

Synthesis and Characterization of a Multifunctional Quercetin-Silk Sericin Hydrogel Formulation

M. Sanika, Greeshma Varma, M Aiswarya, G.Nikhila, S. Lakshmi Priyadarsini *

Department of Zoology, Government Victoria College, Palakkad, Kerala, India

Abstract. Multifunctional wound healing silver-based compounds are of preclinical trials, especially phytocompounds with high antioxidant and wound healing activity. Phytocompounds have been widely used as wound healing agents due to their antimicrobial and anti-inflammatory properties. The primary goal of this project is to develop a natural, effective wound healing agent that controls microbial growth and promotes healing. In this study, silver nanoparticles were reduced by Quercetin, leveraging its natural properties to create a promising wound healing agent which then emulsified in a silk sericin hydrogel matrix. Even though, Quercetin is a widely used natural antioxidant and wound regenerative agent, its bioavailability and biostability is very low. To overcome this, a quercetin Nano formulation with silver has been formulated, a strong antimicrobial agent. The nanocomposite was characterized through Dynamic Light Scattering (DLS) Scanning Electron Microscopy (SEM), UVVisible spectroscopy, Raman spectroscopy and FTIR spectroscopy. These nanoparticles were then capped with the protein, silk sericin for an effective fibroblast migration as well as slow and consistent drug delivery. The cytotoxicity of the drug hydrogel composite was then analysed by evaluating hemocompatibility properties. The system demonstrated beneficial properties highlighting its promise as a natural, cost-effective wound healing agent.

Keywords: Nanoparticles, Quercetin, Wound healing agent, Antimicrobial activity, Silk sericin, Spectroscopy

* Corresponding author: lachusuresh2017@gmail.com

1 Introduction

Recent research advancements in modern medical research utilize the healing potentials of natural bioactive agents, metal nanoparticles, natural and synthetic polymers in Diabetic foot cure. Among them, a selective oestrogen receptor modulator, an oestrogen analogue, quercetin-Nano formulations have promising applications due to their combined antimicrobial, regenerative, antioxidant, and anti-inflammatory effects.

The Selective oestrogen receptor modulator Quercetin is reported to be a potent therapeutic drug for diabetic foot ulcer [1]. It has been demonstrated that a macrophage morphological shift from M1 phenotype to M2, macrophage polarization improve inflammation by decreasing the level of pro-inflammatory cytokines (IL-6, TNF- α), increasing IL-10, and

enhancing angiogenesis via upregulation of factors such as VEGF-a and CD31. The compound also found to be modulating cytokine release and collagen production. Integrating Quercetin to Nano formulations. So, the Nano formulations of Quercetin, in the form of hydrogels, provide broad-spectrum wound-healing support ([2];[3]). Even then, clinical trials in this regard may be conducted to explore the potentials of Quercetin from natural resources in wound healing and regeneration. Another important limitation of Quercetin in clinical aspect is its low solubility and low bioavailability [4]. Formulations addressing this issue is of serious concern and several innovative formulations have developed for effective drug delivery; a few examples include quercetin on silver nanoparticles in a Carbopol–aloe-Vera hydrogel [5]. This formulation exhibited good antimicrobial activity, sustained release, and effective wound closure in diabetic mice. Another example is alginate with chitosan [6] for faster wound closure, enhanced collagen deposition. Similarly, quercetin nanocrystal-loaded alginate hydrogels [7] and [8] exhibited antibacterial potency preserving antioxidant function, and improving healing in both normal and diabetic models.

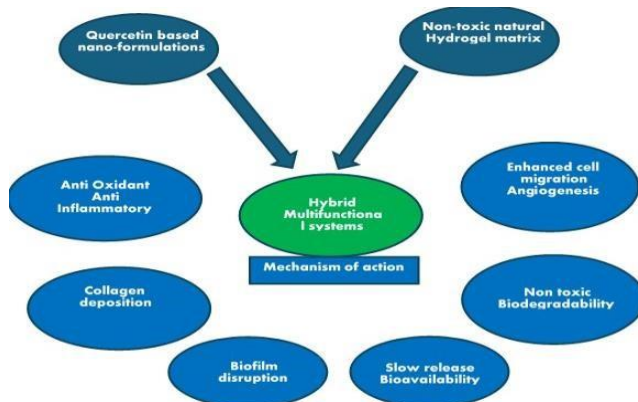


Fig. 1. Schematic diagram representing the mechanisms of action and healing outcomes of various multifunctional Quercetin-formulations

To summarise quercetin with biopolymeric stabilizers demonstrated superior cell migration, controlled release with reduced cytotoxicity and wound healing in both normal as well as diabetic rats targeting multiple wound-healing barriers come out as a promising method of environment-responsive wound therapy. ([9];[10]; and [11]). Even though preclinical efficacy is consistently high, identified gaps such as large-scale clinical trials and comparative benchmarking with current clinical dressings and long-term safety data should be addressed.

2 Materials and Methods

2.1 Materials Used

Silver nitrate, Quercetin Ultra-pure, DMSO, Triton X-100, trisodium citrate, phosphate buffered saline (PBS), Silk cocoons (*Bombyx mori*), chicken blood.

2.2 Methodology

2.2.1 Synthesis and Characterization of Quercetin reduced Silver nanoparticles

Silver nanoparticles were synthesized following a modified green synthesis protocol. A

10 mL solution of 100 μM AgNO_3 was prepared in distilled water and adjusted to pH 8 with NaOH. Separately, a 5 mg/mL quercetin solution was prepared in DMSO. 2ml AgNO_3 from the stock (100 μM AgNO_3) is directly added to 200ml double distilled water and 3ml quercetin solutions were mixed under constant stirring at 70 °C for 30 minutes and then incubated in the dark for 24 hours to allow complete reduction of silver ions. The final reaction volume was 18 mL and the progress of the reaction was visually tracked through a colour transition from pale yellow to brown. The mixture was then centrifuged at 5,000 rpm for 5 minutes to eliminate any unreacted substances, and the resulting supernatant containing AgNPs was collected for characterization. Then the further analysis of the synthesized nanoparticles were subsequently characterized using UV–Visible spectrophotometry, FTIR, Raman spectroscopy, Dynamic Light Scattering, and Scanning Electron Microscopy.

2.2.2 Isolation of Silk sericin Hydrogel

Silk Sericin was freshly extracted from Silk Cocoons of *Bombyx mori* using established protocols [12]. Silk cocoons were cut, weighed (10g), and autoclaved in 100ml distilled water at 121°C for 10–15 minutes, an eco-friendly, chemical-free extraction method. After filtration, the sericin solution was subjected to UV-Visible spectrophotometric analysis for purity check and quantification and used for the preparation of nanocomposite.

2.2.3 Preparation of Silk Sericin Hydrogel nanocomposite

Silk Sericin was freshly extracted as described. To prepare the nanocomposite, 80 μg of quercetin–AgNPs were mixed with 100 μg of sericin solution in 2 mL distilled water. The mixture was stirred at room temperature for 1 hour, followed by sonication for 10 minutes to ensure uniform dispersion. The sericin-capped nanoparticles were collected by centrifugation and resuspended in 5% DMSO to obtain the hydrogel nanocomposite for subsequent experiments.

2.2.4 The in-vitro hemolytic activity assay

The in-vitro haemolytic activity of Silver–Quercetin–Sericin nanoparticles was assessed following the protocol outlined by [13], with slight modifications. Fresh chicken blood was collected in tubes containing 3.8% (w/v) trisodium citrate to prevent coagulation. From the nanoparticle stock solution (1 mM), 200 μL was taken and subjected to a 1:5 dilution. Subsequently, serial half-dilutions were prepared and mixed with 500 μL of a 10% (v/v) red blood cell (RBC) suspension. The samples were incubated at 37 °C for 30 minutes and then centrifuged at 13,500 rpm for 5 minutes. The supernatant obtained was examined for haemoglobin release by measuring absorbance at 620 nm using a microplate reader. Triton X-100 was used as a positive control to induce complete red blood cell lysis, whereas quercetin (10 μL in 5 mL PBS) served as the negative control, indicating no lysis.

3 Results and Discussion

3.1 Green Synthesis of Quercetin reduced Silver nanoparticles

Reduction of silver ions have been carried out by the addition of silver nitrate and quercetin as described in methodology (Fig 2). After synthesis the quercetin reduced AgNPs were centrifuged at 5000 rpm for 5 minutes to discard the macromolecules and collected the supernatant for UV -Visible spectrophotometric analysis (Fig 3), quantified and used for physicochemical characterizations.



Fig. 2. Showing synthesized Quercetin reduced AgNPs

3.2 Characterisation of Silver- Quercetin Nanoparticles Using UV Visible Spectroscopy

The UV–Visible spectroscopic analysis displayed a distinct absorption band between 400 and 450 nm, which is typical of silver nanoparticles due to surface plasmon resonance. As shown in Figure 3, a peak appearing at 420 nm validates the successful synthesis of AgNPs. Absorption bands at 280-350 nm were seen in free quercetin due to the aromatic ring's $\pi \rightarrow \pi$ transitions. The partial obfuscation of the nanocomposite's quercetin peak by the silver SPR indicated an electronic interaction between quercetin chromophores and the silver surface. The observed spectrum overlap suggests that quercetin has been well integrated into the AgNP matrix, providing improved stability and the potential for regulated release. This encapsulation provides benefits like enhanced stability and regulated release, which are essential for biomedical uses such as targeted drug delivery and therapeutic treatments.

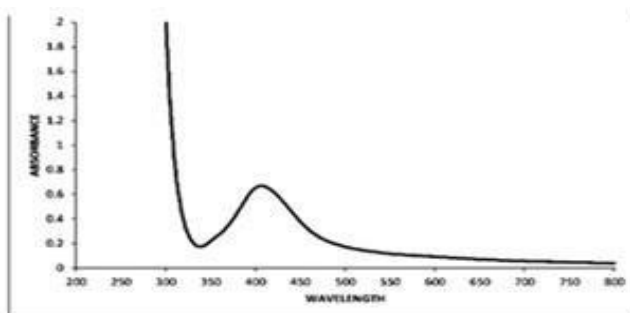


Fig. 3. UV-Visible spectra exhibiting characteristic peak of Quercetin reduced AgNPs

3.3 Extraction and Characterisation of Silk sericin from Silk Cocoon of *Bombyx mori* and Preparation of Quercetin Hydrogel Nanocomposite

Silk sericin was extracted as described in the methodology. Extracted Sericin solution was then subjected to spectrophotometric analysis and quantified. UV- Visible spectrum exhibited a characteristic peak at 280 nm (Fig 4) and the protein solution at 100ug/ml concentration was used for capping as per the methodology part. Sericin capped silver nanoparticle exhibited two peaks. A silver nanoparticle peak at 410 nm and peak of sericin at 296 nm (Fig 5).

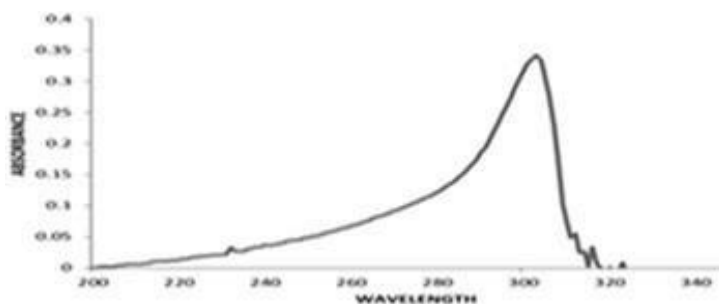


Fig. 4. UV spectrophotometric analysis of silk sericin showing characteristic peak of protein

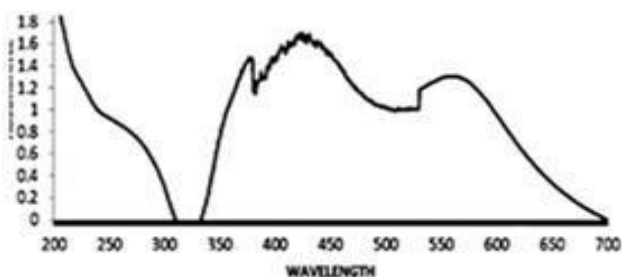


Fig. 5. UV spectrophotometric analysis of silk sericin capped silver nanoparticle showing peak at 410nm

3.4. Dynamic Light Scattering Exhibited Stability and Polydispersity of Particles.

The hydrodynamic diameter and polydispersity index (PDI) of sample were measured using a Zetasizer (Malvern Panalytical, Ver. 8.02). The measurement was conducted at 25 °C utilizing a disposable sized cuvette with water as the dispersant (refractive index 1.330, viscosity 0.8872 cP). The sample was equilibrated for measurement, and the equipment automatically adjusted the scattering angle. The analysis revealed (fig 6) a broad size distribution, as evidenced by a Z-average particle size of approximately 975 nm and a PDI of 0.832. From DLS analysis, the Z-average doesn't represent a true nanoparticle size and PDI shows broad multimodal distribution of particles size. This result indicates either the nanoparticles have aggregation or the minimal usage of the reducing (stabilizing) agent. The relatively large Z average and high PDI indicates significant polydispersity and possible aggregation of the AgNPs. SEM of the silver nanoparticles (fig.7) confirmed that the size of the nanoparticles are in the nanoscale range. Disparity in the DLS result will be the aggregation in the aqueous solution, as is clearly shown by the DLS is more sensitive to larger particles.

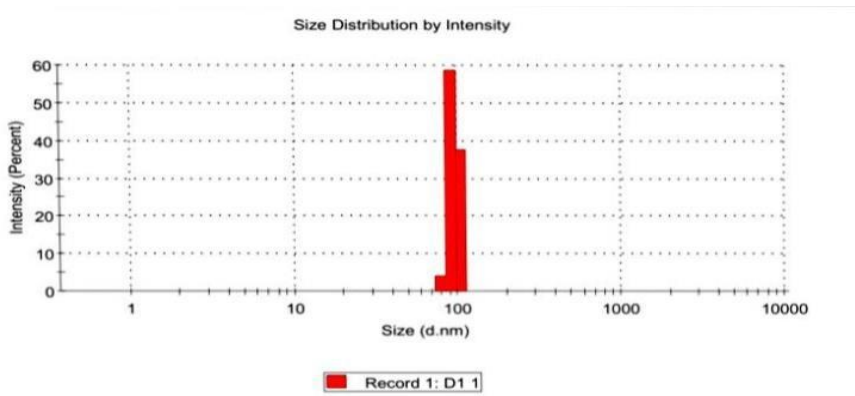


Fig. 6. DLS analysis showing hydrodynamic size and PDI of nanoparticle

3.5. Scanning Electron Microscopy characterisation of Silver-Quercetin nanoparticles.

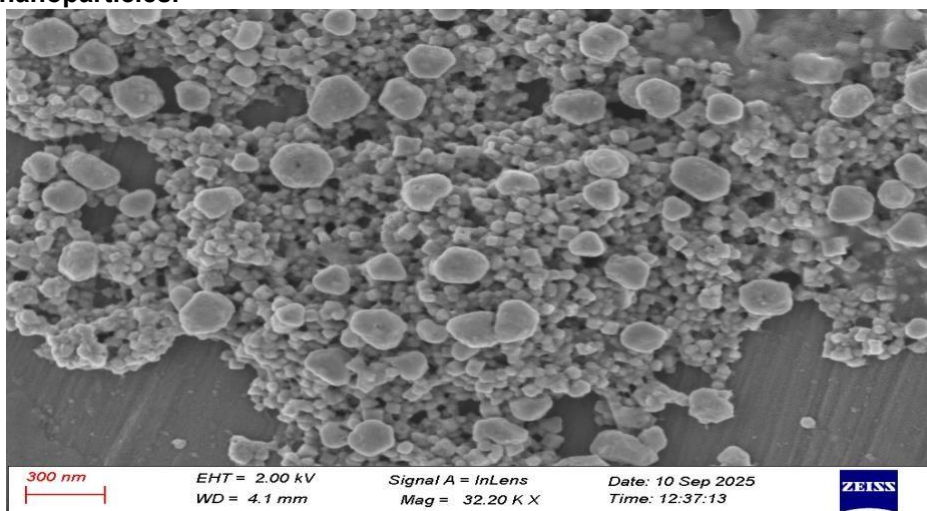


Fig. 7. SEM image of quercetin reduced silver nanoparticle

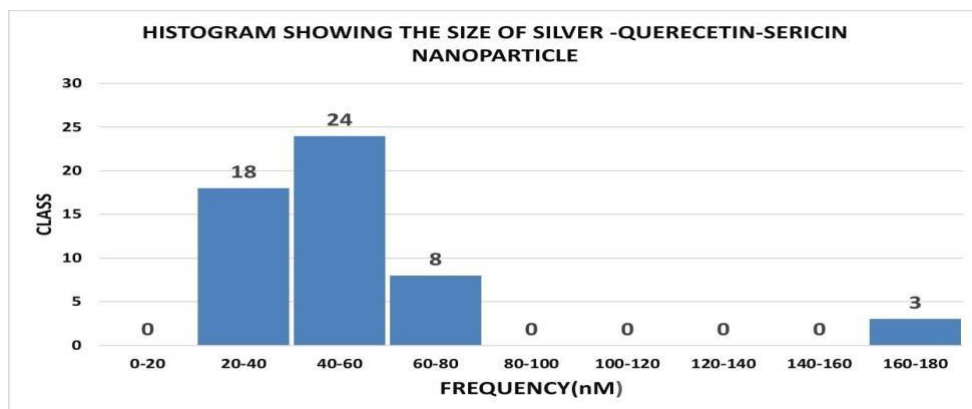


Fig. 8. Histogram showing the size of silver-quercetin nanoparticle

The surface morphology of quercetin-reduced AgNPs was examined using scanning electron microscopy (Fig. 7). Most of the nanoparticles in the micrograph were spherical or nearly so. The particles were distributed across the nanoscale range and had an average size of between 30 and 80 nm (Fig. 8). Agglomerates show that some particles are grouped together, and this is because AgNPs have a lot of surface energy. The stabilizing effect of quercetin, which acts as a reducing and capping agent, helped create unique nanostructures. The SEM analysis shows that silver nanoparticles with nanoscale shapes that are good for biomedical uses were made successfully.

3.6. Raman Spectrophotometry Exhibited Characteristic Peaks of the Quercetin

The sample was subjected to Raman spectrophotometric analysis and spectrum exhibited a clear Raman scattering signal, indicating the presence of Raman-active vibrational modes in the sample. The x-axis represents wavenumber or Raman shift cm^{-1} , which corresponds to the vibrational frequencies of the molecules, while the y-axis represents intensity, indicating the Raman signal strength. As shown in Fig 6, the Raman spectroscopic analysis of Silver Quercetin nanoparticles reveals distinct peaks at 670, 820, 966, 1114, 1205, 1288, 1422, and 1500 cm^{-1} . These correspond to quercetin vibrational modes, including C–O–C stretching (820 cm^{-1}), C–C skeletal vibrations (1114–1288 cm^{-1}), and aromatic C=C stretching (1500 cm^{-1}). Shifts in band positions relative to free quercetin indicate π – π interactions between quercetin rings and the AgNP surface, as well as possible hydrogen bonding with sericin. These interactions stabilize the nanocomposite and alter the vibrational environment of quercetin.

In conclusion, Raman spectroscopic analysis confirms the presence of Quercetin-silver nanoparticles based on characteristic vibrational modes observed in the 1000–1700 cm^{-1} region.

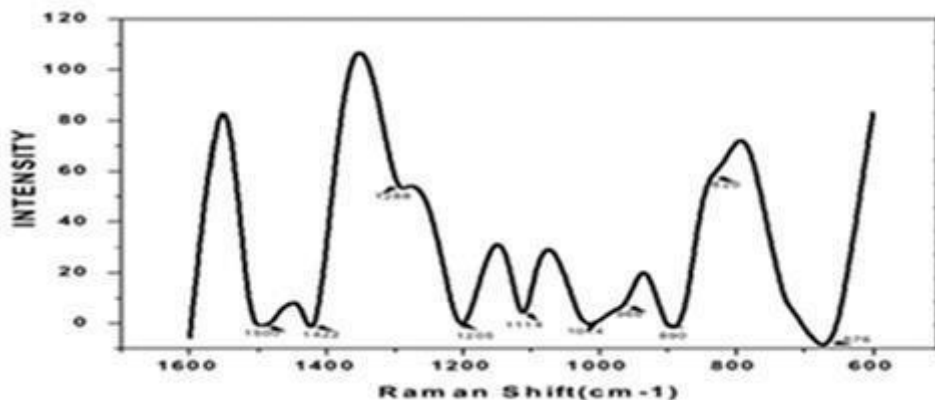


Fig. 9. Showing Raman spectroscopic analysis of Silver-Quercetin nanoparticles

3.7 Fourier Transform Infrared Spectroscopy of Silver Nanoparticle Confirming the Presence of Quercetin

FTIR analysis of silver nanoparticles reveals characteristic absorption bands (Fig 7) related to the functional groups present on the nanoparticle surface, primarily those responsible for capping and stabilization. As shown in the Fig .7, prominent bands are observed in the 3400-3200 cm^{-1} , 1600-1500 cm^{-1} , and 1300-1000 cm^{-1} regions, which can be attributed to O-H stretching, C=O stretching, and CO stretching, respectively.

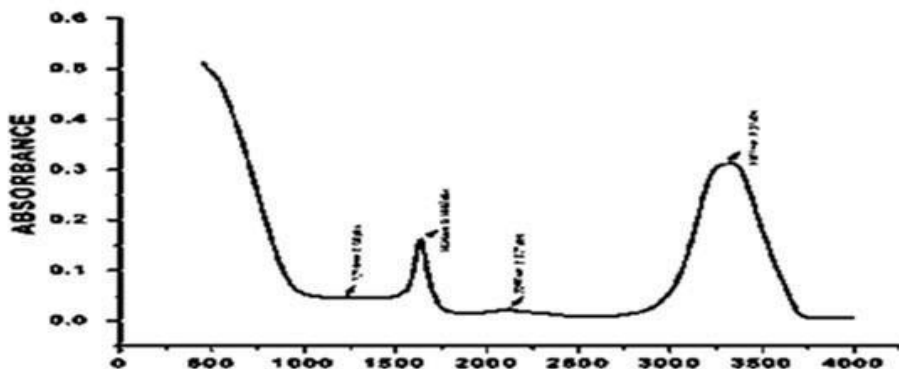


Fig. 10. Fourier Transform Infrared spectroscopy analysis of Silver-Quercetin nanoparticles

- **3400-3200 cm^{-1} :** Broad peaks in this region often indicate O-H stretching vibrations, typically associated with hydroxyl groups from the capping agents or plant extracts.
- **1600-1500 cm^{-1} :** This region may show peaks attributed to C=O stretching (carbonyl groups) or C-N stretching (aromatic amino groups) depending on the capping agent.
- **1300-1000 cm^{-1} :** Peaks in this region can correspond to C-O stretching, indicative of the presence of carbohydrates or other oxygen-containing functional groups in the capping agent.

3.8 The Silver Quercetin Nanoparticles were Found to be Nontoxic by Haemolytic Assay

Haemolytic assay has been carried out to observe the degree of cytotoxicity of the compound, using chicken red blood cells as described in the methodology. The haemolytic assay of Silver–Quercetin–Sericin nanoparticles shows that all tested concentrations exhibited very low absorbance values at 620 nm, close to the PBS negative control (0.1027) and far below the Triton X-100 positive control (1.264), which caused complete red blood cell lysis. The nanoparticles at concentrations ranging from 9 µg/ml to 0.009 µg/ml produced absorbance values between 0.0682 and 0.0836, indicating negligible haemolytic activity. As per research reports Silver nanoparticles at concentrations below 10ug/ml has been reported to be non toxic to mammalian cells and above 25ug/ml exhibits reduced viability and oxidative stress ([14]; [15]). This suggests that Silver–Quercetin nanoparticles are largely nontoxic to red blood cells and show no dose-dependent increase in haemolysis.

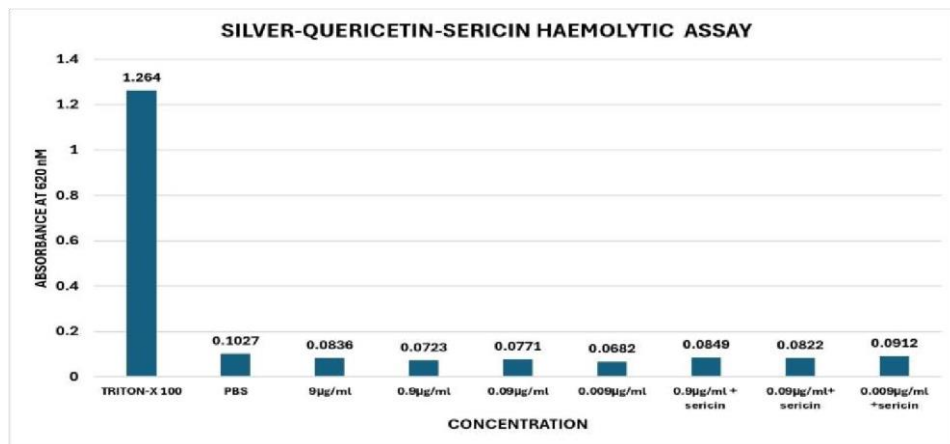


Fig. 11. Showing haemolytic activity of nanoparticle alone and sericin capped silver nanoparticle.

3.9 Analysis of Stability of the Nanoparticle Drug Shows High Stability

UV-visible spectroscopy revealed distinct absorbance spectra for same concentration of Silver-Quercetin nanoparticles at different UV exposure : 24hours , 48hours, 72hours, one week, two week and 3 weeks .This is evident in the Figure 9. Notably, absorbance values within the 400-500 nm range increased progressively with longer UV exposure times. These findings indicate a proportional enhancement in silver coating thickness on the nanoparticles corresponding to extended UV exposure durations.

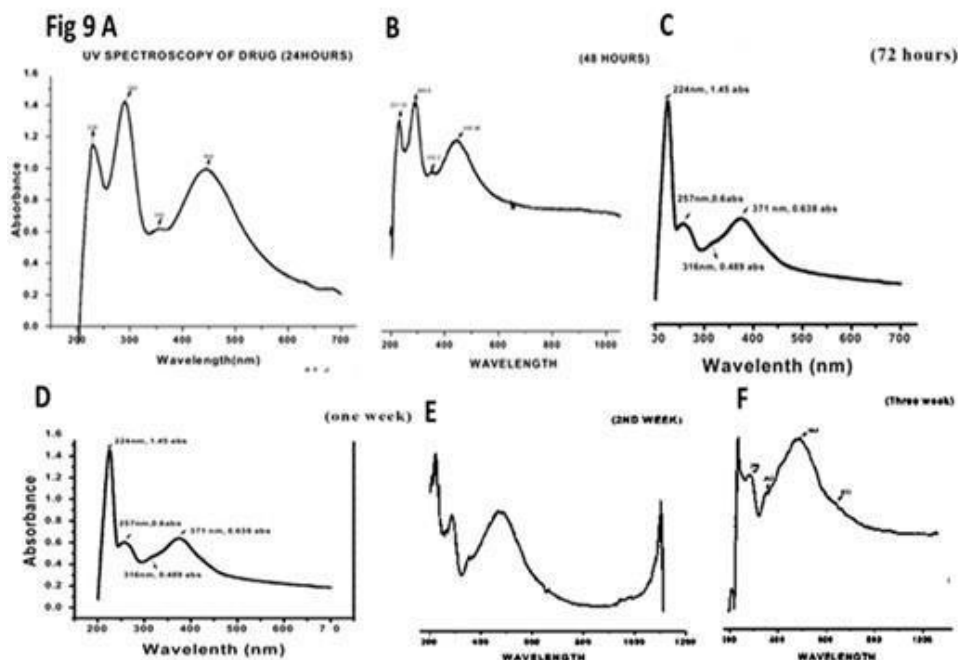


Fig. 12. UV-Visible spectrophotometric analysis of the drug over different time points exhibiting stable peaks without any shift in peaks or degradation, showing high stability

4 Conclusion

Quercetin-reduced AgNPs, capped in a silk sericin hydrogel nanocomposite is synthesized and characterized using various methods. The extraction of sericin was successfully carried out in the laboratory, and its characterization using UV spectroscopy revealed a distinct absorbance peak at 280 nm, confirming its presence. Furthermore, quercetin-reduced silver nanoparticles were synthesized and capped with sericin, leveraging the synergistic benefits of both compounds. The synthesized nanoparticles were analyzed through DLS, SEM, UV-Vis spectroscopy, Raman spectroscopy, and FTIR to determine their physicochemical characteristics. Stability assessment indicated that the particles maintained considerable stability, highlighting their suitability for potential drug applications in wound healing. Remarkably, these nanoparticles exhibit antimicrobial, anti-inflammatory, and wound healing activities, making them strong candidates for inhibiting bacterial growth and enhancing tissue regeneration. In the context of diabetic foot ulcers, where excessive microbial growth can significantly impede the healing process, sericin-coated silver nanoparticles offer a promising, cost-effective and natural solution for managing microorganisms and promoting wound healing by the inherent properties of both Quercetin as well as the natural hydrogel protein Sericin.

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