Brewed and Renewed: Preliminary Characterization and Antibiotic Potential of Chitosan Nanogels Loaded with Arabica (*Coffea arabica*) Spent Coffee Grounds

ABSTRACT

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| --- |
| **Aims:** The study aimed to determine the physicochemical characteristics of the *C. arabica* SCG*-*loaded nanogel, its wound healing potential through antibiotic activity *in vitro,* and its safety and stability through cytotoxicity and pH tests, respectively.  **Study design:** The study is experimental in nature and employed a Research and Development (R&D) research design.  **Place and Duration of Study:** San Isidro College Wet Laboratory, Coffee Wagon (Malaybalay Branch), Natural Products Research and Development Center, Central Mindanao University Microbiology Laboratory, and Mindanao State University, between February and March of 2025.  **Methodology:** SCG extract was obtained through the solvent extraction method and prepared for use. Chitosan-based nanogels were then formulated, utilizing the ionic gelation method. Subsequently, the nanogels were characterized using Dynamic Light Scattering for particle size and UV-Vis spectrophotometer for drug loading capacity and encapsulation efficiency. Antibiotic activity was then measured *in vitro* through the Kirby-Bauer Disk Diffusion Assay, while cytotoxicity was measured using the Brine Shrimp Lethality Assay across five (5) concentrations, and pH testing was replicated four (4) times.  **Results:** The *C. arabica* SCG-loaded nanogels obtained a particle size ranging from 4470 nm to 6280 nm and a low PDI of 0.01, indicating potential aggregation and uniformity of particles, respectively. Meanwhile, the nanogel exhibited a drug loading capacity of 4.76% and an encapsulation efficiency of 99.77%, attributed to having lower mass ratios. For the antibiotic testing, no zones of inhibition were formed. Furthermore, the nanogel was generally non-toxic and stable, with lethality of 11 out of 50 brine shrimp and a pH of 4.5, respectively.  **Conclusion:** Formulation of the loaded nanogel is room for improvement through accessibility of resources. Future studies must focus on enhancing the formulation process and reducing aggregation. Additionally, more characterization parameters can be added for a much more comprehensive result. |

*Keywords: C. arabica, chitosan, nanogel, spent coffee grounds*

1. INTRODUCTION

Nanotechnology has recently become relevant in the field of biochemistry and the field of medicine. In particular, the creation of nanogels, a type of hydrogel with molecules sized in the nanoscale, continues to be an emerging subject for research due to its efficient performance as a drug delivery system (Neamtu et. al., 2017). Subsequently, these cross-linked polymer networks hold promise in the field as it is highly versatile and can withstand physical stability conditions than the standard drugs and medications (Sivaram et. al., 2015).

In addition, the Philippines is a high producer of coffee and coffee products and is a contributor to the agricultural sector (Luat e. al., 2021). Due to this, the byproduct derived from brewing and roasting coffee, or the spent coffee grounds (SCGs), becomes unutilized and eventually would become an environmental nuisance, untreated in landfills (McNutt and He, 2019; Stylianou et. al., 2018). Fortunately, SCGs provide a vast array of uses especially in the medical field, rich in bioactive compounds and is a hotspot for byproduct valorization (Kourmentza et. al., 2018). Meanwhile, chitosan, though an antimicrobial and is a cost-effective and biocompatible polymer, is poorly utilized in the context of wound healing nanogels due to its low solubility (Basak et. al., 2024). In connection, with its versatility, its efficiency as an antibiotic nanogel is subject to exploration, contributing to the emerging research on the utilization of byproducts and naturally-derived resources in the realm of nanotechnology and the significance it would hold in medicine.

The importance of nanotechnology and nanogels in particular is that they are finely tuned and provide minimal side effects brought by the usual drugs and medication through serving as a vehicle and eliminating their accompanying adversities (Attama et. al., 2022). It has a trait of being able to adapt to its desired application, holding characteristics that make its molecule carrier system and its drug delivery highly efficient (Soni et. al. 2016). Additionally, it amplifies the therapeutic potential of phytopharmaceuticals and bioactive compounds such as plant extracts and other natural medicine resources through delivery enhancement, which is also an emerging study (Taha et. al., 2022). Therefore, it is a promising candidate for effective treatments such as eliminating microbial drug resistance much effectively than free antibiotics (Mohammed et. al., 2018).

Being highly regarded as an efficient drug delivery system and molecular carrier, nanogels as an antibiotic treatment have been in practice as well. A study stated that antibiotic nanogels were able to address the issues of antibiotic resistance through enhancing the delivery of such antibiotics and antimicrobials, therefore bringing significant impact to how the prospects are in the medical field in general (Weldrick et. al. 2019; Keskin et. al., 2021). Antibiotic nanogels also wield anti-cytotoxicity and exhibits as an alternative to the common antimicrobial formulations as a response to these medications become lesser effective due to resistance and offering a potentially novel approach (Amasya et. al. 2024; Chung et. al., 2023). On a more specific prospect, the study of Tripathi et. al. (2024) stated that nanogels are efficient in wound care and wound healing, leaning on a more sustainable and patient-centric method. Furthermore, favorable reception and the developed composition of nanogels can be subject to a wide examination against pathogenic (Abdollahi et. al., 2024).

This study aimed to create a nanosized drug delivery system for naturally-derived antibiotics in the form of a chitosan-based nanogel, loaded with spent coffee grounds (SCGs) as the primary active ingredient, as well as a preliminary physicochemical assessment of the nanogels and focused on its particle size and size distribution, drug loading capacity, and encapsulation efficiency (EE). To examine its antibiotic potential, SCG extract was subject to *in vitro* antimicrobial activity against the common bacteria strains *Staphylococcus aureus* and *Escherichia coli* in comparison to the positive controls. The *in vitro* evaluation will utilize the Kirby-Bauer disk diffusion assay. Furthermore, their zone of inhibition was compared to determine if the formulated nanogels performed more efficiently. Additionally, a safety and stability test was conducted to ensure the nanogels' suitability for potential commercialization.

2. METHODOLOGY

**2.1 Research Design**

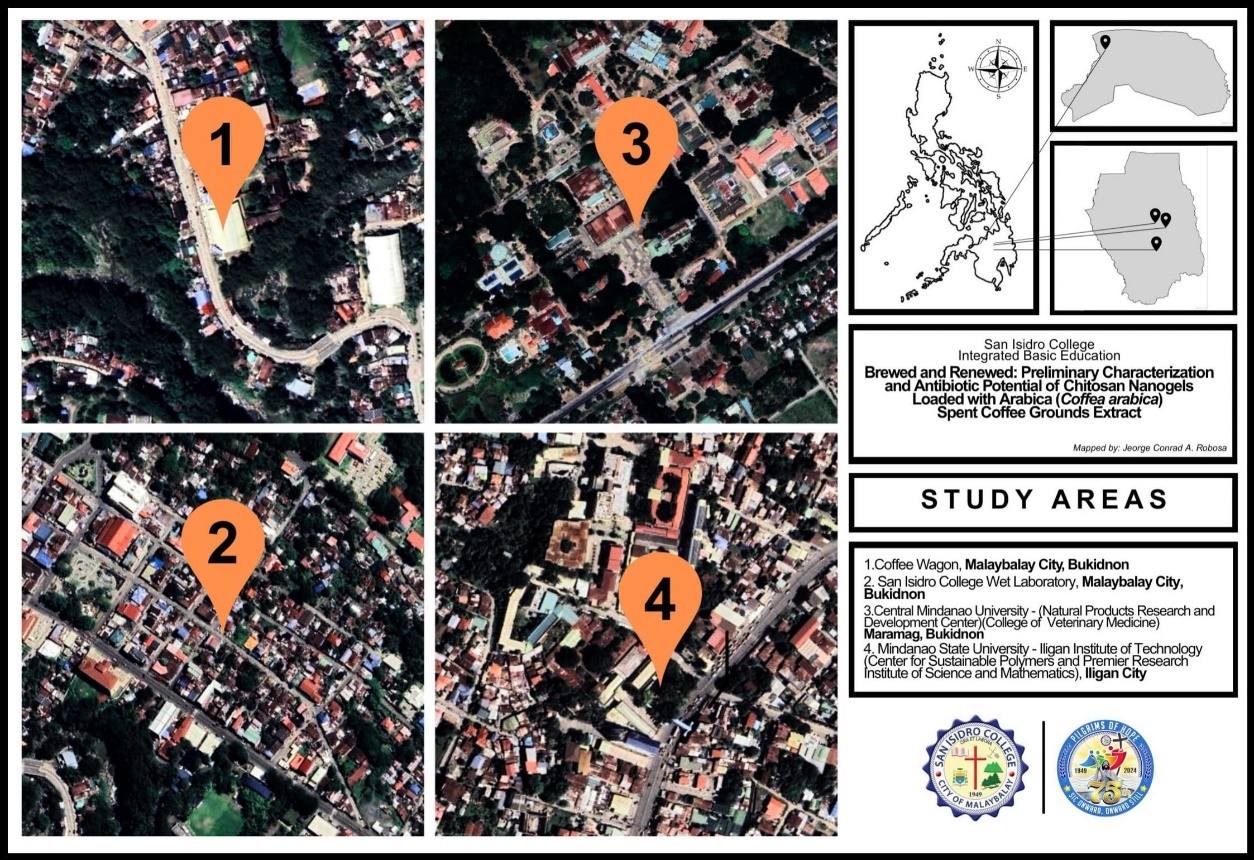
This study utilized a research and development (R&D) research design, which involved the creation of products for innovation and was subject to trials and numerous developments, followed by an experimental approach. The study involved a series of analytical testing through determining the presence of bioactive compounds in the SCG extract; quantification of nanogel particle size and size distribution, drug loading capacity, and encapsulation efficiency; and the antibiotic capability of the formulated SCG-loaded nanogels in comparison to the positive controls.

**2.2 Entry Protocol**

The researchers implemented multiple measures and obtained the necessary permits to ensure the ethicality and professional aspect of the study.

**2.3 Locale of the Study**

Throughout the study, numerous study areas and analytical laboratories were involved. The collection of samples initially took place at Coffee Wagon in Malaybalay City, Bukidnon. Meanwhile, the extraction process and formulation of nanogels were conducted at the San Isidro College Wet Laboratory and sonicated at the Natural Products Research and Development Center. Lastly, the characterization of the formulated nanogels was determined in the laboratories of Mindanao State University – Iligan Institute of Technology, particularly in the Center for Sustainable Polymers (CSP) and PRISM, while antibacterial testing was performed at the Microbiology Research Laboratory of the College of Veterinary Medicine in Central Mindanao University.



**Fig. 1. Map of Research Locale**

**2.4 Collection and Preparation of Samples**

Spent coffee grounds (SCGs) from *C. arabica* specimens were obtained through the pour-over brewing method explained by Chung (2019). Coffee beans were ground into coarse particles using a mortar and pestle and placed in a sieve with a coffee filter. Once the grounds were laid out, briskly boiling water was poured over them slowly, while the resultant coffee was stored in a beaker. After all the grounds had been used up, they were laid out to dry in an oven at a maximum temperature of 60° C for 5 hours. The spent coffee grounds were then subjected to extraction.

**2.5 Extraction Process**

Das et. al. (2014) utilized the solvent extraction method for the extraction of bioactive compounds from *C. arabica* SCG extract with modifications. Specimens of *C. arabica* SCG were further refined using a blender to increase the surface area for extraction and were run through a sieve to collect the more refined parts. Following refinement, the powdered coffee grounds were soaked in 95% ethanol. The ethanol was added to completely submerge the plant material, ensuring all parts of the powdered specimens were saturated. The mixture then underwent maceration for 72 hours. It was filtered using standard filter paper to separate the liquid extract from the coarse plant residues and was repeated until the filtrates were fully separated. Lastly, the extract was run through a water bath at 45–55 degrees Celsius for the evaporation of the solvent and was kept in a dark area for further use.

**2.6 Formulation of Nanogels**

The formulation of nanogels utilized the ionic gelation process, wherein the constituents and procedures of Ansari et. al. (2019) were followed with modifications. A 1 mL solution of sodium triphosphate (TPP) (0.5% w/v) was gradually added to a 7 mL chitosan solution, which consisted of chitosan diluted with 0.06% w/v acetic acid, in a sonicator set to pulse mode to create nanosized molecules. This was followed by the centrifugation of the solution at 15,000 rpm to separate the supernatant. The formulated nanogels were suspended in water and stored in a freezer for further use and characterization.

**2.7 Nanogel Characterization**

The formulated nanogels were preliminarily characterized based on particle size and size distribution, drug loading capacity, and encapsulation efficiency, utilizing the process of a study (Mahdiani et. al., 2024).

**2.7.1 Size and Size Distribution**

The SCG-loaded nanogels underwent size and size distribution analysis through Dynamic Light Scattering (DLS). Molecular distribution followed the polydispersity index (PDI), where values close to 0.1 and 1.0 indicated broad and narrow distribution, respectively.

**2.7.2 Drug Loading Capacity**

Capacity was quantified using a UV-Vis spectrophotometer after the centrifugation of the nanogel and the SCG extract. Quantification followed the formula:

**2.7.2 Encapsulation Efficiency**

Was quantified through the use of a UV-Vis Spectrophotometer. The calculation will use the following formula:

**2.8 Antibiotic Activity Analysis**

The antibiotic potential of the nanogels was evaluated using the process of (Hudzicki, 2009; Abdon et. al., 2024). It involved soaking filter paper discs in the extract for 24 hours and preparing a bacterial suspension of the bacteria subject to testing. The strains were added to 9 mL of sterile distilled water to adjust the turbidity. The bacterial suspension was immersed in Mueller-Hinton agar using the Kirby-Bauer technique, ensuring that the agar fully coated the suspension. This was followed by the immersion of the filter paper discs into the agar, along with the free drug and the paper disc containing the SCG extract alone. The plates were then inverted to record the test specimens' inhibition zones.

**2.9 pH Testing**

*C. arabica* SCG-loaded nanogels underwent pH testing to measure their acidity and alkalinity levels to prevent the precipitation of chitosan, which becomes insoluble at a pH above 6.5 (Qin et al., 2006). This was quantified using a pH meter, where a pH level below 7 was considered acidic and above 7 was considered basic.

**2.10 Cytotoxicity Testing**

The formulated and loaded nano gel was subjected to cytotoxicity testing, utilizing the procedure of Sarah et al. (2017) or the Brine Shrimp Lethality Assay with recalibrations. Ten milligrams of SCG-loaded nano gels were diluted in 1 mL of water to form a stock solution. Once formed, it underwent serial dilution for various concentrations (1000 ppm, 100 ppm, 10 ppm, 1 ppm), which were segregated into five labeled test tubes containing 10 brine shrimp larvae each. Dead larvae were counted 24 hours after exposure to the mixture.

**2.11 Statistical Treatment of Data**

Following expert review, the collected data underwent data analysis. The researchers utilized descriptors for the color reactions of the extracts during phytochemical analysis and applied descriptive statistics, such as percentage, mean, and standard deviation, for the presentation of the quantified data (nanogel characterization, antibiotic activity).

**2.13 Ethical Considerations**

The researchers followed the Data Privacy Act in accordance with the General Data Protection Regulation (GDPR). This study protected the confidentiality of the data collected through robust encryption, secure storage, restricted access, and a clearly defined data retention policy. The researchers ensured that the data remained transparent and controlled, avoiding any potential biases once the data was collected.

3. results and discussion

**3.1 Nanogel Characterization**

One way to describe nanogels is their versatility. They are synthesized based on their tunable architecture and properties that depend on how they are applied. Additionally, their drug delivery system is operated by tuning their physicochemical characteristics as targeting approaches (Anwar et al., 2022). In connection, the preliminary physicochemical characteristics of the nanogels are presented in the tables below.

**Table 1. Particle Size and Size Distribution of SCG Extract Nanogels**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Mean Volume**  **Diameter** | **Mean Area**  **Diameter** | **10th**  **Percentile** | **50th**  **Percentile** | **90th**  **Percentile** | **Polydispersity**  **Index** |
| 5490 nm | 5390 nm | 4470 nm | 5640 nm | 6280 nm | 0.01 |

**Table 2. Characterization of SCG-loaded Nanogel (Drug Loading Capacity**

**and Encapsulation Efficiency)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trial** | **Absorbance** | | **Drug**  **Loading**  **Capacity**  **(%)** | **Encapsulation**  **Efficiency**  **(%)** |
| 1  2  3  4  5 | 0.883  0.883  0.883  0.883  0.883 | | 4.76%  4.76%  4.76%  4.76%  4.76% | 99.77%  99.77%  99.77%  99.77%  99.77% |
| **Total**  **Mean** | | **0.883** | **4.76%** | **99.77%** |

Table 1 presents the particle size, size distribution, drug loading capacity, and encapsulation efficiency of the nanogel loaded with *C. arabica* SCG extract. The particle sizes across all percentile ranks were listed at 4470 nm, 5640 nm, and 6280 nm, respectively, which were higher than the range. The polydispersity index is at 0.01, indicating that the particle size is relatively uniform. This could be attributed to factors such as potential aggregation in storage or molecular swelling, as well as unavailability of equipment in the formulation process (Huang et. al., 2017). Meanwhile, Table 2 presents that the nanogels demonstrated an absorbance of 0.883, therefore exhibiting drug loading capacity across all 5 trials at 4.76%, while encapsulation efficiency is at 99.77%.

Though nanogels carry out the advantages of the conventional drug delivery systems, there is still room for addressing certain limitations, including the scalability of the product as well as the traditional reproduction and manufacturing (Gupta and Sharma, 2025). These factors are then brought about by the availability and quality of materials and the formulation process (Taha et. al., 2022). Moreover, the optimization of nanogel particle size depends on the power and time of the ultrasonic waves transmission (Ruiz et. al., 2022).

Moreover, the average drug loading capacity and the high encapsulation efficiency indicate acceptable incorporation of the SCG extract into the nanogels, Similarly, also in the study of Anwar et. al (2022), the increase in encapsulation efficiency of the nanogels, were observed due to their hydrophilic state as well as of the loaded drug. In addition, the water solubility of nanogels and the drug was also attributed to the high crosslinking and density of the nanogel network. Meanwhile, the low drug loading capacity can be attributed to factors such as the total mass of the extract loaded into the nanogel, which is directly proportional to each other (Shen et. al., 2017).

In the study of Fernandez-Solis et al. (2024), the use of chitosan and other naturally sourced polymers in the synthesis of the nanogels provided an efficient drug delivery system, utilized in the transport of free drugs such as methotrexate, doxorubicin, fluorouracil, and pravastatin. More notably, the delivery of amoxicillin was also explored in the study of Mardikasari et al. (2022) to regulate a slow and sustained drug release.

**3.2 Antibiotic Activity of SCG-Loaded Nanogels**

Nanogels have been linked to a more efficient delivery of antibiotics, substantial in the aspects of wound healing, cell proliferation, and tissue regeneration. Moreover, it has been studied through the loading of the drug in the nanogel structure (Rusu et. al., 2022). The results of the *in vitro* antibiotic activity of the nanogels in comparison to the control drugs have been quantified below.

**Table 3. Mean zone of inhibition of SCG-loaded nanogels and the positive controls**

|  |  |  |
| --- | --- | --- |
| **Bacteria** | **Zone of Inhibition (mm)** | |
| **SCGNanogel** | **Control** |
| *S. aureus*  *E. coli* | 0  0 | 25 (Tetracycline)  24 (Enrofloxacin) |
| **Descriptor** | Resistant | Susceptible |

The results in Table 2 show the antibiotic activity of the nanogels and the control drug. The zone of inhibition of the nanogel across both S. aureus and E. coli was reported at 0 mm, indicating resistance of bacteria to the sample. Meanwhile, the positive controls (tetracycline and enrofloxacin) demonstrated zones of inhibition of 25 mm and 24 mm, respectively. Though not demonstrating antibiotic activity, the factors that can be attributed are due to the sustained and prolonged drug release characteristic of the nanogels; therefore, the burst diffusion mechanism required for the Kirby-Bauer method is uncontained (Vijyalakshmi et. al., 2025; Hafeman et. al., 2010), highlighting the potential use of other methods in determining antibiotic activity.

Migration of drug molecules can be inefficient due to viscosity. The tuned drug release network of nanogels and the density of the polymer networks can impede the diffusion of the molecules. Additionally, inhibition zones are poorly detected despite the presence of an antimicrobial agent, resulting in a more pronounced activity over time rather than a burst release mechanism. As a result, the release kinetics of the nanogel are more efficient in wound healing applications and tissue regeneration (Sabee et. al., 2020).

In addition, the low drug loading capacity of the nanogels is a driving attribute contributing to the low or absent zones of inhibition, as it limits the availability of the active pharmaceutical ingredient necessary to exert antimicrobial effects. In the study of Shen et al. (2017), it was also highlighted that nanomedicine often faces challenges such as low drug loading, which diminishes the therapeutic efficacy. While increasing drug loading could enhance activity, it also introduces complications, including potential network degradation and systemic toxicity. Structural modifications to improve drug entrapment, such as altering polymer composition or crosslinking density, may compromise nanogel stability, leading to uncontrolled drug release or cytotoxicity. Therefore, achieving an optimal balance between drug loading capacity, stability, and controlled release is essential to maximize therapeutic potential while minimizing adverse effects.

In the study of Asadi et al. (2024), chitosan nanogels were created, loaded with trinitroglycerin for wound healing applications, and tested in vivo. Subsequently, it demonstrated wound healing activity, exhibiting a complete wound closure ratio, enhanced epithelialization, and accelerated tissue regeneration. The nanogel also showed skin formation by promoting fibroblast proliferation and extracellular matrix deposition, contributing to efficient cell proliferation and supporting new blood vessel formation. This indicated the therapeutic potential and biomedical implications of the nanogels in topical wound healing applications, highlighting their role in enhancing tissue repair and promoting a conducive healing environment. Additionally, wound healing nanogels derived from herbal compounds were created in the study of Singh et. al. (2024). As a result, the incorporation of components in the nanogels promoted significant amounts of wound size reductions, enhanced coagulation, reduced oxidative stress, and proliferated the regrowth of fibroblasts

Furthermore, the demonstration of antibacterial activity of *C. arabica* SCG is known to be prominent on gram-positive bacteria. In the study of Asido et. al. (2024) as an active ingredient in liquid hand soap. The findings stated that the *C. arabica* SCG exhibited zones of inhibition of 14 mm and 18.33 mm against *S. aureus* and *Bacillus subtillis* respectively, but is resisted by gram-negative bacteria *E. coli* and *Pseudomonas spp.*. Additionally, in the study of Monente et. al. (2015), the SCG recorded peak percent inhibition of 92% on gram-positve bacteria.

**3.3 Safety and Stability Test (Cytotoxicity and pH test)**

The production of nanomedicine involves carefully monitoring and ensuring product quality over time. Maintaining stability and safety help prevent issues such as premature drug degradation, aggregation, or loss of bioactivity, which could compromise both safety and performance. By addressing these factors, nanomedicine production aims to enhance long-term product reliability, minimize potential toxicity, and ensure that the medicine remains safe and effective for its intended application (Muthu & Feng, 2009). The results of the cytotoxicity test and pH level assessment are listed in the tables below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cytotoxicity Test** | **Concentration** | | **No. of Live Brine Shrimp (BBS)** | |
| **Day 1** | **Day 2** |
| 0 | | 10 | 10 |
| 1000 ppm | | 10 | 5 |
| 100 ppm | | 10 | 7 |
| 10 ppm | | 10 | 7 |
| 1 | | 10 | 10 |
| **pH** | **R1** | **R2** | **R3** | **R4** |
| 4.5 | 4.5 | 4.5 | 4.5 |

**Table 4. Cytotoxic Activity and pH level of SCG-loaded nanogels**

Table 3 presents the preliminary cytotoxic activity of the SCG-loaded nanogel and its pH levels across 4 replicated tests. In the initial day of the cytotoxicity test, 10 brine shrimps were placed in each concentration and left with 10, 5, 7, 7, and 10 respectively, indicating that the nanogels are generally non-toxic according to the performed assay. Meanwhile, the pH level across all replicates were reported at 4.5, indicating that the nanogel remains stable and not at risk of chitosan precipitation due to low solubility in alkaline solutions (Tang et. al., 2023).

According to the study of Xu and Matysiak (2017), chitosan nanogel networks are highly dependent on its pH level. It impacts the structural integrity and the physicochemical attributes of the network, Moreover, it serves as a driver of suitable applications. Additionally, the study of Huang et. al. (2015) emphasizes that pH concentrations of the nanogels help determine its overall stability and network function.

The study of Manivong et. al. (2022) stated that the biocompatibility of nanogels is driven by factors such as their composition, size, surface charge, and degradation products. Nanogels composed of biodegradable polymers, like chitosan, exhibit favorable biocompatibility, reducing the risk of adverse reactions. For instance, biodegradable nanogels can enhance therapeutic efficacy while minimizing toxicity, making them suitable for treating conditions. Conversely, non-biodegradable nanogels may show risks due to potential accumulation in tissues, leading to more risks in topical applications like inflammation. To alleviate such issues, designing nanogels with stimuli-responsive characteristics, such as pH or temperature sensitivity, allows for controlled degradation and release of therapeutic agents at targeted sites. This approach enhances efficacy and reduces systemic toxicity (Mastella et. al., 2024).

Furthermore, the overall safety of products ensures that treatments show benefits without causing harm. It helps prevent adverse drug reactions, ensuring they are efficient and not detrimental. Additionally, ensuring the safety of medications aligns with the drug's overall performance, regulatory standards, and public health goals, allowing for the safe use of new and established therapies while minimizing legal and financial risks. Ultimately, it is essential for improving health outcomes and maintaining public confidence in healthcare (Brennan et. al., 2024).

4. Conclusion

The nanogel characterization revealed a particle size distribution of 4,470 nm at the 10th percentile and 6,280 nm at the 90th. This relatively large particle size may be attributed to molecular aggregation, swelling, or, more notably, an unoptimized sonication process. Meanwhile, drug loading capacity and encapsulation efficiency were measured at 4.76% and 99.77% across five trials, respectively, suggesting that while the amount of loaded drug was relatively low, encapsulation was highly efficient due to the nanogel-to-extract ratio.

*In vitro* antibacterial activity tests showed no zones of inhibition against *S. aureus* and *E. coli*, likely due to the nanogel's low drug loading capacity and prolonged drug release mechanism—features that may be beneficial for direct wound healing and tissue regeneration applications.

Furthermore, the Brine Shrimp Lethality Assay indicated that the nanogels were generally non-toxic, with 11 out of 50 brine shrimp exhibiting mortality in each distribution. Additionally, the pH was maintained at 4.5, ensuring chitosan solubility, as it is known to be stable in acidic conditions.

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