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**Biosynthesis, characterization and anti-microbial activity of silver nanoparticle based gel hand wash**

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**Abstract:** In the current study, silver nanoparticles (AgNPs) were biosynthesised by microwave irradiation using Azadirachta indica. The formation of AgNPs was confirmed by surface plasmon resonance (SPR) band at 408 nm at UV-visible spectroscopy due to reduction of silver metal into (AgNPs) and further confirmed its particles in nano range. Nine different smart-gel hand wash were prepared by dispersing (AgNPs) in the HPMC and/or Pluronic F-127 polymers. The prepared smart gel hand wash was optimized based on pH, viscosity, spreadability, foamability, clarity. The optimized hand wash (NH5) had pH (6.6 ± 0.33), viscosity (66 ± 0.77, cp), spreadability (24.34 g-cm/s), foamability (100 mL). The optimized hand wash (NH5) showed a superior efficacy against pathogenic organisms in comparison to germ protection based marketed hand wash.

**Keywords:** silver nanoparticles; smart-gel hand wash; anti-microbial efficacy; stability

1 Introduction

Hand washing is a religious and cultural habit, the relation between handwashing and infectious disease was recognized only two centuries ago by a Hungarian doctor Ignaz Semmelweis, known as the father of hand hygiene [1,2]. He noticed the spread of infection and mortality of new born given birth in his hospital, which was relatively higher than the adjacent midwife-run maternity hospital. In his investigation, he concluded that doctors with bare hands after performing surgery or autopsy visited the maternity ward were the cause to transmit the infection in new-borns and causes death [3]. In the present scenario challenges of the 21st century include infectious diseases; the second leading cause of death that kills 17 million people in a year worldwide [4]. Majorly transmission of infection takes place by contaminated hands, therefore hand plays a vital role in the spread of infectious diseases in the healthcare setups, industrial setting related to the food manufacturing and also in the community to larger extent [5,6]. Hand hygiene as an intervention to prevent the spread of infections, the term hygiene was derived from the Greek healing goddess Hygeia. In 1961 the Centre for disease control and prevention (CDC) recommended hand wash for health care workers. The recent guideline recommends, use of waterless antiseptic agents instead of soap and water for hand wash [7,8]. Hand hygiene preparation with soap may damage the skin and increases microbial growth over the time. Usage of soap in hand wash may damage the skin which could be more prone to microbial colonization [9,10].

Reports on Triclosan an active antiseptic ingredient commonly used in hand wash proved ineffective than using plain soap [11]. Nowadays researchers pay more attention in natural medicine due to its safety and dependability as compared with synthetic medicine that causes deleterious effects. Nature is the reliance of remedies for all ailments of mankind. Until now, 1340 plants and over 30,000 antimicrobial constituents have been isolated from plant sources [12,13]. *Azadirachta indica* (Neem) leaf showed antimicrobial and antifungal activity due to quercetin, beta-sitosterol, polyphenolic flavonoids [14]. *Azadirachta indica* is an evergreen plant native of India and found in most tropical countries belongs to the family Meliaceae [15]. Metallic nanoparticles are emerging as novel class of biomedical material applied for hygiene in daily to day life. Silver nanoparticles (AgNPs) were the clusters of silver (Ag)
atoms ranged from 1-100 nm showed predominant robust
cytotoxicity to different bacterial cells by interacting with
functional group on the bacterial cell membrane and
inactivate bacteria. Silver in the presence of water converts
to level 1 (Ag+) which could be reactive and binds to protein
of the bacteria act as bactericidal [16-19].

Thus the present study aims to biosynthesis AgNPs
by microwave irradiation using silver nitrate and neem
leaf extract solution. The AgNPs synthesis was confirmed
by surface plasmon resonance (SPR) band and formed
AgNPs was incorporated in smart-gel hand wash by using
different polymers.

2 Materials and methods

2.1 Materials

Silver nitrate, Hydroxypropyl Methyl Cellulose (HPMC),
Pluronic-F-127, Sodium lauryl sulphate were purchased
from Sigma-Aldrich, USA. Fresh neem leaves of Azadirachta
indica were collected from the local area. Glycerine and
triethanolamine obtained from SD Fine Chemical Ltd.
Agar was purchased from Hi-Media laboratories, other
chemical were used of analytical grades.

2.2 Microwave irradiation assisted green
synthesis of silver nanoparticles

Locally available Azadirachta indica leaves, authenticated
by botanist were selected and rinsed thoroughly many
times with tap water followed by ion free water. 5 g of
young leaves was subjected for size reduction in ball mill
by adding 100 mL of distilled water then aqueous extract
was filtered through Whatman No. 1 filter paper. 5 mL
filtrate as reducing agent was added in 100 mL of 1 mM of
silver nitrate. Then the solution was taken in a closed round
bottom flask and mounted on a ring placed in microwave
oven (“Samsung Model ME0113M1”) operated at power
output 900 W, irradiated by microwave heat until the
solution turned to colloidal dispersion of yellowish brown
colour indicates the formation of silver nanoparticles. The
formation of AgNPs was confirmed by UV-Visible absorption
Surface Plasmon Resonance (SPR) spectrum [20,21].

2.3 Formulation of smart hand wash
entrapped with bio silver nanoparticles

The microwave irradiation assisted biosynthesised AgNPs,
subjected to ultracentrifugation at 10000 rpm for 4 h. The
sediment was collected and dried at room temperature,
and utilized for the preparation of smart hand wash, nine
sets of gel hand wash (NH1-NH9) were prepared as per
the composition mentioned in the Table 1. The smart-gel
hand wash were prepared by dispersing in hydrophilic
thermoresponsive polymer (Pluronic F-127) in the sufficient
amount of purified water with continuous stirring at
1000 rpm, then add sodium lauryl sulphate (SLS) 1% and
2% glycerine. Desired quantity of triethanolamine then
added to adjust pH. Finally fragrance was added and final
volume is made up with distilled water up to 100 mL and
mixed at 300 rpm under ultrasonication for 10 min [22,23].

2.4 Characterizations of bio AgNPs
entrapped in smart hand wash

2.4.1 UV-Visible measurements

Spectral analysis of the fabricated nanoparticles was
carried out using JASCO 730- UV–Vis spectrophotometer.

| Formulation code* | AgNPs (mg) | HPMC (% w/v) | Pluronic(F127) (% w/v) | SLS (% w/v) | Glycerine (% w/v) | Fragrance (% w/v) |
|------------------|------------|---------------|------------------------|-------------|-----------------|------------------|
| NH1              | 1.5        | 1             | –                      | 1           | 2               | 0.5              |
| NH2              | 1.5        | 1.5           | –                      | 1           | 2               | 0.5              |
| NH3              | 1.5        | 2             | –                      | 1           | 2               | 0.5              |
| NH4              | 1.5        | –             | 1                      | 1           | 2               | 0.5              |
| NH5              | 1.5        | –             | 1.5                    | 1           | 2               | 0.5              |
| NH6              | 1.5        | –             | 2                      | 1           | 2               | 0.5              |
| NH7              | 1.5        | 1             | 1                      | 1           | 2               | 0.5              |
| NH8              | 1.5        | 1             | 1.5                    | 1           | 2               | 0.5              |
| NH9              | 1.5        | 1             | 2                      | 1           | 2               | 0.5              |

*Each formula consists of distilled water up to 100 mL. All formulations were neutralized by triethanolamine to pH=6.8
The spectra were scanned in the range of 300 to 700 nm wavelength with resolution of 1 nm at room temperature. The surface Plasmon resonance band (SPR) at visible range of wavelength was monitored to confirm AgNPs formation.

2.4.2 Particle size measurement

Biosynthesized AgNPs were suspended in distilled Milli Q treated water (pH 7) and characterized for particle size by Nanotrac particle size analyzer (Microtrac, S3500) [24]. The colloidal solution of AgNPs was analysed at 25°C with an angle of detection of 90°. Each measurement was done in triplicate.

2.4.3 pH measurement

Formulated smart hand wash were evaluated for pH to check the skin compatibility by using a pH meter (SensoDirect, Lovibond- Germany) the results were tabulated in Table 2.

2.4.4 Viscosity measurement

The flowability of the prepared formulation was determined by Brookfield DV-I Prime viscometer (Brookfield, Massachusetts, USA). The sample adapter (spindle), rotated at an optimum speed, the sample is placed in flow jacket to maintain a constant room temperature and allowed to settle for 5 min prior to taking the reading. The rheological results showed in Table 2.

| Formulation Code | pH           | Viscosity (cp) | Spreadability g-cm/s | Foamability (mL) |
|------------------|--------------|----------------|----------------------|------------------|
| NH1              | 6.4 ± 0.13   | 150 ± 0.23     | 22.13                | 95               |
| NH2              | 6.6 ± 0.17   | 280 ± 0.45     | 20.65                | 95               |
| NH3              | 6.3 ± 0.21   | 445 ± 0.41     | 19.67                | 90               |
| NH4              | 6.8 ± 0.14   | 40 ± 0.33      | 26.87                | 100              |
| NH5              | 6.6 ± 0.33   | 66 ± 0.77      | 24.34                | 100              |
| NH6              | 6.4 ± 0.74   | 89 ± 0.63      | 22.32                | 90               |
| NH7              | 6.5 ± 0.86   | 310 ± 0.23     | 21.98                | 95               |
| NH8              | 6.6 ± 0.11   | 420 ± 0.16     | 20.03                | 85               |
| NH9              | 6.4 ± 0.12   | 880 ± 0.23     | 18.54                | 85               |

2.4.5 Spreadability measurement

Multimer [25] method used to determine the spreadability by placing the smart-gel hand wash under investigation in between two glass plates of 05x20 cm. 500 g weight was placed for 5 min to disperse the content, the weight was removed and the 50 g of weight was then attached to the pulley connected to the upper layer. The bottom glass plate was fixed to a wooden table. The upper glass plate rolls over the bottom plate the time required to travel for 10 cm was noted and spreadability was measured by the below formulae and presented in g-cm/s. The spreadability was determined by special fabricated apparatus and it was calculated using the formula (Eq. 1):

$$ S = \frac{ml}{t} $$

where:
- $S$ – spreadability
- $m$ – weight tied to the upper slide
- $l$ – length of the glass slide
- $t$ – time taken in seconds.

2.4.6 Foamability measurement

Smart-gel hand wash (5 g) was taken in 100 mL measuring cylinder, agitated for 10 times in 50 mL of tap water, and final volume was measured in mL, the results were given in Table 2.

2.4.7 Clarity measurement

The smart-gel hand wash was evaluated for clarity by placing the three different concentrations (10% w/v, 25% w/v and 50% w/v) of content in 1 cm quartz cuvette and scanned at 400 nm using UV-Visible spectrophotometer. The % transmission was of the sample noted to measure the clarity of the product.

2.4.8 In vitro anti-microbial activity

The antimicrobial activity of the smart-gel hand wash (NH5) was aseptically evaluated by dip well agar diffusion technique against Staphylococcus aureus and Candida albicans [26]. A well was excavated (0.85 cm) in the plates containing 15 mL of Muller-Hilton agar medium. 100 μL (15 mg/L) aqueous suspension of AgNPs entrapped in
smart-gel hand wash and germ protection based marketed hand wash was introduced in the different wells of petri plates. The dip wall filled with normal saline served as the control. The plates were incubated overnight at 37°C in the incubator. The efficacy of the developed smart-gel hand wash was determined by measuring the zone of inhibition in diameter (mm).

2.4.9 Stability study

The stability studies was performed as per the ICH guidelines [27] by keeping the product in screw tight vial in a humidity chamber at RH 75 ± 5% and temperature 40 ± 2°C for a period of 1 month and examined for change in colour and pH.

3 Results and discussion

3.1 Fabrication mechanism of bio silver nanoparticles

The hypothetical mechanism involved in the fabrication of bio silver nanoparticles was the presence of reducing enzymes in neem aqueous extract filtrate that reacts with silver ion of AgNO₃, henceforth used as scaffolds in the fabrication of Silver nanoparticles, further terpenoids present in azadirachta indica extract acts as a surfactants to stabilized the synthesized AgNPs.

3.2 Characterizations of bio AgNPs entrapped in smart hand wash

3.2.1 UV-Visible measurements

The formation of microwave irradiated green AgNPs in the aqueous neem extract was observed due to colour change to yellowish brown which was confirmed by UV-Visible spectrum (Figure 1). A sharp and intense SPR band observed around wavelength 408 nm due to transverse and longitudinal plasmon vibration and difference in dielectric properties between SPR and inters band transition in Ag [28].

3.2.2 Particle size measurement

The mean particle size of synthesized AgNPs was measured as 50.47 nm with a very low polydispersity index 0.326. Narrow dispersion of particles recognized as monodisperse as could be seen in Figure 2.

3.2.3 pH measurement

The pH of all smart-gel hand wash (NH1-NH9) were found to be in the range of 6.3 ± 0.21 to 6.8 ± 0.14 which is considered to be compatible with the skin to avoid the irritation during hand wash.

3.2.4 Viscosity measurement

The rheological study of smart-gel hand wash (NH1-NH9) indicated that the viscosity of HPMC based hand wash increased by increasing the concentration of the polymer the same pattern was observed for Pluronic F-127 polymer. The viscosity of NH7-NH9 was relatively high as compared with another set of smart-gel hand wash. The smart-gel hand wash (NH4-NH6) containing thermo responsive polymer (Pluronic F127)

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**Figure 1:** UV-Visible spectrum of biosynthesised silver nanoparticles.

**Figure 2:** Particle size distribution of biosynthesised silver nanoparticles.
showed increased in viscosity during hand wash due to physiological temperature 37°C, that can enhance the antimicrobial efficacy of synthesized of AgNPs by prolonged contact time.

3.2.5 Spreadability measurement

The results of spreadability of prepared smart-gel hand wash (NH1-NH9) were tabulated in Table 2. The spreadability of was smart-gel hand wash was found in the range of 18.54 to 26.87 g-cm/s. The results were in the agreement of viscosity, as the viscosity increased the spreadability was found to be decrease and the product with least viscosity value is said to spread more on the palm during application of smart-gel hand wash.

3.2.6 Foamability measurement

The results of foamability prepared smart-gel hand wash (NH1-NH9) were documented in Table 2. The maximum production of foam were observed for pluronic 127 based smart-gel hand wash and least foam was generated by NH8 & NH9 smart-gel hand wash composed of both HPMC and Pluronic F-127 polymers. It was also observed that less amount of water consumed if the formulation has maximum foamability. Formation of foam was due to 1% SLS surfactant added into the smart-gel hand wash. Foam or lather of surfactants act by reducing the surface tension between the water and oil or sebum on the hand and washed away the dirt or other visible stains.

3.2.7 Clarity measurement

The prepared smart-gel hand wash (NH1-NH9) were assessed for its clarity by measuring % Transmittance as compared to transparent clear products and the results revealed that the smart-gel hand wash (NH4) has highest transmittance and clear solution due to the lowest concentration of Pluronic polymer. However, smart-gel hand wash (NH9) showed the lowest clarity due to the high concentration and combination of HPMC and Pluronic polymer in the ratio of 1:2 respectively. Clarity % transmittance decreased with increased in the solution concentration and polymer ratio due to turbidity formed by micelle formation (Figure 3). From the above physical characterization smart-gel hand wash (NH5) was found to be relatively excellent in all parameters hence considered to be promising for further exploitation in antimicrobial studies.

3.2.8 In vitro anti-microbial activity

The antimicrobial activity of the smart hand wash was tested against bacterial *Staphylococcus aureus* and fungal pathogenic organisms *Candida albicans*. The three dip well in each microorganism petri plate were filled with 100 μL (15 mg/L) of NaCl (F0), optimized smart hand wash (NH5) and MH germ protection based marketed hand wash (Figure 4). The order of zone of inhibition was found to be significantly higher for the developed smart-gel hand wash (NH5) as compared to control (NaCl) and marketed germ protection detergent based marketed product (Table 3). The antibacterial activity of optimized smart-gel hand wash (NH5) probably due to the change

| Microorganisms       | Zone of inhibition (mm) |
|----------------------|-------------------------|
|                      | F0          | NH5          | MH           |
| *Staphylococcus aureus* | 5 ± 1.2   | 50 ± 4.5     | 8 ± 1.9      |
| *Candida albicans*    | 7 ± 1.4    | 38 ± 2.4     | 6 ± 1.2      |
in membrane permeability and degradation of bacterial enzymes [29].

3.2.9 Stability study

The results of colour and pH before and after stability testing were assessed, no significant changes were observed after 1 month of study.

4 Conclusion

In the present study, it can be concluded that the formulation NH5 which is composed of thermoresponsive Pluronic F-127 polymers has shown the excellent pharmaceutical performance and antimicrobial activity, in addition, during hand wash consistency of smart-gel hand wash may be increased that in turns increases the antimicrobial activity, henceforth smart-gel hand wash (NH5) considered to be the best smart-gel hand wash. Hence, it was concluded that the developed smart-gel hand wash benefits from its nano size and found biocompatible, efficacious and cost-effective for sanitizing the hand for day to day activities. AgNPs loaded hand wash in gel form can therefore be good replacement for the conventional hand wash.

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