ORIGINAL ARTICLE

Development and Evaluation of Silver Nanoparticles and its Applications in Topical Drug Delivery Systems

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Abstract

Background: Nanotechnology is finding new applications in the field of pharmaceuticals and various other fields. Increase of the surface area of a drug by reaching to the nano size offers more than one beneficial effects and applications. The same drug molecule in the nano size can work more efficiently, reach the target organ in the desired concentration but in lowered therapeutic dose, with less side effects and thus can give better treatment. This science is explored in wound healing process using silver nanoparticles (SNPs). Silver in its nano size shows better wound healing effect. Nano size sliver can be synthesized by various reduction methods in which silver nitrate is reduced to give silver atoms. Atomic silver enters into wound healing process more efficiently than silver sulfadiazine a well-known silver compound for wound healing. Various reducing agents from chemical origin have been successfully tried for synthesis of SNPs, but these tend to leave their traces behind, and could be toxic in wound healing process. Many phytochemicals have redox potential and are successfully used in creation of metal nanoparticles. In this project SNPs are developed using phytochemicals of turmeric, which is not reported as yet. The in-vitro and in-vivo evaluation of SNPs developed in this project show promising results. **Aim:** To develop Silver Nanoparticles using phytochemicals from turmeric and evaluate these using in-vitro and in-vivo methods. **Method:** In this project, a new method is explored for synthesis of SNPs using hydroalcoholic turmeric exctract and curcumin as reducing agents. Curcumin and other ingredients of turmeric called curcuminoids, owing to their structures (Keto enol moieties) can act as reducing agents. 1 mM $AgNO_3$ was incubated with turmeric extract and curcumin separately for various time intervals. The temperature , time and proportion of reagents was optimized to gets maximum concentration of SNPs.SNP production using glucose as reducing agent was used as standard.Extract of turmeric proved better redox reagent than curcumin alone may be due to presence of other curcuminoids in the extract apart from curcumin. SNPs prepared using turmeric extract were evaluated by physical methods of characterizations such as scanning electron microscopy, Zeta potential, and particle size analysis. 0.02 % SNPs were loaded in 1% carbopol 934p gel and were evaluated for wound healing activity using burn wound model. **Results:** SNPs prepared using turmeric extract were evaluated for particle size analysis, PDI-Polydispersibility index, Zeta potential, SEM . All results indicated formation of SNPs (average particle size 235nm) compared with standard glucose reduction method (average particle size 895nm). Stability study showed no aggregation of SNPs. The in vivo study showed better wound healing activity than standard used namely silver sulphadiazine marketed cream. **Conclusion:** Synthesis of SNPs using turmeric extract and curcumin is a new, green method and not reported yet, as per literature survey done for this project. Successful synthesis and evaluation of SNPs was proved by the in vivo and in-vitro study.

Key words: Curcumin, curcuminoids, silver nanoparticles, turmeric extract, wound healing

INTRODUCTION

Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into their nano size, which drastically changes the chemical, physical, and optical properties of metals. Nanoparticles are the clusters of atoms in the size range of 1-100 nm. Metallic

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Received: 23-07-2014
Revised: 04-04-2015
Accepted: 13-04-2015

silver in the form of silver nanoparticles (SNPs) has made a remarkable comeback as a potential antimicrobial agent. These nanoparticles are most promising as they show good antibacterial properties due to their large surface area to volume ratio.[1]

SNPs were synthesized using an extract of turmeric. Turmeric is a spice derived from the rhizomes of *Curcuma longa*, family (Zingiberaceae). Curcumin, the principal curcuminoid found in turmeric, is generally considered its most active constituent.[2] It has generally used to treat a topical infection like wounds and shows better wound healing activity.[3]

Silver is generally used in the nitrate form to induce antimicrobial effect, but when SNPs are used, there is a huge increase in the surface area available for the microbe to be exposed to. The nano size of the particles also increases the penetration potential of the silver particles hence aiding in better utilization of metal properties.[4] The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins and the SNPs interact with these proteins in the cell as well as with the phosphorus-containing compounds such as DNA. When SNPs enter the bacterial cell, it forms a low molecular weight region in the center of the bacteria leading to cell death. The nanoparticles release silver atoms in the bacterial cells, which enhances their bactericidal activity.

The surface of the body is entirely covered by the skin. It is one of the most extensive and readily accessible organs of the human body. The skin of an average adult body covers a surface area of approximately 2 m^2 and receives about one-third of the blood circulating through the body. The skin can function in these many ways starts with understanding the structure of the three layers of skin - the epidermis, dermis, and subcutaneous tissue.[5]

First, the drug has to pass the delivery system then through stratum corneum, epidermis, and then dermis. Each of these layers has different barrier properties due to their differences in the composition of different layers as shown in Figure 1.

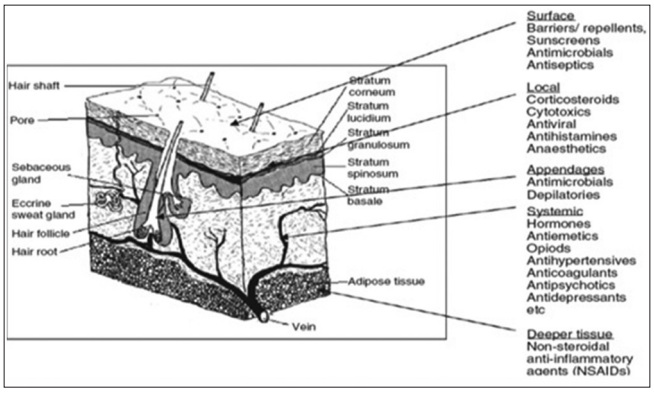

Figure 1: Structure of skin showing the potential targets for cosmetics and drugs[6]

Wound healing

Wound healing is a complex biological process which results in the restoration of tissue integrity. Disruption of the integrity of the skin, mucosal surfaces, or organ tissue results in the formation of a wound. At the time of healing, multiple cellular and extracellular pathways are activated, in a tightly regulated and coordinated fashion, with the aim of restoring tissue integrity. This process of wound healing is divided into four distinct phases; hemostasis, inflammation, proliferation, and tissue remodeling. Lots of cellular and molecular biological studies demonstrated that many cytokines, growth factors, and proteases are closely involved in the wound healing process to complete normal tissue repair after damage.[7,8]

Various factors involved in wound healing to promote the process like nutrition in which vitamin A (involved in epidermal growth) and omega-3 fatty acids(modulate arachidonic acid pathway). Oxygen is important for cell metabolism, especially energy production by means of adenosine triphosphate, and is critical for nearly all wound healing processes. It prevents wounds from infection, induces angiogenesis, increases keratinocyte differentiation, migration, and re-epithelialization, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction. Immunosuppression in which patients with human immunodeficiency virus, cancer, and malnutrition all have a degree of immune suppression which can lead to delayed wound healing. This explains the need for efficient wound healing therapy.[9]

MATERIALS AND METHODS

Glucose (Research Lab Fine Chem.), poly vinyl pyrrolidone (PVP K30) (BASF, Mumbai), and silver nitrate ($AgNO_3$) (Research Lab Fine Chem.) were procured for the preparation of SNPs. Chemical reduction method was used for the preparation of SNPs. Methods reported in research papers and patents were not reproducible and were modified and optimized. The methods used were determined to be optimum after evaluating the prepared nanoparticles for particle size, scanning electron microscopy (SEM), and zeta potential.

Chemical reduction method

Uniform SNPs were obtained by reduction of ($AgNO_3$) at 37°C under atmospheric pressure in an incubator for 48 h. PVP K30 was used as a stabilizer. SNPs synthesized by dissolving $AgNO_3$ (158 mg), and PVP K30 (5 g) were dissolved in 100 ml of 40% (w/w) of glucose syrup. The reaction completion for all the ionic silver to be converted to nanoparticles was indicated by the generation of light orange color.[10]

SNPs prepared using turmeric and curcumin extract

Preparation of turmeric extract

An amount of 30 g of dried powder of turmeric was boiled in 100 ml of distilled water for 30 min. After cooling at room temperature, it was centrifuged at 4000 rpm for 10 min and filtered. The filtrate was stored at 4°C for further experiments. The filtrate used as a reducing agent and stabilizing agent for 1 mM of $AgNO_3$. The same method was used for the preparation of curcumin reagent. This process was adapted from the nanoparticles prepared using Triphala (*Terminalia chebula*) extract and Neem leaf (*Azadirachta indica*) extract.[11] Preparation of SNPs using turmeric extract and curcumin is not reported as yet.

Preparation of SNPs

The extract of turmeric (18.0 ml) was mixed with 50 ml of 1 mM ($AgNO_3$) solution in 1:2.78 ratio in a conical flask under aseptic conditions. The flask was kept in a shaking water bath at 37°C in dark for 48 h. A change in color was observed indicating the formation of SNPs. The same procedure was done for SNPs using curcumin reagent (0.846 g curcumin in 50 mL $AgNO_3$).

CHARACTERIZATION OF SNPS

UV (ultraviolet)-visible spectroscopy

SNPs were characterized by UV-visible spectroscopy monitored by sampling the aqueous component (2.0 ml). The UV-visible spectra of this sample were measured on UV Jasco V-630 spectrometer which showed spectra in range 300-600 nm. Distilled water was used to adjust the baseline.

Particle size determination

Mean particle size and size distribution of SNPs was determined by photon correlation spectroscopy using Nanophox at room temperature. Before measurement, batch was diluted. The SNPs solutions were homogeneous throughout with no sedimentation and were light orange in color. The width of the size distribution was indicated by the polydispersity index (PDI) using following formula-PDI = $(X_{90}-X_{10})/X_{50.}$

SEM

The SNPs which showed higher particle size were subjected to SEM analysis to know about the shape and surface morphology of the SNPs.

Zeta potential determination

The SNPs were subjected to zeta potential analysis to determine the surface charge of the nanoparticles, so as to predict their aggregation behavior.

In-vitro antimicrobial activity

The antimicrobial activity of SNPs was determined by agar plate method. The diameter of the zone of inhibition was measured as antimicrobial efficacy SNPs.

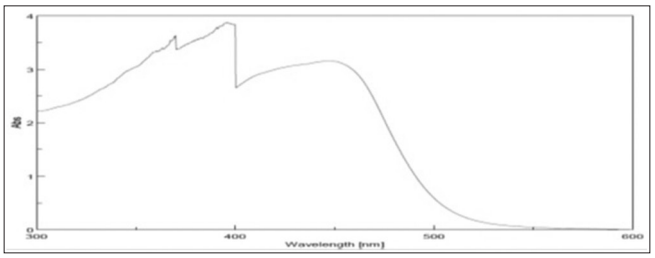

Figure 2: Ultraviolet analysis of silver nanoparticles

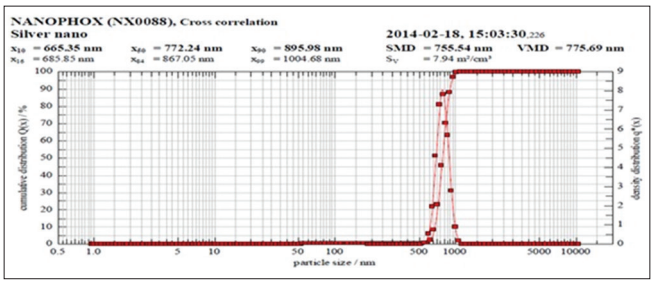

Figure 3: Particle size determination of silver nanoparticles: Glucose and poly vinyl pyrrolidone

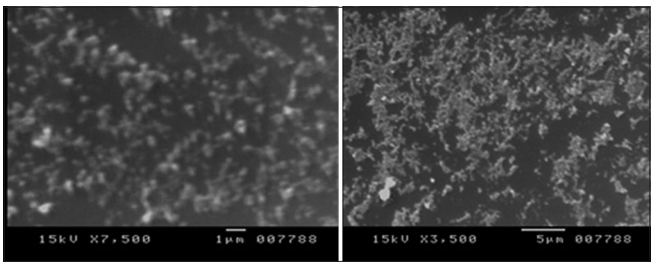

Figure 4: Scanning electron microscopy images of silver nanoparticles: Glucose and poly vinyl pyrrolidone K30

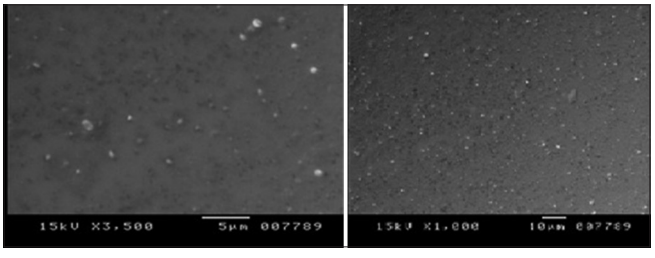

Figure 5: Scanning electron microscopy images of silver nanoparticles: Turmeric

RESULTS AND DISCUSSION

UV-visible spectroscopy

The synthesized SNPs showed the following absorption spectrum at the wavelength range of 300-600 nm. The surface plasmon resonance peak at around 450nm confirmed the formation of SNPs as shown in Figure 2 UV analysis of SNPs.

Particle size determination

The particle size determination is a very important criterion for the evaluation of nanoparticulate systems. Since it is difficult to distinguish between nanoparticles and micro particles, the particle size analysis gives the idea about the nanosizing of the SNPs (Figure 3).

SEM

The SEM analysis results are shown in Figures 4 and 5. These figures clearly reveal that all the SNP were spherical in shape and smooth in nature on the rough background of PVP particles.

Zeta potential determination

Values of Zeta potential showed that prepared SNP have sufficient charge and mobility to inhibit aggregation of nanoparticles.

Zeta potential was found to be positive for glucose+PVP SNPs in Figure 6 and negative for turmeric SNPs in Figure 7 of the

Table 1: Zone of inhibition in cm

Sample	Concentration (mg/ml)	Zone of inhibition (in cm)
SNP	2	3.0
Silver sulfadiazine	2	2.2
$AgNO_3$	2	1.2

$AgNO_3$: Silver nitrate, SNP: Silver nanoparticles

Table 2: Observations of % closure or wound remain

Formulation applied	Observation (wound area in cm^2)				Last day % closure of wound remaining
	0 day	4th day	8th day	12th day	
Test gel	1.8	1.7	1.1	0.8	20
Mar SNG	1.8	1.6	1.0	0.7	16
Mar SSDC	1.9	1.7	1.4	1.1	34
Control (plain carbopol gel)	1.8	2.0	2.2	2.4	100

MAR SSDC: Silver sulphadiazine cream (marketed), Mar SNG: Silver nano gel (marketed)

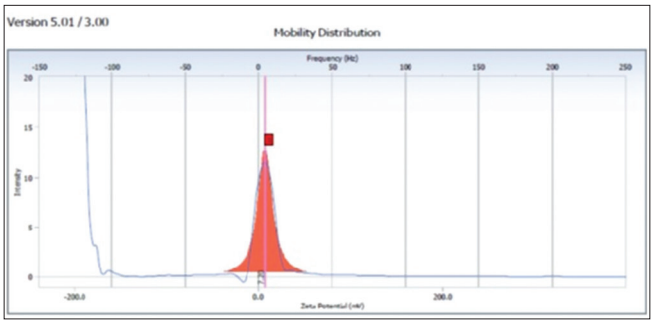

Figure 6: Zeta potential of silver nanoparticles: Glucose and poly vinyl pyrrolidone K30

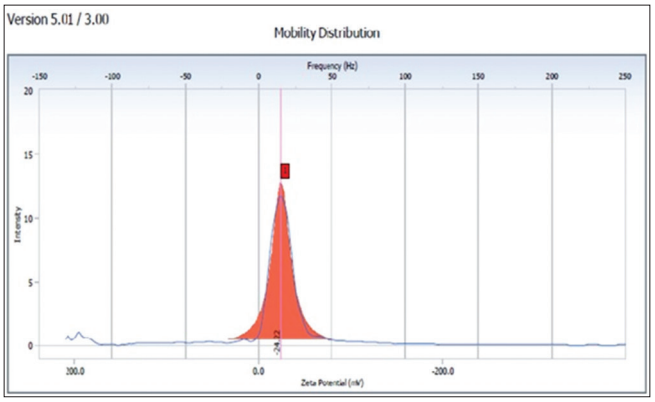

Figure 7: Zeta potential of silver nanoparticles: Turmeric

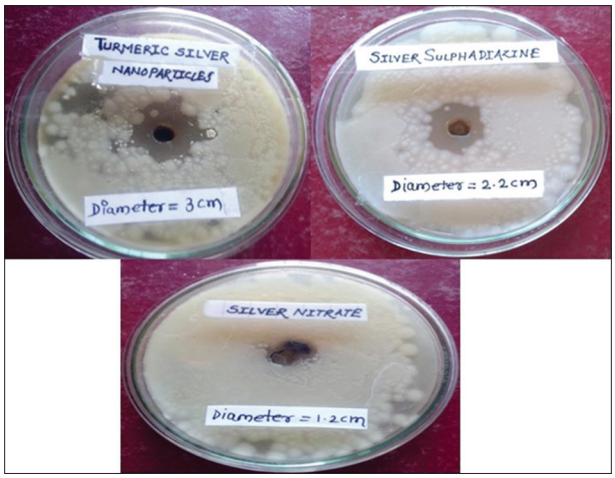

Figure 8: Zone of Inhibition of silver nanoparticles: Turmeric and silver sulphadiazine plain drug and silver nitrate

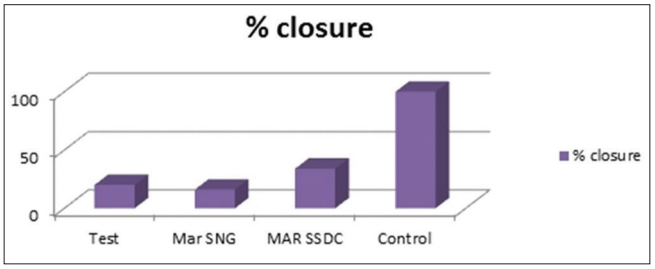

Figure 9: Graph: Percentage closure of wound and formulations used for wound healing

optimized batch. Since the Zeta potential of nanoparticulate suspension was found to be negative, it automatically reveals the stability of nanoparticles against aggregation potential. The repulsion of negatively charged particles leads to behave as separate entities in a nanoparticulate suspension.

Determination of *in-vitro* antimicrobial activity

The SNP was tested for their antimicrobial property against gram positive *Staphylococcus aureus*. The results of the antimicrobial activity are shown in Table 1. The antimicrobial effect of SNP prepared using turmeric was found to be more prominent than the effect of plain silver sulfadiazine drug showed in Figure 8. Zone of inhibition of SNPs: Turmeric and silver sulfadiazine plain drug and ($AgNO_3$).

Determination of *in vivo* animal wound healing study

Percentage closure or wound contraction

The percentage of wound contraction in test, standard, and control was measured showed in Table 2 and Figure 9.

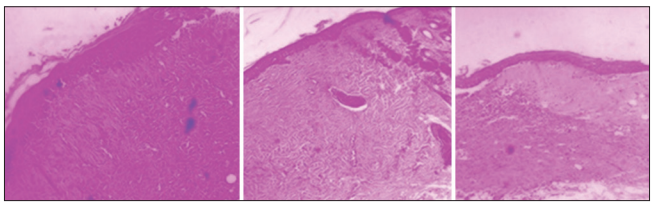

Figure 10: Hematoxylin and eosin staining of granulation tissue on test formulation (silver nanoparticles: turmeric gel) on 12th day

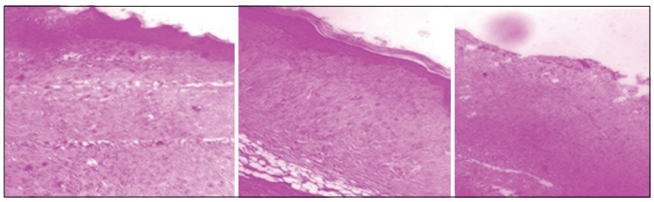

Figure 11: Hematoxylin and eosin staining of granulation tissue on standard group (marketed silver nano gel formulation) on 12th day

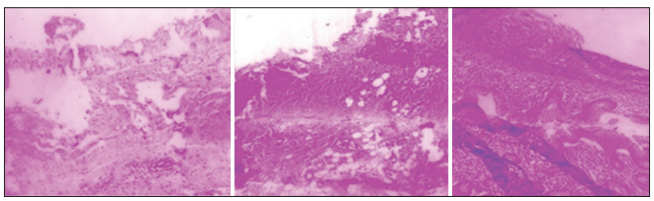

Figure 12: Hematoxylin and eosin staining of granulation tissue on control group (plain carbopol gel formulation) on 12th day

The degree of wound healing was calculated as percentage closure in wound area from original wound area using formula:

$$\text{Percentage closure} = 1 - AD/AO \times 100$$

Where:
AO - Wound area on day 0
AD - Wound area on corresponding days

Histopathological studies

Histopathological studies were carried out on sections from regenerated tissues. The sections were observed under a light microscope for re-epithelialization and granulation tissue thickness. Hematoxylin and eosin stained sections of the epidermal layer were examined for cellular infiltration, neo vascularization, and epithelial regeneration. A well-advanced organization of granulation tissue and on-going epithelization was observed in treated rats than control on the 8th day as shown in Figures 10 and 11. Complete epitheliazation, vascularization, and hair follicles were observed in treated rats on day 12, whereas the organization of granulation tissue was in progress in the case of control showed in Figure 12.

CONCLUSION

From the present study, it can be concluded that the preparation of SNPs: Turmeric using chemical reduction method proved to be a new and successful approach to obtain stable SNPs. The optimized batch of SNPs showed optimum particle size of nanoparticles. SEM study revealed the formation of nanoparticles with spherical and smooth nature on the rough background of PVP particles. Zeta potential analysis result reveals the stability of optimized SNPs formulation against aggregation.

Thus, SNPs proved the potential for topical delivery over the conventional formulations. Preparation of SNPs using turmeric and curcumin is not yet reported, and thus, it may be concluded that a new green method of synthesis of SNPs is devised in this project. Further it can be concluded that topical drug delivery system for burn wound healing using SNPs prepared by the new method has been successfully developed.

ACKNOWLEDGMENT

The authors acknowledge Sinhgad Institute of Pharmacy Narhe, Pune for analysis of particle size distribution and zeta potential, Diya labs, Mumbai for SEM analysis, Plus Pathology Lab, Pune for Histopathology analysis and Sinhgad

Technical Education Society and Principal, Dr. S. D. Sawant of STES's Shrimati Kashibai Navale College of Pharmacy Kondhwa, Pune for providing the facilities to carry out this research work.

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Source of Support: Nil. **Conflict of Interest:** None declared.