Silver Nanoparticles For Cosmetic Use: Synthesis, Characterization, Antimicrobial Properties And Preservative Challenge Test In formulation

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Abstract - Introduction: ***This article describes new research investigating in the manufacturing of nanoparticles in laboratory, characterization, testing for their properties particularly in relation to microbial properties and formulating Silver nanoparticle ointment. This Research shows some of the most exciting research undertaken currently and fits within a dynamic research program, which is global in scope and which attempts to unravel these complex areas.***

Method: silver nanoparticles were prepared by reducing silver nitrate with oxalic acid. Formulated silver nanoparticles were first characterized then tested for its antimicrobial properties and ointment formulation.

Results: The Silver Nanoparticles were characterized by UV-visible spectrophotometer, particle size analyzer (DLS), scanning electron microscopy (SEM) and transmission electron microscope (TEM). Silver nanoparticles obtained showed significantly higher antimicrobial activities against Escherichia coli (E. coli), S. aureus and P.acne in comparison with tetracycline. Silver nanoparticle ointment was prepared without preservative as silver nanoparticles itself acts as preservative.

Conclusion: Prepared Silver Nanoparticles showed that the nanoparticles possess antimicrobial properties and forms a stable ointment. Thus these nanoparticles can be used in preventing bacterial contamination in cosmetic preparation as these nanoparticles act as a preventive method for microbial contamination. Therefore we can say that these Silver nanoparticles can be used as a preservative in cosmetic preparation.

Keywords: Silver Nanoparticles, SEM, TEM, DLS, antimicrobial, TPC and preservative.

I. INTRODUCTION:

The long history of silver and its use by humans provides a consolidated knowledge about its effects on the environment and human health. Silver as a substance is considered as a nontoxic for humans.[1] Silver is mostly used because of its antibacterial activity in medical and consumer products for its broad range of activity and lower toxicity compared to other bactericides.[2] Silver Nanoparticles are widely applied nowadays in shampoos, soaps, detergents, cosmetics, toothpaste as well as in medical and pharmaceutical products and are hence directly encountered by human systems. [3] The broad spectrum of silver nanoparticles which includes microorganisms in general, as Gram-positive and Gram-negative bacteria, filamentous fungi, yeasts, and viruses. Its most prominent property is to have a large surface area. The antifungal activity of silver nanoparticles was also reported by several researchers.[4][5] However, despite its efficiency already well known, the mechanism of action of silver nanoparticles is not well understood yet. [6] The inhibitory effect of silver nanoparticles on bacteria has been proposed to explain the mechanism action of it. The high affinity of silver towards sulfur and phosphorus is assumed to be the key element of the antimicrobial effect. Silver nanoparticles can react with sulfur-containing amino acids inside or outside the cell membrane because of sulfur-containing proteins on the bacterial cell membrane in abundance, which in turn affects bacterial cell viability. [7] The growth of Gram-positive B. subtilis bacteria is inhibited by Silver nanoparticles, and exerting toxicity by damaging cellular membranes, lowering reductase activity, degrading chromosomal DNA, and reducing protein expression.[8] The protective effect of the cell wall is determined by the ability to reject the stain (in gram negative cells). It does not retain the stain because of the thin peptidoglycan layer in gram-negative cells which is located between the inner cytoplasmic membrane and the outer membrane of the bacteria. The interest in developing new antibacterial systems which are more resistant to antibiotics has been channeled through gram-negative bacteria. The permeability of the bacterial wall using Silver Nanoparticles, especially for gram-negative types has been reported. The nanoparticles adhere and accumulated to the cell surface, causing changes in its structure. The ability of Silver Nanoparticles to penetrate the bacterial membrane increases with reduced diameter. The nanoparticle shape and the quantity are the other factors influencing this property. [9]

Nanoparticle technology is increasingly used in the cosmetics industry, in items ranging from sun cream to skin care products. In the field of cosmetics, they are used for a variety of purposes including reflecting light, as preservatives and to deliver active ingredients through several layers of skin. The silver nanoparticles are also used for wound dressings and other medicinal purposes.

[10] There are various methods for synthesis of silver nanoparticles including chemical [11], electrochemical [12], photochemical [13], Microwave irradiation [14] etc [15]. Although green synthesis method is widely used in a research laboratory for the preparation of silver nanoparticles but the chemical method is also extensively used in the industry and mostly preferred. The beauty industry is another sector in which nanotechnology is used. It is very important to protect the products against microbial contamination in the field of cosmetic, which may occur during production of cosmetics or during their storage. Before the use of nanotechnology in the cosmetic industry, organic compounds such as parabens and phenoxyethanol had been used to control unwanted microbial flora. Studies revealed the irritant effects of these types of preservatives, especially parabens, in relation to the epidermis. What is more, their increased susceptibility to UV light has been confirmed. The harmful preservatives have been partially replaced by metal nanoparticles, in particular, silver nanoparticles. [16] Silver also possesses healing properties apart from its anti-bacterial feature. The skin regenerating function of dermis cells is activated thus speeding up wound recovery. Silver also helps in the formation of a more even structure of the skin fiber cells, minimizing the formation of uneven scars and smoothing the skin surface. [17] This study reports the successful synthesis of silver nanoparticles by using a chemical method where oxalic acid was used as reducing agent and CTAB as a stabilizing agent. [18] In this study we have developed a convenient method for synthesis of silver nanoparticles from silver nitrate by using a different concentration of oxalic acid to get the different size of silver nanoparticles. Further, in this study, synthesized silver nanoparticles were characterized and studied in details for their respective properties.

The antimicrobial properties of synthesized silver nanoparticles were evaluated by the well diffusion method and this synthesized silver nanoparticle was used in ointment to compare for its preservative activity. Thus silver nanoparticles can be used to prevent the bacterial contamination in cosmetic preparation, especially for dermal use and can also be used as a preservative in cosmetic formulation. Along with its antimicrobial properties it can also be used for speeding up the skin regeneration as it has got the healing properties.

II. EXPERIMENTATION:

SYNTHESIS OF SILVER NANOPARTICLES:

Silver Nanoparticles were prepared by using oxalic acid as a reducing agent, for reducing silver nitrate in presence of CTAB, which were used as a stabilizing agent. Six solutions were prepared. First, three solutions were used to get Silver nanoparticles of 100 nm and last three solutions were used to get the 200 nm size of silver nanoparticles.

0.03 gm of silver nitrate was dissolved in 15 ml Distilled water.

0.03 gm of CTAB was dissolved in 30 ml Distilled water.

0.15 gm of oxalic acid was dissolved in 30 ml Distilled water.

0.03 gm of silver nitrate was dissolved in 15 ml Distilled water.

0.06 gm of CTAB was dissolved in 30 ml Distilled water.

0.20 gm of oxalic acid was dissolved in 30 ml Distilled water.

The solution of (CTAB) was added dropwise to the solution of silver nitrate. Followed by Oxalic acid solution was added dropwise to the solution of CTAB and silver nitrate. After mixing oxalic acid solution, the colour of solution tends to change from colourless to light yellow colour. Finally, the colours of the result solutions become dark yellow.

UV- VISIBLE ANALYSIS:

The blackish brown coloured sample powder of prepared Silver Nanoparticles was dissolved in deionized water and sonicated. Then this solution was taken in cuvette and exposed to UV-visible radiation and the absorbance of the solution was recorded (Beckman – model No. DU – 50, fullerton, CA, USA). Because of the surface plasmon resonance phenomena resonant peak occurs at different wavelength for different nanoparticles solution and as per the theory of resonance maximum wavelength is absorbed at resonant wavelength.

SEM ANALYSIS:

In this research work, Scanning Electron Microscopic (SEM) analysis was done using [SEM – JEOL 6380 A] machine (microscope) to characterized particle size and morphology. The powdered silver nanoparticles were sonicated with alcohol. Thin films of the sample were prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid, extra sample was removed using a blower and then the film on the SEM grid were coated with palladium layer in auto fine coater coating unit [JEOL JFC-1600] to make the sample conductive. The accelerating voltage of microscope was kept in the range 15 kV.

Note: although the silver is a good conductor but at nanosize, there are changes of oxide formation to avoid the oxidation silver was coated with palladium layer.

TEM ANALYSIS:

The size of synthesis Silver nanoparticles was determined by transmission electron microscopy (TEM). TEM analysis was done using [Philips model CM 200]. Before analyzing the powdered sample of prepared silver nanoparticles were sonicated with water for 10 minutes, and then a drop of diluted sample was placed on grid coated with carbon. A liquid was allowed to evaporate at room temperature. Operating voltage for testing was 20-200 kV with Resolution 2.4 AO.

ZETA POTENTIAL MEASUREMENTS:

The long-term stability of colloidal Silver nanoparticles was monitored spectroscopically by measuring zeta potential, which indicates the changes in surface charge with time. Such method is mostly used to control the stability of colloidal metal nanoparticles. A large positive or negative zeta potential of the metal nanoparticles tend to repel each other and they do not show any disposition to come together.[19] The experiment was carried out in computer controlled analyzer [Zetasizer Ver. 6.32 Serial Number: MAL1068498]

PARTICLE SIZE DISTRIBUTION:

In order to find out the particles size distribution, the Silver nanopowder was dispersed in water by an ultrasonic processor. Then experiment was carried out in computer controlled particle size analyzer [Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0011 RC1] to find out the particles size distribution.

EVALUATION OF ANTIMICROBIAL ACTIVITY:

The synthesized Silver nanoparticles were tested for anti-bactericidal activity by agar well diffusion method [20] against both Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli and Propionibacterium acnes. The dilution of the microbial culture were added to each medium and mixed thoroughly. The seeded media so prepared were poured into Petri dishes. The dishes were immediately covered and were allowed to solidify. All operation was carried out in laminar flow. After the media solidified in the prepared Petri dishes, small cups (4-5) of diameter Wells of 5 mm were punched into nutrient agar plates using sterilized cork board. By using a micropipette, nanoparticle (20, 40, 60 and 80 µg/ml) solution were poured into each well on all plates and tetracycline as control in one of the well. Plates were then incubated at 35±2°C for 24 h and the level of zone of inhibition of bacterial growth was measured [21]

FORMULATION OF OINTMENT BASE:

Ointments are soft, semisolid dermatological preparations used for application to skin for therapeutic and protective action. Ointments are formulated to deliver drug into the skin for treating dermal disorders, with the skin as the target organ.

HYDROPHILIC OINTMENT BASES:

Water-soluble bases do not contain oleaginous components. They are mostly formulated from (polyethylene glycols). They are completely water washable and are greaseless. They soften greatly with addition of water and thus hydrophilic ointment base was selected to use for the study.

ADVANTAGES

a. Water solubility: Easily removed from skin.
b. Good absorption by the skin: As water soluble easily penetrates through skin for the drugs which are poorly soluble.
c. Good solvent properties.
d. Freedom from greasiness.
e. Compatible with many dermatological medicaments

PROCEDURE FOR PREPARATION:

a. Stearyl alcohol and white petrolatum was melted on a hot plate.
b. This mixture was heated to 70°C.
c. Remaining ingredients was dissolved in water and solution was heated to 70° C.
d. Oleaginous phase was added slowly to the aqueous phase, and stirred constantly.
e. Solution was removed from heat and stirred the mixture until it congeals.[22]

ANALYSIS OF FINISHED PRODUCTS:

The analysis of finished products was carried out by fallowing methods [23],

1) Physical method

2) Microbiological method

1. PHYSICAL METHOD:

APPEARANCE:

Appearance of the ointment was determined by the visual observation for texture and clarity.

pH :

The pH value represents the acidity or alkalinity of the solution. The pH determination of ointment was carried

out at temperature 27 ^{0} C. pH of the ointment was determined in the laboratory using digital pH meter.[24]

CENTRIFUGE TESTING:

The dispersed phase (of an oil-in-water ointment) has a tendency to separate and rise to the top of the emulsion forming a layer of oil droplets. This phenomenon is called creaming. Creaming is one of the first signs of impending ointment instability and should be taken seriously. This test method is mostly used to determine the accelerated deterioration of ointment. For which 6 gm of ointment was heated to 50 ^{0} C (122F) and was filled in 10 ml graduated centrifuge tube and centrifuged for thirty minutes at 3000 rpm. This was then observed the resultant product for signs of creaming. [25]

2. MICROBIOLOGICAL METHOD:

TOTAL PLATE COUNT OF THE PREPARED OINTMENT:

The microbial analysis of ointment was carried out to determine that the ointment is within the limits according to the BIS specification of skin cream. So that it should not cause any microbial infection to the skin, therefore the microbial analysis for TPC is carried out. The man interest to find the total plate count was to check whether silver nanoparticle can act as preservative in the formulation. This test was done after 6 month of ointment preparation stored at normal temperature 25 ^{0} C/ 60% RH where no phase separation was observed even after 6 month of storage.

PROCEDURE:

1. A pre-weight amount of the sample approximately 1 – 10 gms was transferred to 9mL of water. Mix it thoroughly and let it stand for 5 min.

2. With the sterile pipettes 1mL of the above solution was dispensed into sterile plates.

3. Nutrient agar was transferred into one plate and Sabourataud dextrose agar was transferred into another plate by shaking gently.

4. After the agar solidifies the plates were inverted and were incubated at 33+2 ^{0} C for 48h in the case of nutrient agar for bacterial enumeration and 28+2 ^{0} C for 3-5 days in the case of Sabourataud dextrose agar.

5. Plates were then counted for the number of colonies formed in it with the help of colony counter.

6. Average was then taken out and total pate count was then calculated with dilution factor. [26]

Formula:

TPC = P1+P2 /2 X 90/W

P1 – Total plate count for the count

P2 - Total plate count for the second plate

W – Weight of the sample taken

III. RESULT AND DISCUSSION

SYNTHESIS OF SILVER NANOPARTICLES:

Silver nanoparticle was synthesized according to the protocol discussed in the ‘Material and Methods which exhibit yellow color in aqueous solution due to the excitation of surface Plasmon resonance. On mixing the oxalic acid to the aqueous solution of Silver ion complex, a change in the color from colorless to yellow and dark brown was observed. It was due to the reduction of Silver ions which indicates the formation of Silver Nanoparticles. [27] Moreover, the slide color changes occurred could be due to the difference in the relative activity in the reduction of silver nitrate ions to metal nanoparticles. [28]

After complete addition of solution, a sufficient amount of the precipitate was observed after a week and it was separated by centrifugation. The separated solid mass was washed. After complete washing, the solid mass was kept for drying. The complete drying of this solid mass resulted in a gray black colored material as showed in figure which was powdered and sampled for characterization purpose. [29]

FIGURE 1: synthesized silver nanoparticles in powder from.

SILVER NANOPARTICLES CHARACTERIZATION V-VISIBLE ANALYSIS:

SPR was observed for the sample solution to occur at the wavelength of 421 nm which confirms the presence of Silver nanoparticles in the prepared solution. The absorbance of surface Plasmon resonance is sensitive to the nature, size, and shape of particles present in the solution and also it depends upon the surrounding media and the inner particle distance. It is reported in the literature that typical Silver Nanoparticles shows the characteristic SPR at the wavelength in the range of 200-600 nm [30],[31]

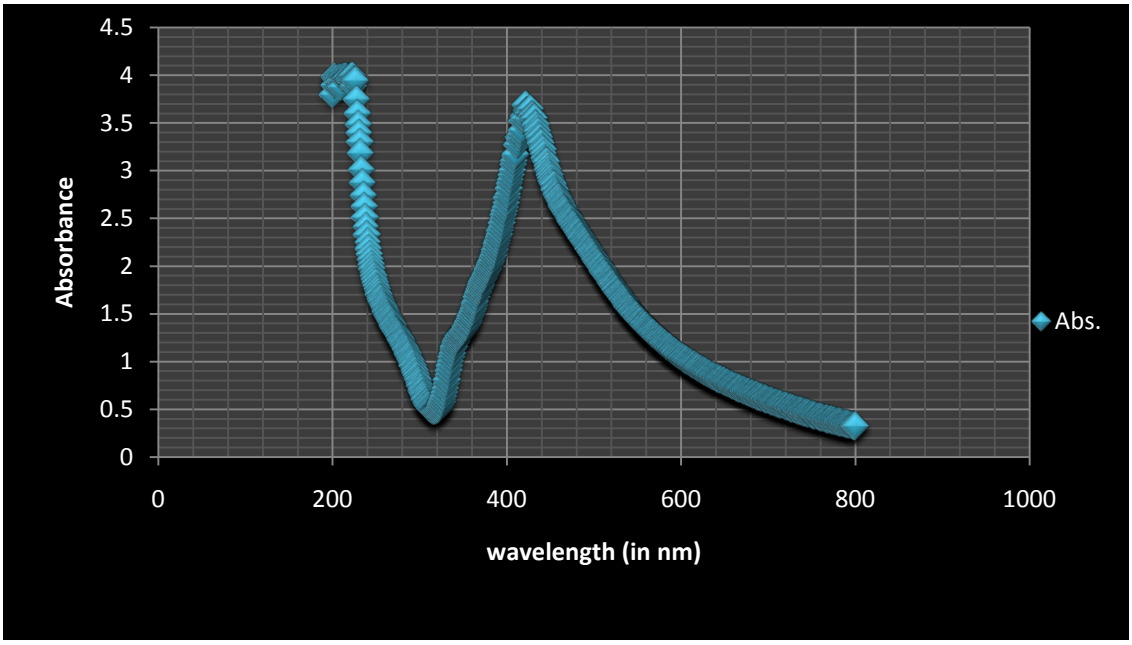

FIGURE 2: shows the SPR for prepared silver nanoparticles sample solution between wavelength of 200 nm and 600 nm.

SEM ANALYSIS:

The SEM image of silver nanoparticles is shown in Figure 3. Clearly, indicates that, in the room temperature synthesized samples are roughly spherical and cuboidal in shape.

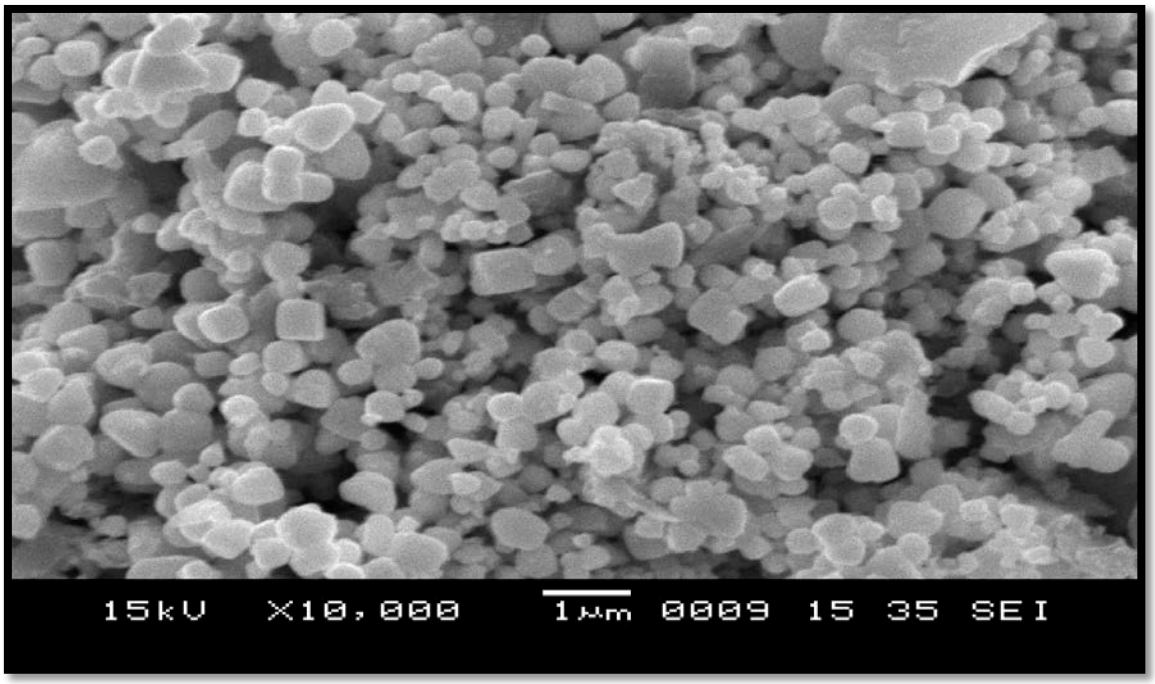

FIGURE 3: Shows the SEM images of prepared silver nanoparticles which are in somewhat spherical and cuboidal in shape.

TEM ANALYSIS:

To understand and to provide more information about the size of silver nanoparticles, TEM investigation was conducted. TEM image shown in figure 4 and 5 of prepared silver nanoparticles indicates that its size ranges brtween 100 and 200 nm in the preparation and most of the silver nanoparticles are roughly Spherical in shape.

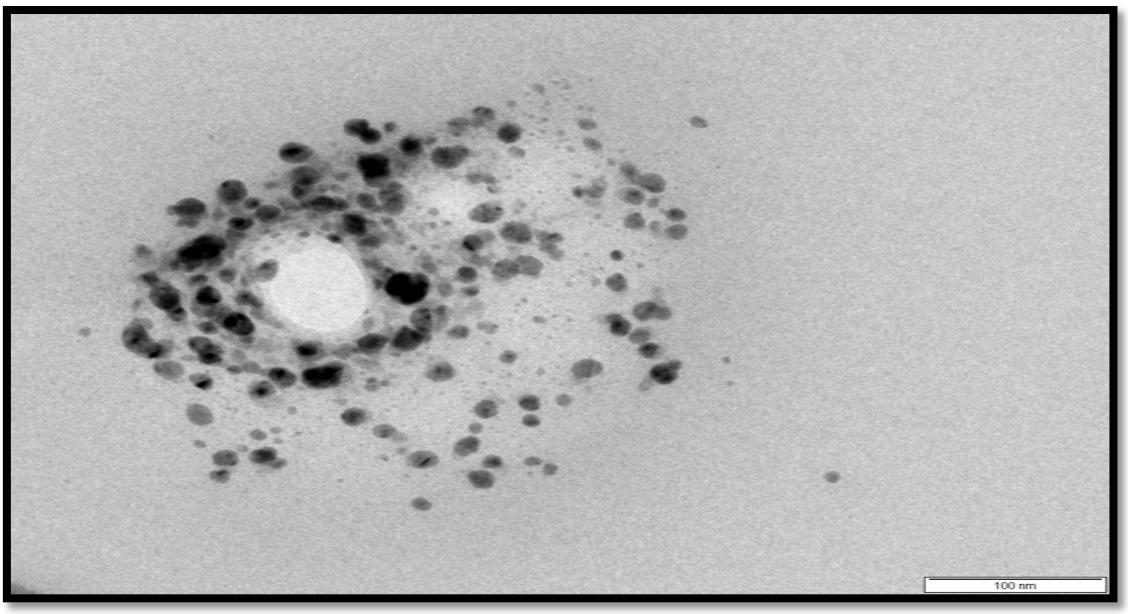

FIGURE 4: TEM images of prepared silver nanoparticles ranging 100 nm was obtained from Sophisticated Analytical Instrument Facility (SAIF) IIT Bombay.

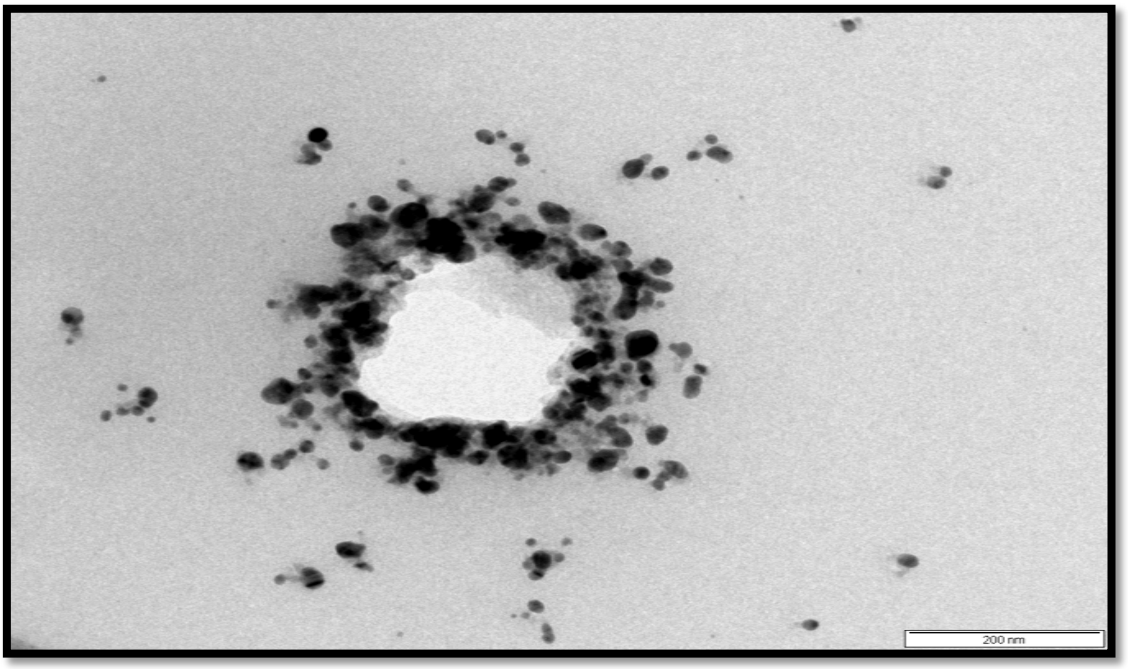

FIGURE 5: TEM images of prepared silver nanoparticles ranging 200 nm was obtained from Sophisticated Analytical Instrument Facility (SAIF) IIT Bombay.

ZETA POTENTIAL MEASUREMENT:

Zeta potential can be used as one of the indicators for the stability of the prepared Silver Nanoparticles. The change in surface charge with time indicates long-term stability of silver nanoparticles which was monitored by zeta potential, which is widely used to control the nanoparticles stability. The zeta potential magnitude gives an indication of the potential stability of colloid. Different studies relate the quantum of zeta potential with the stability of nanopartical colloid. Values slightly vary in case of metal nanoparticles. It should be noted that the particles with zeta potential values more positive than +30mV or more negative than −30mV are considered to be highly stable. [32]. The large

positive and large negative zeta potential of metal nanoparticles tends to repel each other which do not show any aggregation and flocculation preventing agglomeration. The Zeta potential value that ranges from ±0-10 mV shows a highly unstable colloid, with ZP value of ±10 – 20 mV, ±20 - 30 mV and > ± 30 mV shows relatively, moderately, and highly stable colloid in the respective order gives the information on how stable the prepared particles are.[33] Figure 6 and 7 shows that the zeta potential of prepared silver nanoparticles is -22.9 mV and -21.0 mV for 100 and 200 nm respectively. These values of zeta potential indicate a moderate stability of the nanoparticles, which could be due to the production of nanoparticles using CTAB which lead to the stability of nanoparticles.

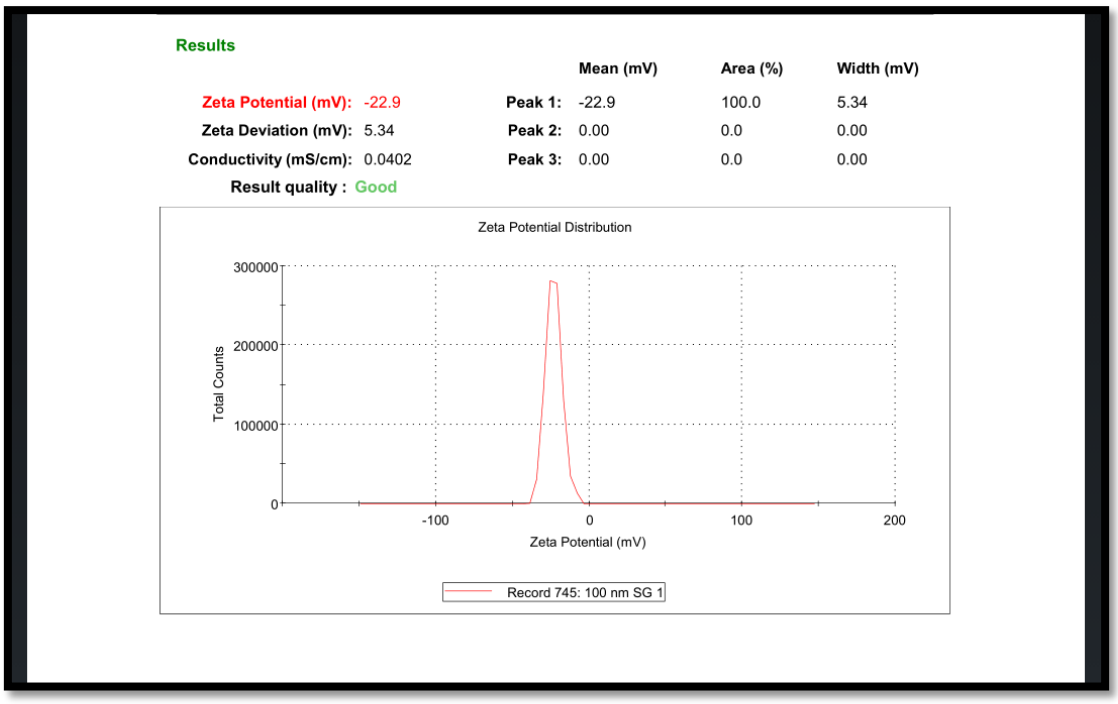

FIGURE 6: Image of zeta potential of prepared silver nanoparticles (100 nm) was obtained from Sant Gadge Baba Amravati University, Amravati.

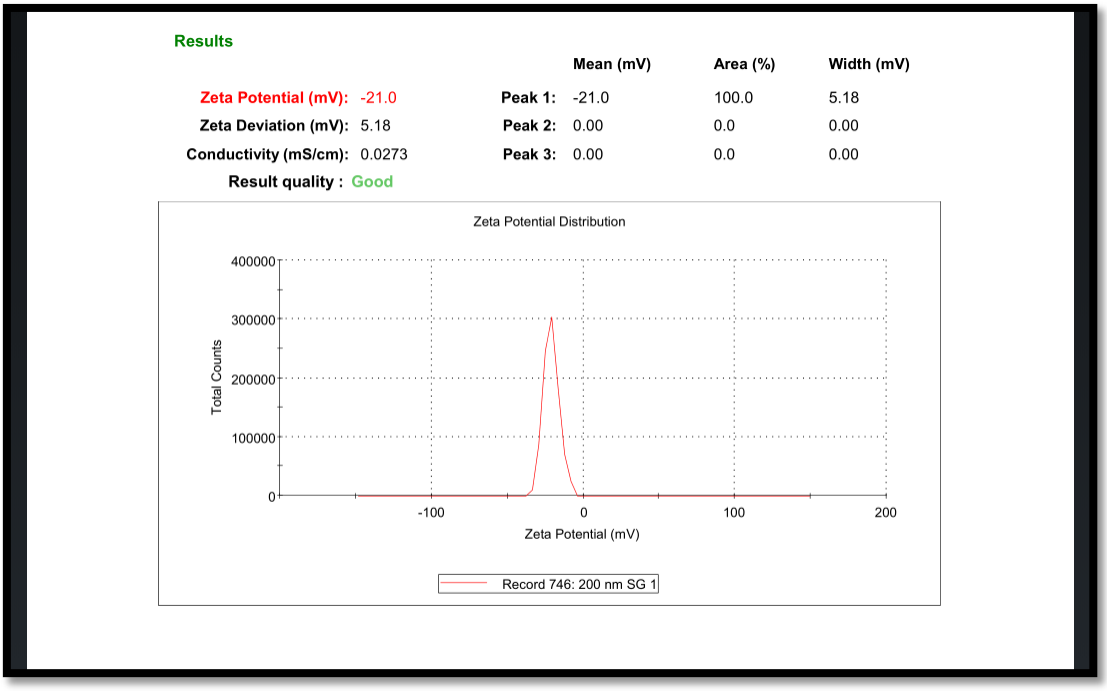

FIGURE 7: Image of zeta potential of prepared silver nanoparticles (200 nm) was obtained from Sant Gadge Baba Amravati University, Amravati

PARTICLE SIZE DISTRIBUTION:

The Figure 8 and 9 shows the particle size of the Silver nanoparticles samples. After analyzing data, it was found that most Silver nanoparticle have size in the range of 40–100nm and 100–200 nm. However, very few have below 40 and above 200 nm range. The highest fraction of Silver nanoparticels present in the solution was of 60nm and 150 nm respectively. From the graph we can say that the powder consist of nanoparticles having various sizes which are indeed in agreement of the result obtained by SEM analysis and TEM analysis.

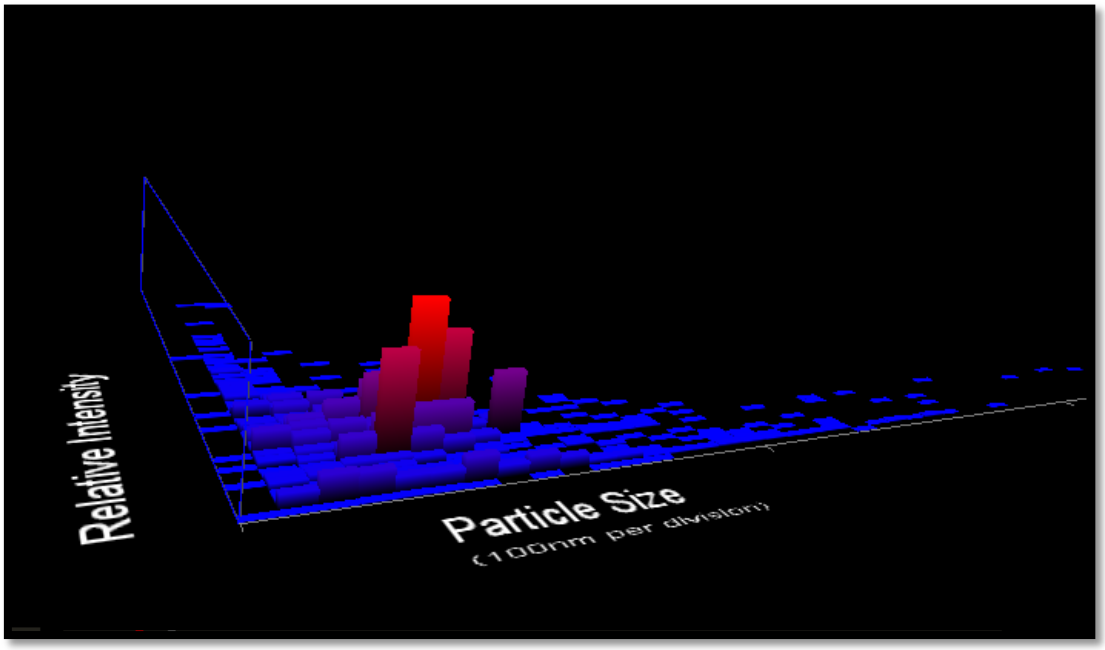

FIGURE 8: Image showing the distribution of particle size 100 nm was obtained from Sant Gadge Baba Amravati University, Amravati.

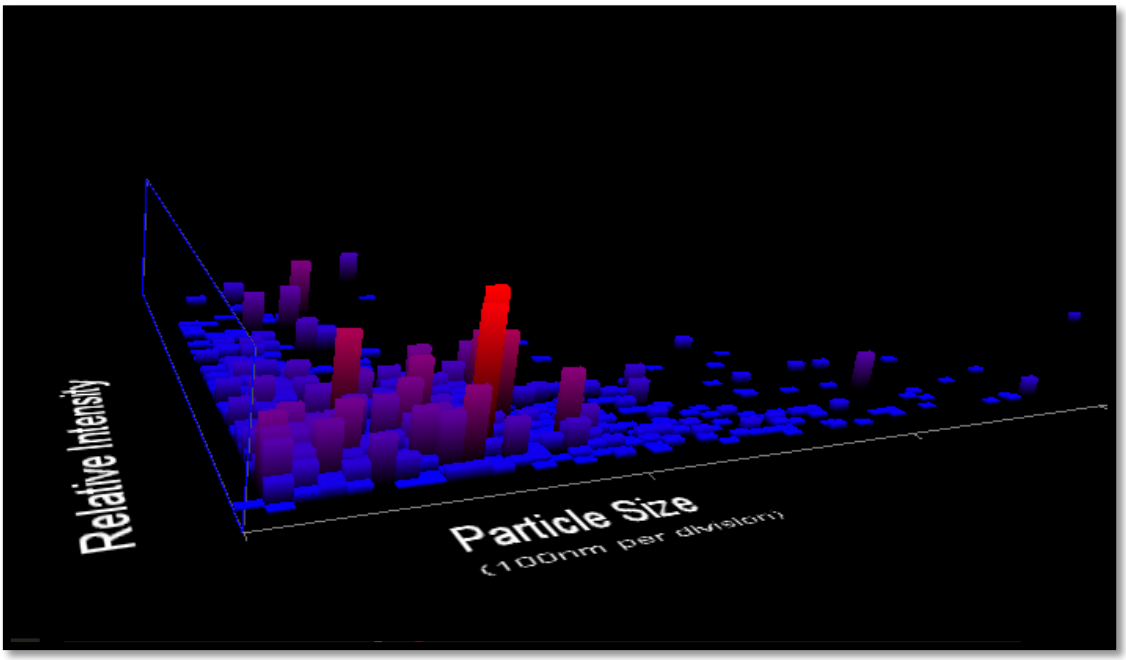

FIGURE 9: Image showing the distribution of particle size 200 nm was obtained from Sant Gadge Baba Amravati University, Amravati

EVALUATION OF ANTIMICROBIAL ACTIVITY:

In this study, the antimicrobial property of silver nanoparticles was examined by agar well diffusion method.[34] The area of the zone of inhibition observed was due to antimicrobial properties of silver nanoparticles rather than to a solvent effect. Tetracycline was used as the reference (positive control) for bacteria E.Coli, S.aureus and

P.acne respectively. Result obtained in the previous studies [35],[36] also gives the potential of silver nanoparticles as antimicrobial. In this study results indicate that the highest zone of inhibition was induced against P.acne and the least against E.coli. The inhibitory effect of silver on microorganisms is partially known. The electrostatic interaction is the reason for antimicrobial activity as the silver ion having a positive charge which attracts the cell membrane of microorganisms which are negatively charged. Thus the silver nanoparticles can easily penetrate the nuclear content of bacteria because of the greater surface area and their unique size. The susceptibility of the microorganism against compound thus followed the sequence: Escherichia coli< Staphylococcus aureus< P.acne.

It is important to note that the diameter of zone for all studied microorganisms are larger in case of samples of silver nanoparticles ranging in 100 nm than corresponding values for 200 nm, showing that smaller nanoparticles have strong antimicrobial activity.

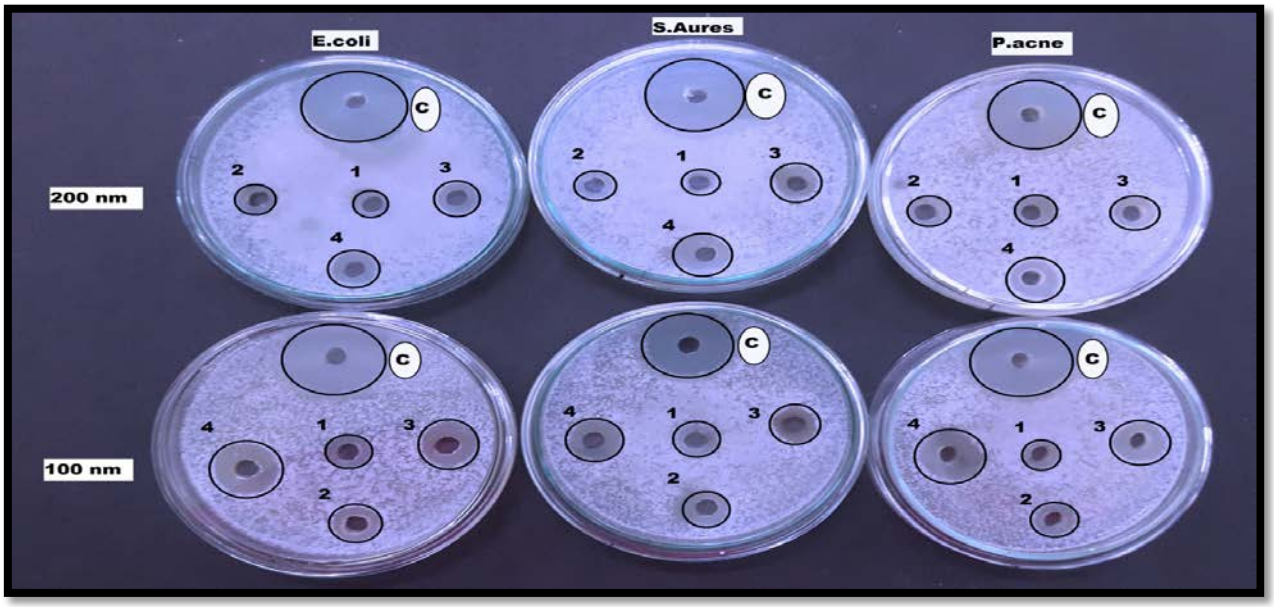

FIGURE 10: Image showing zone of inhibitions of 200 and 100 nm

TABLES 1: Silver nanoparticels have shown prominent antimicrobial activity.

Bioactive agent		**Zone of inhibition (Diameter, mm)**					
			E. coli		**S. aureus**		**P. acne**	
Silver nanoparticles 100/200nm		**µg/ml**	200nm	100 nm	200 nm	100 nm	200 nm	100 nm
		20	10mm	13mm	11mm	14mm	12mm	12mm
		40	12mm	14mm	11mm	15mm	12mm	14mm
		60	14mm	20mm	15mm	19mm	13mm	21mm
		80	15mm	24mm	16mm	23mm	17mm	25mm
		Control	32mm	32mm	35mm	31mm	31mm	32mm

FORMULATION AND DEVELOPMENT OF OINTMENT:

SELECTION OF BASE WITH ACTIVE IS AS FOLLOWS:

TABLE 2: Selection and incorporation of active into base.

Ingredients	**F1 (%)**	**F2 (%)**	**F3 (%)**	**Final (%)**	**Uses**
Oil Phase					
Steryl Alcohol	25	15	15	15	Emollient, emulsifier and thickener.
White petrolatum	25	20	20	20	Oil base of o/w emulsion
Isopropyl myristate			4	4	Imparts velvety emollience to products

Water Phase					
Sodium lauryl sulfate	1	1	1	1	Emulsifying agent easily removed from the skin
propylene glycol	12	6	6	6	increased viscosity
Water	37	58	54	53.5	Solvent.
Active (silver nanoparticle)				0.5	Antimicrobial and healing agent

Note: Preservatives are not added as Silver Nanoparticles itself acts as a preservative.

MODIFICATION AND OBSERVATIONS:

First base formulated was very hard thus water was increased and propylene glycol percentage was reduced but in second formulation appearance was dull thus Isopropyl myristate was added in third formulation to give velvety appearance to the ointment and was finalized, for final formulation 0.5% active silver Nanoparticle was added by reducing water quantity by 0.5% percent.

FIGURE 11: Prepared Silver Nanoparticle Ointment.

ANALYSIS OF FINISHED PRODUCT:

APPEARANCE:

Prepared ointment was slight gray in color due to the black color of the active. Texture was velvety and shiny due to Isopropyl myristate along with the semisolid creamy consistency.

pH:

pH of the ointment was determined by digital pH meter and was found to be 5.12 initially and after 3 month was found to be 5.64 and after 6 month it was found to be 5.82. When the ointment was stored at 25 ^{0} C/ 60% RH. pH of the ointment stored at 40 ^{0} C/ 75% RH was found to be 5.12 initially, 5.31 in first month, 5.87 in second month, 6.09 in three month. After third month pH was not determined as phase separation was observed in the end of fourth month. pH of ointment stored at 5 ^{0} c was 5.12 initially, 5.61 in third month, and 5.73 in sixth month.

CENTRIFUGE TESTING:

In this test ointment was found to be stable as no phase separation was observed when stored at 25 ^{0} C/ 60% RH even after 6 month. Whereas the ointment stored at 45 ^{0} C/ 75% RH phase separation was observed after 3 month hence further tested was not done. No phase separation was observed when ointment stored at 5 ^{0} c. OTAL PLATE COUNT:

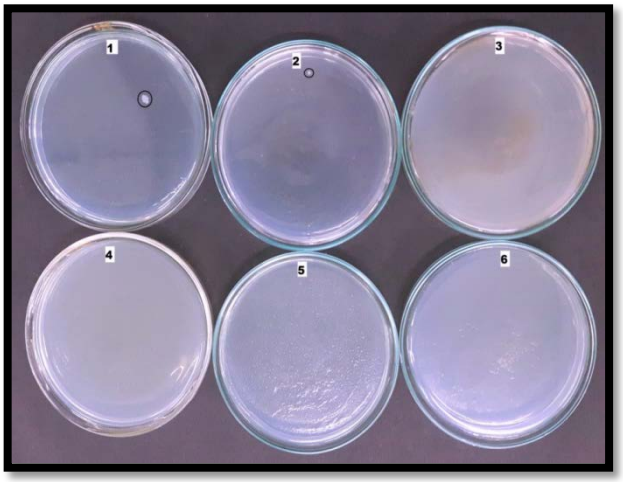

FIGURE 12: Nutrient Agar plate.

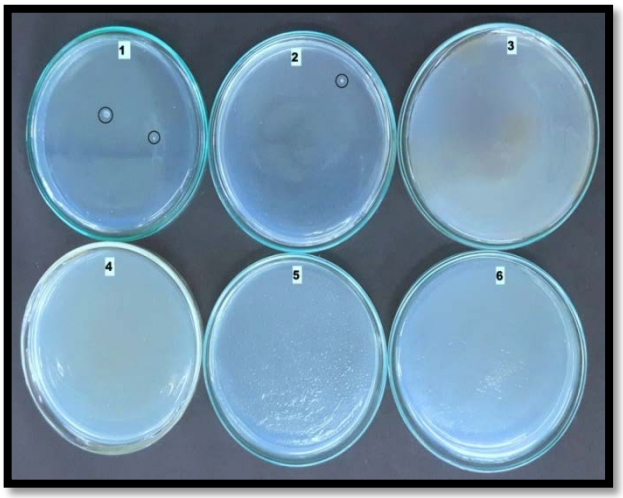

FIGURE 13: Sabourataud dextrose agar plate.

(1+2) / 2 x 90 / 10
= 3/2 x 90 / 10
= 13.5

The ointment passes the requirement for the total plate count as per BIS specification of skin cream which in less than 1000 cfu/g.[24] The total plate count of the ointment was done after 6 months of storage at 25 ^{0} C/ 60% RH condition and was found to be in good condition, Although few colonies were found as shown in the figure, which were within the acceptable range , whereas, no colonies were found in the ointment stored at 5 ^{0} c temperature. It must be noted that the ointment does not contain any added antimicrobial or preservative. In absence of the preservative system it is Silver nanoparticles that must have acted as preservative in ointment. Therefore it can be used as preservative in other cosmetic preparation. Although detailed study on preservative action needed to be done.

CONCLUSION:

Chemically prepared silver nanoparticles was confirmed by UV visible spectroscopy and SEM and TEM result showed that the formulated silver particles where 100 and 200 nm in size along with somewhat spherical in shape. Zeta potential result showed that formulated particle is well stabled. These silver nanoparticles were then tested for antimicrobial properties which indicate that the concentration 60 ug/ml and above showed clear zone of inhibition which indicates its antimicrobial properties. Apart from these the ointment containing prepared silver nanoparticles formulation confirms that it can also be used as preservative in cosmetic formulation as it showed the stable formulation.

REFERENCES

[1] Andrea Porcari, Alessandro Inguscio, Elvio Mantovani, Silver nanoparticles: situation and perspective for industrial application in the Lombardia region, AIRI/Nanotec IT - Italian Association for Industrial Research (AIRI), Italian Centre for Nanotechnology (Nanotec IT), 2013, 1-28, http://www.nanotec.it/public/wpcontent/uploads/2014/04/AIRI_Report_PerspectiveNanosilver_Overview.pdf

[2] https://ec.europa.eu/health/scientific_committees/docs/citizens_silvernanoparticles_en.pdf

[3] P. Banerjee. Leaf extract mediated green synthesis of silver nanoparticles ferom widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresources and Bioprocessing a Springer open journal 2014, 1:3. http://www.bioresourcesbioprocessing.com/content/1/1/3

[4] R. Ravishankar, Nanoparticles and their potential application as antimicrobials, www.formatex.info/microbiology3/**book**/197-209.pdf

[5] Kim, Keuk-Jun, Antifungal Effect of Silver Nanoparticles on Dermatophytes, J. Microbiol. Biotechnol. (2008), 18(8), 1482–1484.

[6] R. Salomoni, Antibacterial Activity of Silver Nanoparticles (AgNPs) in Staphylococcus aureus and Cytotoxicity Effect in Mammalian Cells, www.microbiology5.org/microbiology5/**book**/851-857.pdf

[7] Eduardo José J. Mallmann, Antifungal Activity Of Silver Nanoparticles Obtained By Green Synthesis, Rev. Inst. Med. Trop. Sao Paulo 57(2):165-167, March-April, 2015 http://dx.doi.org/10.1590/S0036-46652015000200011

[8] Yi-Huang Hsueh, The Antimicrobial Properties of Silver Nanoparticles in Bacillus subtilis Are Mediated by Released Ag+ Ions, PLOS ONE | DOI:10.1371/journal.pone.0144306 December 15, 2015, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4682921/pdf/pone.0144306.pdf

[9] Alexandru Grumezescu, Inorganic Nanoarchitectonics with anti-infective potential, Nanobiomaterials in Antimicrobial Therapy: applications of Nanobiomaterials, Volume-6, page no: 30-31. https://books.google.co.in/books?id=fZJCCQAAQBAJ&pg=PA30&lpg=PA30&dq=antimicrobial+and+anti+infective+properties+of+silver+nanoparticles&source

[10] Chris BARKER, Scientists develop silver nanoparticles that can outshine gold, 09Sep2013, http://www.cosmeticsdesign.com/content/view/print/8183871/ .

[11] Natalia L. Pacioni, Synthetic Routes for the Preparation of Silver Nanoparticles, Springer International Publishing Switzerland 2015, DOI 10.1007/978-3-319-11262-6_2

[12] Lucas Blandón, Electrochemical Synthesis of Silver Nanoparticles and their Potential Use as Antimicrobial Agent: a Case Study on Escherichia Coli, Portugaliae Electrochimica Acta 2012, 30(2), 135-144 DOI: 10.4152/pea.201202135, http://peacta.org/articles_upload/PEA_30_1_2012_135_144.pdf

[13] M. Jannathul Firdhouse, Novel synthesis of silver nanoparticles using leaf ethanol extract of Pisonia grandis (R. Br), Scholars Research Library Der Pharma Chemica, 2012, 4(6):2320-2326. http://derpharmachemica.com/vol4-iss6/DPC-2012-4-6-2320-2326.pdf

[14] Siby Joseph, Synthesis of Silver Nanoparticles by Microwave irradiation and investigation of their Catalytic activity, Research Journal of Recent Sciences ISSN 2277-2502 Vol. 3(ISC-2013), 185-191 (2014) http://www.isca.in/rjrs/archive/special_issue2013/34.ISCA-ISC-2013-4CS-108.pdf

[15] Cheah, biosynthesis of nanoparticles and silver nanoparticles, Bioresour nd bioprocessing a springer open journal (2015), DOI 10.1186/s40643-015-0076-2.

[16] Jolanta Pulit-prociak, Marcin Banach, Silver Nanoparticles – a material of the feature…?, August 10, 2015; DOI: 10.1515/chem.-2016-0005.

[17] JOYONA Micro Silk® Nano Gold Complex Cream), JOYONA ; www.joyona.de.

[18] Dr. Mukhils M. Ismail. Spectral study of silver Nanoparticle prepared by chemical method 8/12/2013 Engg. Tech journal, Vol 32, part (B) no.5, 2014.

[19] Amr T. M. Saeb, Production of Silver Nanoparticles with Strong and Stable Antimicrobial Activity against Highly Pathogenic and Multidrug Resistant Bacteria, Hindawi Publishing Corporation, the Scientific World Journal Volume 2014, Article ID 704708, 9 pages http://dx.doi.org/10.1155/2014/704708

[20] Mendhulkar Vijay D, Photochemical decolorization of Methyl Violet dye using Azadirachta indica (Neem) mediated synthesized silver nanoparticles, Scholars Research Library Der Pharmacia Lettre, 2016, 8 (7):119-128 (http://scholarsresearchlibrary.com/archive.html)

[21] Mohammad Oves, Antibacterial and Cytotoxic Efficacy of Extracellular Silver Nanoparticles Biofabricated from Chromium Reducing Novel OS4 Strain of Stenotrophomonas maltophilia, journal PLoS One. 2013; 8(3): e59140. Published online 2013 Mar 21. doi: 10.1371/journal.pone.0059140, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3605433/
[22] https://pharmlabs.unc.edu/labs/ointments/prep.htm
[23] Stability testing of active pharmaceutical ingredients and finished pharmaceutical products, © World Health Organization, WHO Technical Report Series, No. 953, 2009.
[24] Indian Standard "skin cream specification (Second Revision)" by Bureau of Indian Standards IS 6608:2004.
[25] http://www.makingcosmetics.com/Stability-Testing-of-Cosmetics_ep_59.html
[26] Neusely da Silva, Marta H. Taniwaki, Valeria C. A. Junqueria, Neliane F. A. silveira, Maristela S. do. Nascimento, Renato A.R. Gomes editors, "Microbiological examination method of food and water: A laboratory manual", chapter 3: Basic plate count techniques for the enumeration of microorganisms, (2013), page no: 23-37.
[27] R. Anbarasu, Pharmacological Potential of Silver Nanoparticles (AgNPs) derived from Evolvulus Alsinoides, International Journal of Recent Research and Applied Studies, Volume 3, Issue 6 (5) June 2016. http://www.ijrras.com/v3_i6/Paper%205.pdf
[28] S. Iravani, H. Korbekandi, S.V. Mirmohammadi, and B. Zolfaghari, Synthesis of silver nanoparticles: chemical, physical and biological methods, Research in pharmaceutical science, 2014 Nov-Dec; 9(6), 385–406.
[29] GM Nazeruddin, NR Prasad In-Vitro Bio-Fabrication of Silver Nanoparticle Using *Trigonella foenum* Seed Extract. Research Journal of Pharmaceutical, Biological and Chemical Sciences. *ISSN: 0975-8585*
[30] Sobhy M Yakout, A novel green synthesis of silver nanoparticles using soluble starch and its antibacterial activity, Int J Clin Exp Med 2015;8(3):3538-3544 www.ijcem.com/ISSN:1940-5901/IJCEM0003909, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4443080/pdf/ijcem0008-3538.pdf
[31] Magdalena Stevanović, A new, simple, green, and one-pot four-component synthesis of bare and poly(α, γ, L-glutamic acid)-capped silver nanoparticles, NIH Public Access, Colloid Polym Sci. 2012 February 1; 290(3): 221–231. doi:10.1007/s00396-011-2540-7., http://digitalcommons.chapman.edu/cgi/viewcontent.cgi?article=1258&context=pharmacy_articles
[32] Zeta Potential An Introduction in 30 Minutes, https://caliscc.org/images/presentations/Morante_Zeta_Potential.pdf
[33] H K Ardani, C Imawan, W Handayani, D Djuhana, A Harmoko and V Fauzia, "Enhancement of the stability of silver nanoparticles synthesized using aqueous extract of Diospyros discolor Willd. leaves using polyvinyl alcohol", IOP conference series: Material science and engineering 188, (2017), page no: 1-5.
[34] Indian Standard, "Method of test for Microbiological Examination of Cosmetics", Bureau of Indian Standards, IS 6356 : 1993.
[35] Khushboo Singh, Antibacterial Activity of Synthesized Silver Nanoparticles from Tinospora cordifolia against Multi Drug Resistant Strains of Pseudomonas aeruginosa Isolated from Burn Patients, journal of Nanomedicine & Nanotechnology 2014, volume 5 issue 2, http://dx.doi.org/10.4172/2157-7439.1000192
[36] Priti Paralikar, Biogenic Synthesis of Silver Nanoparticles Using Leaves Extract of Epiphyllum Oxypetalum and its Antibacterial Activity, Austin Journal of Biotechnology & Bioengineering, December 08, 2014, http://austinpublishinggroup.com/biotechnology-bioengineering/fulltext/ajbtbe-v1-id1032.php