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RESEARCH ARTICLE
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Stabilization Of Silver Nanoparticles Using Whey Proteins

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ABSTRACT

Introduction: Silver nanoparticles (AgNPs) are widely recommended as a substitute for antibiotics because the antibiotics are becoming ineffective and the dosages are increasing day by day.

Materials and methods: A nanoparticle formulation was prepared by adding 100 ml of water and 0.32 g of silver nitrate and then heating the mixture. Then, 10 ml of whey protein was slowly drizzled into this concoction and obtained a red hue.

Results: The particle formation was seen using UV spectroscopy. SEM analysis visualised the formation of the nanoparticles and they were formed to be a uniform size. MTT assay confirmed the viability of the cells. The antibacterial and anti-inflammatory properties of AgNPs were confirmed.

Conclusion: Silver nanoparticles synthesized from whey protein are stable and consistent in size.

Keywords: *Silver nanoparticles, whey protein, cell culture, anti-inflammatory*

INTRODUCTION

Antibiotics played a crucial role in the mid-20th century, as they are effective in treating patients against microbial infections, which were fatal during that period. Silver nanoparticles (AgNPs) are widely recommended as an antibiotic substitute because antibiotics are becoming ineffective and overdoses are increasing [1]. AgNPs are prepared using different parts (fruit, seed, leaves, root, etc.) of the herbal plants, including Terminalia chebula,

Emblica officinalis and Curcuma longa [2] [3]. Various efforts were made in the synthesis of AgNPs using animal-based products, including buttermilk, peptone, and bovine serum albumin [4] [5] [6].

The milk by-product whey is a complex protein. Lactoferrin, beta-lactoglobulin, alpha-lactoglobulin, glycomacropeptides, and immunoglobulins are some of the components. Multiple health benefits, including those of an antioxidant, anti-hypertensive, hypolipidemic, and chelating agent, are attributed to it. As a supplement to the diet, it has been shown to reduce the risk of cardiovascular disease and osteoporosis. In the present study, we have formulated AgNPs with the aid of whey proteins from milk. The anti-inflammatory and cytotoxic behaviour of the prepared silver nanoparticles were analysed.

MATERIALS AND METHODS

Preparation of whey liquid

Cow milk was obtained from the local vendor in Chennai. The milk was heated and allowed to boil. The milk was coagulated by adding a few drops of lemon juice to it. The coagulated contents were removed by filtering, and the remaining solution was used for the nanoparticle preparation.

Formulation of silver nanoparticles

Silver nitrate solution (10 mM) was placed in a beaker covered with aluminium foil. The solution was heated for 15 min at 60 °C. Then 5 mL of the prepared whey liquid was added and the reaction was allowed to proceed for 1 h.

Characterization of silver nanoparticles

The preliminary confirmation of the formation of silver nanoparticles stabilised with whey protein (w-AgNPs) using a UV-visible spectrophotometer (JASCO 760D). The prepared w-AgNPs were examined using a field emission scanning electron microscope (JEOL, JSM-IT800) attached with an energy dispersive X-ray spectroscopy (EDX, Oxford) to determine the morphology and elemental composition.

Evaluation of Cell viability

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to assess the cell viability as reported in the previous literature [12]. In short, MG-63 cells were added to 96-well culture plates at a seeding density of 50,000 cells/well along with the different concentrations of the epi-HAp for 24 h. Then MTT solution was added to each well and incubated for 4 h followed by the addition of dimethyl sulfoxide (DMSO). The optical density was measured at 570 nm wavelength by the Elisa Reader (read well). The cells were visualized under a light microscope.

RESULTS AND DISCUSSIONS

Figure 1 shows the UV-visible spectrum of w-AgNPs. The addition of whey liquid to the heated silver nitrate solution led to a light red colour change. After continuous stirring of 1 h, it is observed a red precipitate settled at the bottom. The surface plasmon peak observed at 257 nm is the preliminary indication for the silver oxide nanoparticle formation, whichmatches the previous literature [7]. No other peaks were seen along with the silver oxide nanoparticles peak representing the purity of the nanoparticle formation. The isoelectric point of whey protein is at a pH of 5.2. The sweet curdling had occurred at pH 6.0, therefore the whey proteins possess a net negative charge that aided the reduction of silver ions into nanoparticles. The hydrophilic polymers in the whey water that contain the functional groups including hydroxyl and carboxylic groups can support both the reduction and stabilization of AgO NPs. The whey protein encapsulates the nanoparticles and their overall negative charge inhibits the aggregation of the nanoparticles [8].

The prepared whey water-mediated silver oxide nanoparticles were analysed with SEM attached to the EDX and the obtained data were given in Figure 2. The spherical-shaped nanoparticles were observed with an average diameter of 23 nm. The surface area to volume ratio of the silver nanoparticles is inversely proportional to the dissolution (i.e) the nanoparticles with smaller size (5 nm) dissolute faster than the large nanoparticles (50 nm). Therefore, the sustainability of the nanoparticles on the site is quite higher [9]. The large particles seen in certain places are the added whey proteins. The EDX showed the occurrence of silver, oxygen, carbon and aluminium. The oxygen presence indicates that silver is in its oxide state. The carbon traces are from the whey water. The

occurrence of the aluminium peak is due to the coating of the whey water-mediated silver oxide nanoparticles on the aluminium foil.

TABLE 1: Zone of inhibition measured from the bacterial culture plates

S. No.	Micro-organism	Zone of inhibition (mm)	
		25 µl	**100 µl**
1.	Escherichia coli	-	25
2.	Staphylococcus aureus	15	16
3.	Enterococcus faecalis	10	12
4.	Candida albicans	Negative	Negative

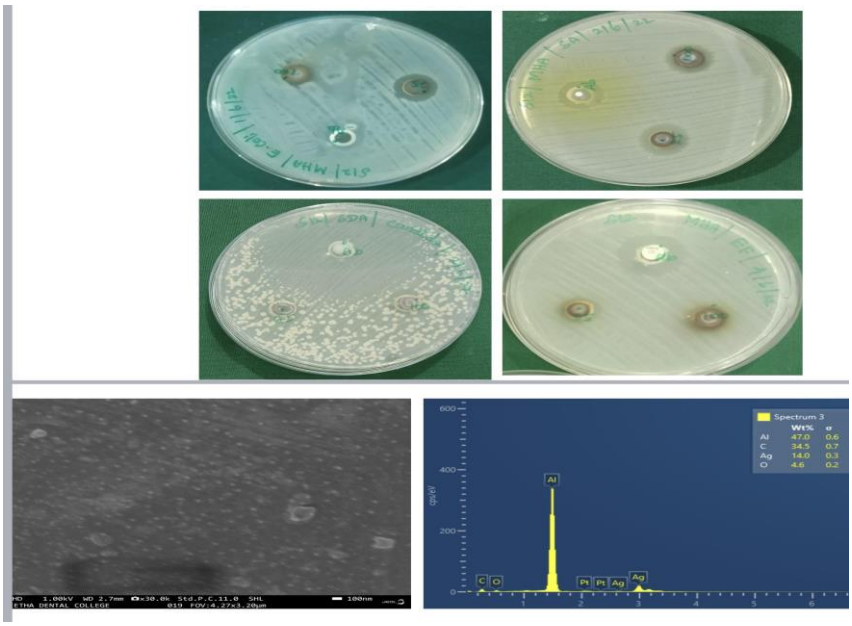

FIGURE 1: UV spectrum of whey water-mediated silver oxide nanoparticles

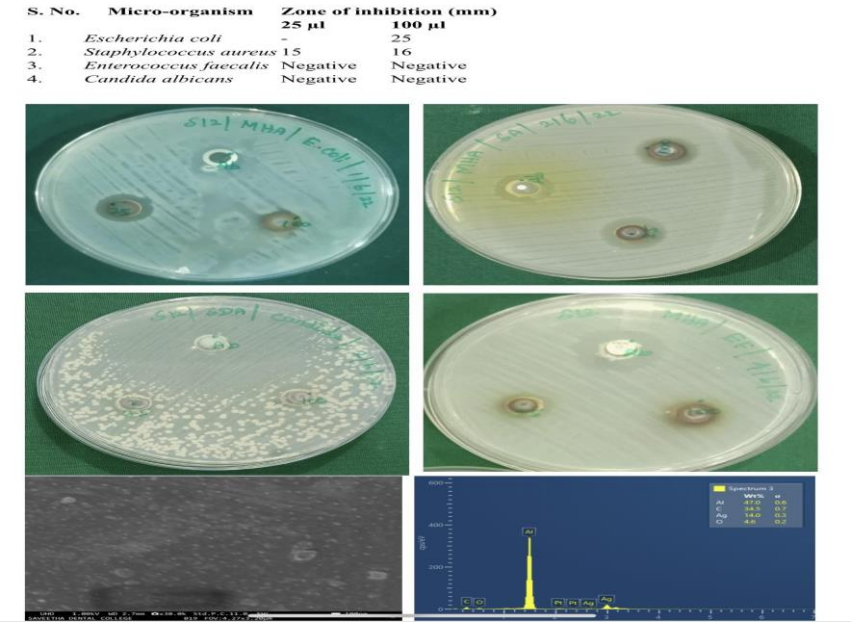

FIGURE 2: SEM image and EDX profile of whey water-mediated silver oxide nanoparticles

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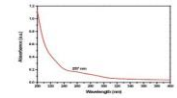

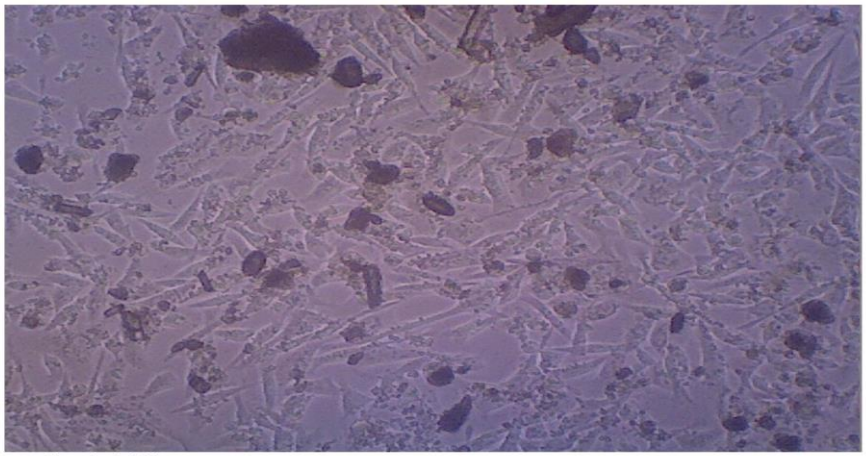

Cell viability = 78.54%

FIGURE 3: Nutrient Agar plates showing antimicrobial activity of w-AgNPs

The antimicrobial activity of the prepared w-AgNPs was analysed against various microorganisms including Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, and Candida albicans. Figure 3 and Table 1 detail the zone of inhibition data obtained from the microorganism. E. coli showed no zone of inhibition at 25 µl and 100 µl, it displayed a 25 mm zone. Staphylococcus aureus exhibited 15 and 16 mm for 25 and 100 µl. There is not much variation in the zone of inhibition at a wide range of concentrations. Enterococcus faecalis showed 10 and 12 mm as inhibition zones for 25 and 100 µl, respectively. Candida albicans showed no zone of inhibition. To be precise, the w-AgNPs were effective at the concentration of 100 µl against Escherichia coli, Staphylococcus aureus and Enterococcus faecalis.

w-AgNPs were added to MG63 cells and incubated for 24 h and given as Figure 4. The cell viability rate was around 78.4 %, which is lower than the control. However, as per the ASTM standards, cell viability above 70 % is considered biologically compatible. Hence, the w-AgNPs can be employed for the use of bio-applications. Our team has extensive knowledge and research experience that has translate into high quality publications(Sathivel et al. 2008; Sekar et al. 2019; Rajeshkumar et al. 2019; Lakshmi et al. 2015; Felicita, Chandrasekar, and Shanthasundari 2012; Thejeswar and Thenmozhi 2015; Saravanan et al. 2021; Menon and Thenmozhi 2016; Sahu, Kannan, and Vijayaraghavan 2014; Wang et al. 2019).

CONCLUSION

Whey protein isolated from milk was used for the synthesis of silver nanoparticles that were both stable and of a consistent size. The cell compatibility of w-AgNPs was highlighted using an MTT assay. The novel applications of this formulation could be demonstrated even further through the use of more descriptive tests, such as antioxidant assays. Additional studies would enhance our understanding of these silver nanoparticles and bring us one step closer to applying that understanding.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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