing poorly, suggesting that this intermediate concentration can enhance adhesion in some cases but lacks stability across all samples. At 5 phr, adhesion becomes more consistent, ranging from 1.12 N to 2.30 N, but overall strength decreases, likely due to oversaturation of mucin disrupting the silicone matrix or weakening its interaction with the substrate (10).

The lowest adhesion force was 1.12N, and the highest was between 5.42N and 6.92N. Theoretical expectations suggest the silicone-adhesive film should have an adhesion force between 0.9N and 2.7N, but measurements showed forces from 1.12N to 6.92N. This study will focus on achieving higher adhesion forces (4.13N to 6.92N), due to the unique adhesive properties of snail mucin from *Achatina Fulica*, which enhances surface wettability and intermolecular interactions like hydrogen bonding, leading to stronger adhesion (11). The highest adhesion forces (4.00N to 6.90N) were seen with 1 phr and 3 phr of snail mucin, particularly at 3 phr (samples 1, 4, 9, 13). The adhesion force depends on the amount of snail mucin and factors like mixing and curing times. Sample 1, with 3 phr of mucin, showed the highest adhesion, suggesting that 3 phr enhances adhesion in hydrophilic silicone. Lower adhesion in sample 4 (0.4N at 3 phr) was due to a shorter heating time (50°C for 65 minutes), indicating that longer heating periods increase adhesion force.

3.2 Water absorption as response 2

To achieve favourable results, hydrophilic silicone is expected to exhibit significant water absorption properties. As shown by the water absorption test, the silicone adhesive absorbs water, increasing the water absorption percentage to at least 1% (12). Silicone adhesives with higher water absorption percentages are preferred. The data collected from this experiment is presented in Table 2 and Figure 2.

Table 2: Result of the water absorption of silicone patches with freeze-dried snail mucin from *Achatina Fulica*

Run	Mixing Time (min)	Curing Time (min)	Loading of Freeze-Dried Snail Mucin (phr)	Water Absorption (%)
1	20	80	3	2.57
2	20	65	5	5.18
3	20	65	1	3.62
4	15	65	3	3.51
5	15	80	1	2.21
6	15	50	5	6.11
7	15	65	3	3.43
8	15	80	5	6.75

9	10	50	3	4.33
10	15	50	1	1.89
11	10	80	3	3.36
12	10	65	5	4.29
13	20	50	3	2.01
14	15	65	3	3.43
15	10	65	1	2.22

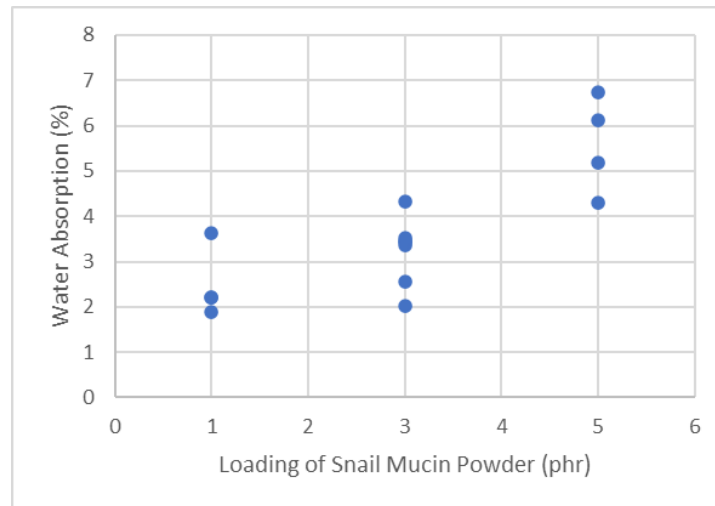


Fig. 2: Water absorption (%) and loading snail mucin powder from *Achatina Fulica*

The silicone adhesive uses different formulas with varying snail mucin powder loadings, mixing times, and curing times, resulting in 15 samples. Figure 2 shows water absorption ranging from 1.89% to 6.75%, depending on snail mucin loadings (1–5 phr). The table highlights that water absorption depends on factors such as the amount of snail mucin powder, mixing time, and curing time.

At a low loading of 1 phr, water absorption ranges from 1.89% to 3.62%, showing moderate hydrophilic behavior, but the variation suggests uneven dispersion of mucin powder in the silicone. At 3 phr, water absorption stays steady between 2.01% and 4.33%, indicating that the mucin's effect on water absorption becomes limited. At 5 phr, water absorption increases significantly from 4.29% to 6.75%, showing that higher mucin concentrations improve the silicone's hydrophilic properties and increase water uptake (13).

The water absorption spectrum ranges from 1.89% to 6.75%, exceeding the theoretical minimum for silicone of 1%. To achieve hydrophilic properties, the study focuses on silicone formulas with water absorption between 4.29% and 6.75%, observed in samples loaded with 3 phr and 5 phr of snail mucin powder (samples 2, 6, 8, 9, and 12). Sample 9 showed the highest water permeation at 3 phr, while samples 2, 6, 8, and 12 performed best at 5 phr. The results suggest that higher snail mucin content enhances water interaction, improving water absorption and moisture retention (14). Lower mucin loading, such as 1 phr, results in minimal water penetration (1.89% to 3.62%), while variations at 3 phr may occur due to long curing times. This supports the hypothesis that water penetration increases with higher mucin content, shorter curing times, and lower processing temperatures.

3.3 Antimicrobial activity

After an incubation period of 4 days and 3 nights, longer than originally planned due to lab limitations, the results from each agar plate were analyzed. Both plates, assigned to sample A and sample B, showed significant bacterial growth, including beneath the samples.

As shown in Figure 3, sample A which was exposed to *E. coli*, extensive bacterial growth was observed on the sub-cultured agar plate. This is expected since sample A, being a hydrophilic silicone without an antimicrobial ingredient, supports bacterial growth. The hydrophilic silicone material lacks properties to prevent bacterial proliferation. Sample B also showed bacterial growth beneath it, which was unexpected. This issue arose from improper storage of the snail mucin powder. Poor storage can lead to contamination,

degradation, and loss of antimicrobial efficacy. The unsealed packaging exposed the powder to air, moisture, and contaminants such as bacteria and fungi (15).

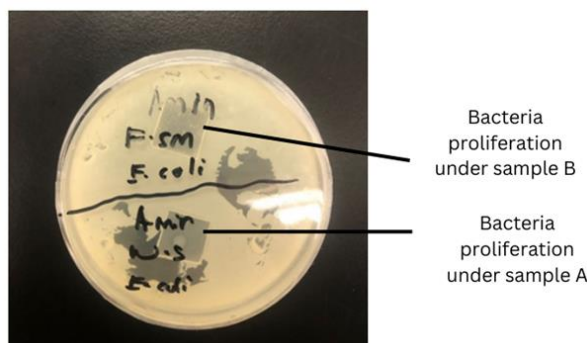


Fig. 3: Medium agar plate containing sample A and B by using *E. coli* from the bottom view

For the sample exposed to *S. epidermidis* as shown in Figure 4, sample A again showed noticeable bacterial growth underneath, consistent with expectations since it contains only hydrophilic silicone. Sample B also showed bacterial growth, which was unexpected. Again, improper storage of the snail mucin powder caused this issue. Moisture from high humidity or improper sealing can foster an environment for bacterial, mold, and fungal growth, leading to contamination. Even though snail mucin has natural antimicrobial properties, improper storage can reduce or eliminate its effectiveness.

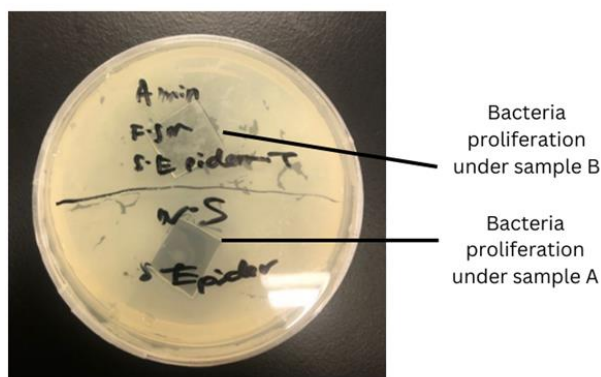


Fig. 4: Medium agar plate containing sample A and B by using *S. epidermidis* from the bottom view

Compared to the theoretical standard treatment with ampicillin, both Sample A and Sample B showed much lower effectiveness in preventing bacterial growth from *E. coli* and *S. epidermidis*. Ampicillin, a common antibiotic, effectively stopped bacterial growth, proving its reliability as an antimicrobial agent (16).

Sample A, made of hydrophilic silicone without any antimicrobial additives, showed significant bacterial growth for both *E. coli* and *S. epidermidis*, as expected. The hydrophilic silicone lacks antibacterial properties, allowing bacteria to grow without resistance.

Sample B, containing snail mucin, was expected to have antimicrobial properties due to its natural components like glycoproteins and peptides. However, bacterial growth was still seen, likely due to improper storage of the snail mucin powder. Poor storage conditions, such as exposure to air, moisture, and contaminants from unsealed packaging, likely degraded its antimicrobial properties.

In contrast, the ampicillin standard completely prevented bacterial growth in both cases. This highlights the importance of using effective antimicrobial agents and proper storage to preserve the effectiveness of bioactive materials. Without proper storage, snail mucin may lose its ability to act as a natural antimicrobial (17).

In conclusion, pure hydrophilic silicone cannot prevent bacterial growth without active ingredients. Similarly, the snail mucin powder, due to improper storage, could not prevent bacterial growth, as exposure to moisture caused contamination and loss of its antimicrobial properties.

4. Conclusion

The goal was to improve silicone patches for wound healing by adding extracted snail mucin. The chemical properties of the patches were optimized with 15 adhesive film samples tested for adhesion and hydrophilicity. The aim was to find the best formula for an adhesive film that incorporates snail mucin for wound healing. Key properties like peel adhesion force and water absorption were optimized. A sample with mixing time of 10 minutes, curing time of 50 minutes and load of freeze-dried snail mucin of 5 phr as the best-performing option, demonstrating strong adhesion while efficiently absorbing water. The optimized silicone patches were then developed using this formula with an outer layer. To confirm the film's effectiveness in holding snail mucin, tests including antimicrobial evaluations was conducted. The antimicrobial evaluation of silicone patches containing freeze-dried snail mucin from *Achatina Fulica* revealed bacterial growth for both *E. coli* and *S. epidermidis*. This contamination likely resulted from improper storage of the snail mucin powder, highlighting the need for stricter handling and storage protocols to maintain its antimicrobial integrity. The results confirmed that the selected adhesive film formulation successfully retained active ingredients, absorbed water, and applied the right force to the skin.

Acknowledgement

Communication of this research is made possible through monetary assistance by Universiti Tun Hussein Onn Malaysia and the UTHM Publisher's Office via Publication Fund K534 FRGS.

Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: **study conception and design:** Muhammad Aminuddin, Aliff Hisyam; **data collection:** Muhammad Aminuddin; **analysis and interpretation of results:** Muhammad Aminuddin, Aliff Hisyam; **draft manuscript preparation:** Muhammad Aminuddin, Aliff Hisyam

References

- [1] Jia, B., Zhang, B., Li, J., Qin, J., Huang, Y., Huang, M., ... & Du, J. (2024). Emerging polymeric materials for treatment of oral diseases: design strategy towards a unique oral environment. *Chemical Society Reviews*.
- [2] Di Filippo, M. F., Dolci, L. S., Bonvicini, F., Sparla, F., Gentilomi, G. A., Panzavolta, S., Passerini, N., & Albertini, B. (2024). Influence of the extraction method on functional properties of commercial snail secretion filtrates. *Scientific Reports*, 14(1). <https://doi.org/10.1038/s41598-024-72733-0>
- [3] Oyinloye, T. M., & Yoon, W. B. (2020). Effect of freeze-drying on quality and grinding process of food produce: A review. *Processes (Basel, Switzerland)*, 8(3), 354. <https://doi.org/10.3390/pr8030354>
- [4] Huang, S., Wu, Z., Johannessen, B., Long, K., Qing, P., He, P., Ji, X., Wei, W., Chen, Y., & Chen, L. (2023). Interfacial friction enabling $\leq 20 \mu\text{m}$ thin free-standing lithium strips for lithium metal batteries. *Nature Communications*, 14(1). <https://doi.org/10.1038/s41467-023-41514-0>
- [5] Bartlett, M. D., Case, S. W., Kinloch, A. J., & Dillard, D. A. (2023). Peel tests for quantifying adhesion and toughness: A review. *Progress in Materials Science*, 137, 101086. <https://doi.org/10.1016/j.pmatsci.2023.101086>
- [6] Dyson, E., Sikkink, S., Nocita, D., Twigg, P., Westgate, G., & Swift, T. (2023). Evaluating the irritant factors of silicone and hydrocolloid skin contact adhesives using Trans-Epidermal water loss, protein stripping, erythema, and ease of removal. *ACS Applied Bio Materials*. <https://doi.org/10.1021/acsabm.3c00874>
- [7] Starolis, M. W. (2024). The contamination monitoring toolbox: Best practices for molecular microbiology testing. *Clinical Microbiology Newsletter*, 47, 21–27. <https://doi.org/10.1016/j.clinmicnews.2024.07.001>
- [8] Pipite, A., Lockhart, P. J., McLenachan, P. A., Christi, K., Kumar, D., Prasad, S., & Subramani, R. (2022). Isolation, antibacterial screening, and identification of bioactive cave dwelling bacteria in Fiji. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.1012867>
- [9] Wu, X., Deng, J., Jian, W., Yang, Y., Shao, H., Zhou, X., Xiao, Y., Ma, J., Zhou, Y., Wang, R., & Li, H. (2024). A bioinspired switchable adhesive patch with adhesion and suction mechanisms for laparoscopic surgeries. *Materials Today Bio*, 27, 101142. <https://doi.org/10.1016/j.mtbio.2024.101142>
- [10] Roth, R. (2019). *Functionalized calcium carbonate based peptide formulation: aspects of the development for oral delivery to the buccal and intestinal mucosa*. <https://doi.org/10.5451/unibas-007136785>
- [11] Cui, C., & Liu, W. (2021). Recent advances in wet adhesives: Adhesion mechanism, design principle and

- applications. *Progress in Polymer Science*, 116, 101388. <https://doi.org/10.1016/j.progpolymsci.2021.101388>
- [12] Miao, W. (2020). *The Mechanism of Accelerated Hydrolytic Aging on Silicone Adhesive*. Michigan State University.
- [13] Bayer, I. S. (2022). Recent advances in mucoadhesive interface materials, mucoadhesion characterization, and technologies. *Advanced Materials Interfaces*, 9(18). <https://doi.org/10.1002/admi.202200211>
- [14] Ahmady, A., Anuar, N. K., Ariffin, S. A., & Samah, N. H. A. (2024). Mucoadhesive enhancement of gelatine by tannic acid crosslinking for buccal application. *Biopolymers*, 116(1). <https://doi.org/10.1002/bip.23646>
- [15] Masotti, F., Cattaneo, S., Stuknytè, M., & De Noni, I. (2019). Airborne contamination in the food industry: An update on monitoring and disinfection techniques of air. *Trends in Food Science & Technology*, 90, 147–156. <https://doi.org/10.1016/j.tifs.2019.06.006>
- [16] Ramzan, M., Raza, A., Nisa, Z. U., Abdel-Masih, R. M., Bakain, R. A., Cabrerizo, F. M., Cruz, T. E. D., Aziz, R. K., & Musharraf, S. G. (2024). Detection of antimicrobial resistance (AMR) and antimicrobial susceptibility testing (AST) using advanced spectroscopic techniques: A review. *TrAC Trends in Analytical Chemistry*, 172, 117562. <https://doi.org/10.1016/j.trac.2024.117562>
- [17] Liegertová, M., & Malý, J. (2023). Gastropod Mucus: Interdisciplinary perspectives on biological activities, applications, and strategic priorities. *ACS Biomaterials Science & Engineering*, 9(10), 5567–5579. <https://doi.org/10.1021/acsbiomaterials.3c01096>