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ANTIBACTERIAL APPLICATION STUDIES OF NANOSILVER INCORPORATED PRODUCTS

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Abstract: Silver is known to kill 650 types of microorganisms. Nanosilver technology is widely applied to impart bactericidal effect on to the products such as soaps, plastics, clothes, etc. Development of Nanosilver technology for various products is a research by itself, since single technology cannot be used for all products. In the present paper, Nanosilver was prepared by chemical reduction method in colloidal and powder form. They were characterized using Physio-chemical techniques like XRD, TEM, Chemical analysis, etc. In this study Nanosilver was incorporated in various products like ointment, room-freshener, 5.0 µm filter, plastic cups. The antibacterial studies were carried out using standard procedure and the concentration of silver required in each product to control the bacterial growth was optimized.

Keywords: Nanosilver, Application, Room-sprayer, Ointment, 5.0 µm filter.

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INTRODUCTION

Silver is well-known to kill bacterial growth and it was being used by our ancestors as preservative by way of making silver utensils. Silver has broad antimicrobial activity while exhibiting low toxicity towards mammalian cells[1-3]. Recently, Silver in the Nano particle size has attracted various scientists and industrialists[4-6] for providing hygiene hygienic way of life to people. Eventhough a few patents are available on nanosilver incorporated products, systematic investigation, revealing the method of preparation, variation of antibacterial activity with concentration of silver are not available[7-10]. Therefore, the present investigation aims to study some critical application of nanosilver in pharmaceutical field. Now-a-days it is quite common to see the office premises, hospitals, shops fitted with air-conditioners, which demands for closed environment. In such cases, there is easy chance of spreading infectious diseases through air from one - to - one. Apart from this, the Clean-room cannot use room-sprayer. But, it contains 5 µm filter for removing the dust particles. Hence, by coating Nanosilver on this filter, the growth of microorganisms in the clean-room can be controlled. Another application to be investigated is replacement of silver suphadiazine by Nanosilver in ointment. The disadvantages of silver sulphadiazine are (i) some, people are allergic to sulpha drugs, (ii) the availability of effective silver for curing the affected area is less and (iii) being organic in nature, the active ingredient (silver sulphadiazine) tends to degrade over a period of time. Hence, it is aimed to investigate the efficiency of nanosilver, in the form of powder to replace the silver sulphadiazine. The nanosilver powder is also incorporated in plastic-cups which can be used for storing food - items, thus, preventing degradation by microorganisms.

MATERIALS AND METHODS

Preparation of Nano Silver Colloid (NSC)

Silver can be easily reduced from ionic form to metallic silver using reducing agents. Such reduction invariably leads to agglomeration of particles and the matrix support plays a vital role in keeping these particles of silver apart and increases the stability of the colloidal solution. In the present study gum - acacia was selected for suspending Nano particles of silver in solution.

Preparation of Nano silver colloid involved dissolving gum - acacia in distilled water and silver nitrate solution was added drop-wise under vigorous stirring. To the above contents diethanolamine dissolved in water was added drop-wise and stirring continued for 15 minutes. Finally, glucose dissolved in water was added, when the dark yellow nanosilver colloid was formed.

Preparation of Nanosilver Powder (NSP)

Nanosilver powder was prepared by taking the hydrophilic fumed silica (Aerosil - 200) in a container and stirred vigorously with distilled water to form slurry. To the above mixture, silver nitrate dissolved in distilled water was added. After 30 minutes, calculated quantity of diethanolamine was added and stirring continued. The dilute colloid becomes viscous and a small quantity of distilled water was added to reduce the viscosity. After 30 minutes, calculated quantity of formaldehyde was added and stirring continued for 45 minutes. The colloidal powder is filtered and dried. Typical composition, identification of nanosilver colloid and powder are given in Table 1.

Table 1: Composition and identification of nanosilver colloid and powder

S.N.	Sample ID	Description	Support (Wt. %)	Silver content	Appearance
Nanosilver Colloid					
1.	NSC-GA-2000	Nanosilver colloid	Gum-acacia (0.25%)	2000 ppm	Dark Yellow
Nanosilver Powder					
2.	NSP-FS-10	Nanosilver Powder	Fumed Silica (hydrophilic – Aerosil 200) (90%)	10 %	Brownish yellow

Powder X-ray diffraction data for the samples were collected on a GE Analytical XRD (XRD 3003 T/T). The TEM-EDAX was recorded using Philips CM12 transmission electron microscope with Energy Dispersive Spectroscopy detector for micro-analysis.

Preparation of products incorporated with Nanosilver

The Nanosilver colloid was prepared with a concentration of 2000 ppm of silver. Beyond this concentration, silver nano particles were found to be unstable beyond 4 weeks. This was diluted to different concentrations with distilled water. An aerosol spray can was made using carbon dioxide gas as the propellant.

The colloidal silver with 10 ppm, 50 ppm and 100 ppm was coated on the High Energy Particulate Arrestor High Efficiency Particulate Air filter (5 µm filter) by spraying. This is fixed in the AHU of clean-room.

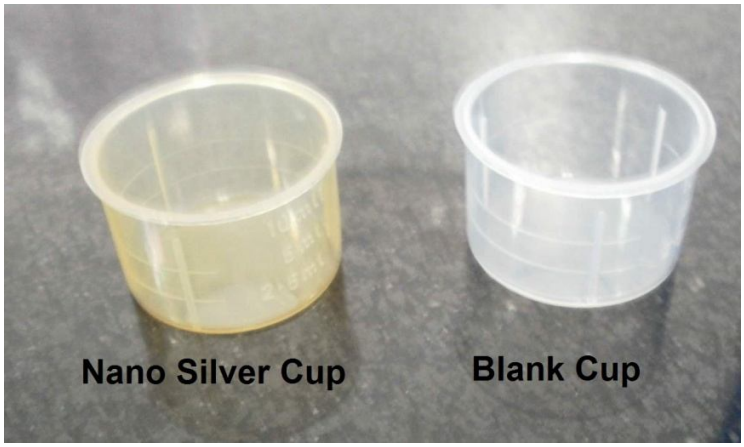
In Ointment: Blank ointment was made by using required raw materials (proprietary) except the active ingredient. Conventionally, 1% Silver sulphadiazine will be present in the ointment

which is used to apply externally for cuts, burns and wounds. NSP-FS-10 was selected for the present study. The weight of the Nanosilver powder and base cream used for the analysis are given in Table 2. For comparison, using silver sulphadiazine, an ointment was prepared. The antibacterial activity of Nanosilver ointment was evaluated and compared with that of silver sulphadiazine ointment.

Table 2: Composition of nanosilver ointment taken for analysis

S.N.	Sample code	Weight of the ingredients in ointment		Silver content, Wt.%
		NSP-10, grams	Base cream, grams	
1.	NanoSil – 1	0.1	9.9	0.1
2.	NanoSil – 2	0.5	9.5	0.5
3.	NanoSil – 3	1.0	9.0	1.0
4.	SSD-Ointment	1.0	9.0	1.0

In Pvc Cups: The Nanosilver powder with 1 % silver is blended with PVC at high temperature to form master-batches containing 1,000 ppm. This is further blended with PVC to form cups containing 50, 100 and 200 ppm of silver.



Antibacterial analysis procedure

For Gram positive bacteria - *Staphylococcus aureus* (ATCC No. 6539 P) and Gram negative bacteria - *Escherichia coli* (ATCC No. 8739) were used for the studying antibacterial activity.

Room Spray Technique: The antibacterial reduction efficiency of room-spray technique was evaluated by selecting a Clean room of 10 x 10 sq. area. Soyabean casein digest agar was prepared by sterilizing at 121°C (15 lbs) for 20 minutes. After sterilization about 20 ml of media was poured into sterile disposable petriplates in a Laminar air flow. The media was allowed to solidify. After solidification, the lid of petriplate was opened and placed in the centre of the room for about 30 minutes and the lid of the petriplate was closed after exposure. The petriplates were incubated at 30 - 35°C for 48 - 72 hrs. Meanwhile, using the sprayer, the nanosilver colloid with required concentration of silver was sprayed all over the room. The room was closed for about 30 minutes. Once again a fresh sterilized soyabean casein digest agar plate was placed in the centre of the room for 30 minutes with lid open. The plate was closed and incubated at 30 – 35°C for 48 - 72 hrs. After 72 hrs of incubation, the number of colonies in both the plates was counted using digital colony counter. The reduction in the number of colonies in petriplate (after applying room-spray) was reported in terms of "percentage of CFU reduction / exposure".

Coated on 5 μm filter: A clean-room containing 5 μm filter of size 610 X 610 mm was selected for the study. The AHU is switched on and the air to flow is allowed for a minimum of about 1 hour. A sterilized petriplate containing soyabean casein digest agar was kept open near the air flow for 30 minutes. The exposed plate is closed with the lid and incubated by inverting at 30 - 35°C for 48 - 72 hrs. This plate was used as control. Different concentrations of the Nanosilver colloid were coated on the 5 μm filter using atomizer. The reduction in bacterial colonies in the air was analyzed by repeating the above procedure. The results were compared with the control and given as percentage reduction of CFU.

In Ointment: The antibacterial efficiency of Nanosilver ointment was evaluated by using sterilized 100 ml Soyabean casein digest medium into a sterilized petriplate inside the Laminar air-flow. About 1 ml of ointment sample loaded with required quantity of Nanosilver powder was added and mixed well. In another sterilized petriplate, about 1 ml of blank ointment base (cream without nanosilver powder) was introduced along with about 20 ml of sterilized soyabean casein digest agar to the above plate at 50–55°C (Control sample). The 2 plates were rotated in clockwise and anticlockwise direction without spillage for the uniform mixing of sample and media. The medium was allowed to solidify and incubated by inverting at 30 - 35°C for 48 - 72 hrs. After Incubation, the petriplates were removed from the incubator and number of colonies grown was counted using digital colony counter and compared. The CFU/mL reduction in nanosilver ointment was calculated based on the control sample.

In PVC cups: The un-coated plastic-cup was sterilized in the autoclave at 121°C for 20 min and 15 lbs pressure. After sterilization the cup was cooled to room temperature and 10 ml of tap-water was added. Immediately, 1 ml of the tap-water from the cup was transferred into a

sterile petriplate and 20 ml of sterile Soyabean casein digest agar was added at 45 – 55°C. It was then cooled, labeled as '0' min sample and incubated at 30 – 35 °C for 48 – 72 hrs. Number of colony forming units (CFU/ml) was counted after the Incubation period. This was taken as control plate. The experiment is repeated in Nanosilver incorporated plastic cup. The cfu/ml was measured for every one hour and reduction in the colonies was compared with control.

RESULTS AND DISCUSSION

The XRD pattern of the NSP-10 is presented in the Figure 1. A major peak at 2θ = 38, and another peak at 2θ = 44, shows presence of metallic silver (PDF No.87-0719).

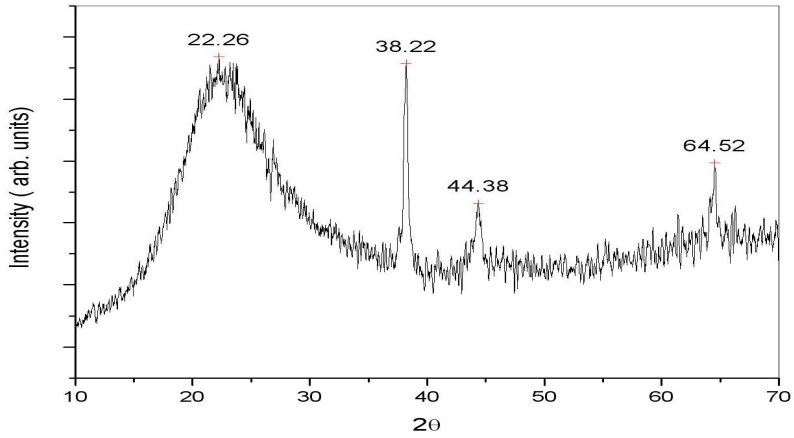


Figure 1: XRD pattern of NSP-10.

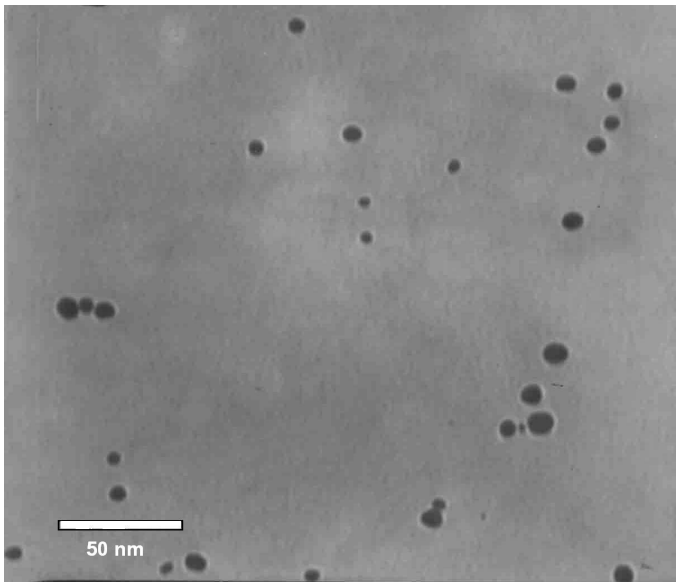


Figure 2: TEM image of NSC-GA-2000

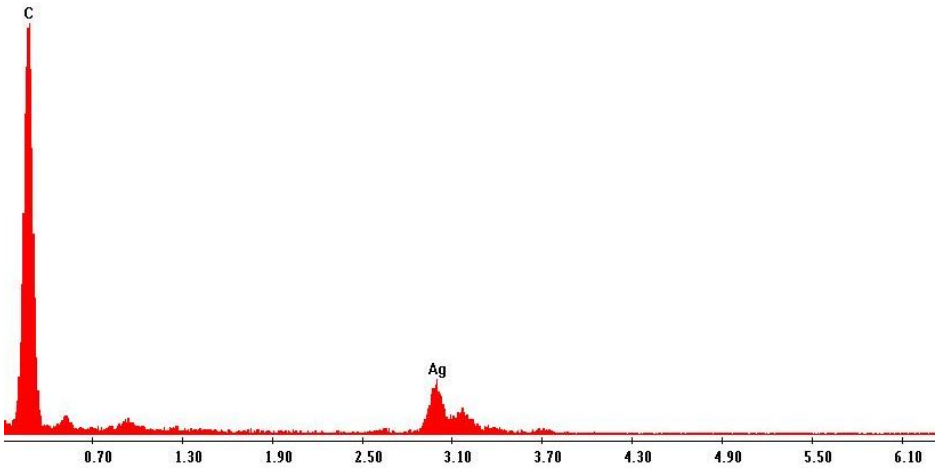


Figure 3: EDAX analysis of NSC-GA-2000 sample

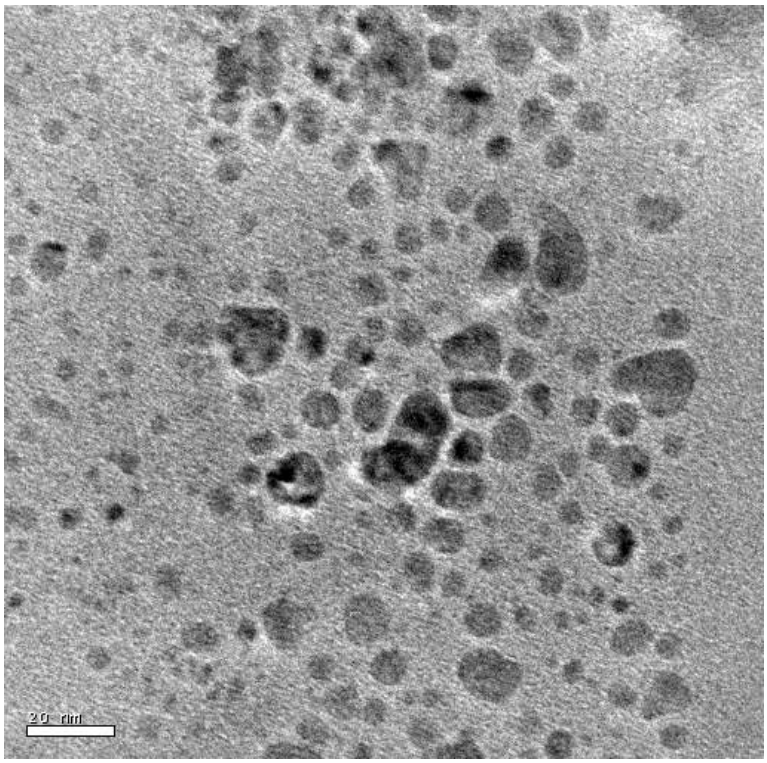


Figure 4: TEM image of NSP-FS-10

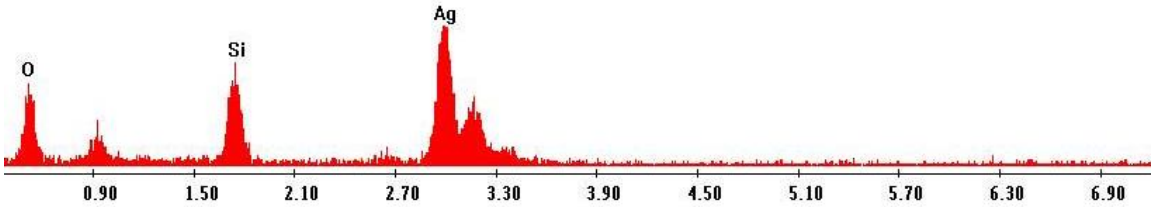


Figure 5: EDAX analysis of NSP-FS-10

The TEM image of both NSC-GA-2000 and NSP-FS-10 revealed deposition of silver nano particles on to the support. The average particle size was found to be around 5 – 10 nm. The chemical analysis of the above two samples were carried out with help of EDAX analysis on the particle and the results showed absence of impurities.

The antibacterial analysis of the Nanosilver colloid as room-sprayer with varying concentrations of silver is given in Table 3. With the increase in silver concentration the antibacterial activity increased. Appreciable antibacterial activity was observed with silver concentration of 100 ppm. Hence, it can be concluded that nanosilver colloid containing around 100 ppm of silver will be effective against spreading of bacteria in air. It has to be noted that, the results were evaluated for single exposure. On repeated application of nanosilver sprayer, the bacteria in the air can be controlled completely. The petriplates containing the bacterial strain developed from the air is shown in Figure 6.

Table 3: Antibacterial analysis results of nanosilver room-sprayer.

S.N.	Concentration of silver, ppm	Time of exposure	CFU/mL Reduction (%)
1.	0 (Control)	30 min.	0
2.	10	30 min.	0
3.	50	30 min.	37
4.	100	30 min.	63

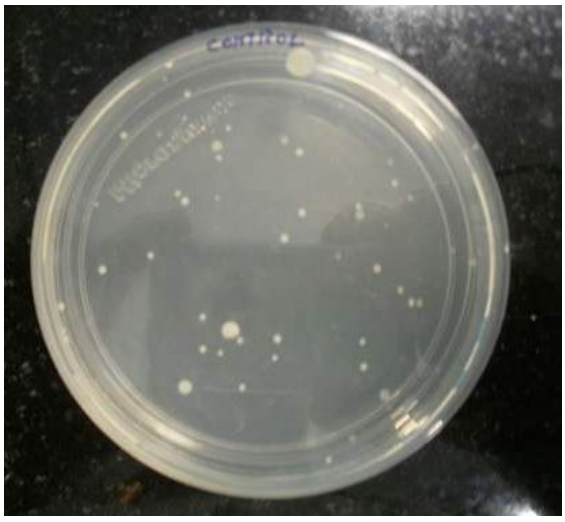


Figure 6.1-A: Bacterial growth exposed in air

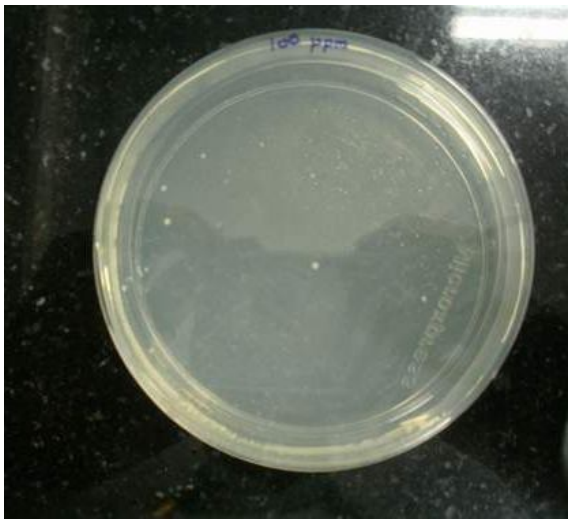


Figure 6.1-B: Reduction in bacterial growth using nanosilver room-sprayer

The results of bacterial colonies present in the clean-room, after installing the Nanosilver coated 5µm filter was analyzed and the results are given in the Table 4. The bacterial reduction efficiency increases with increase in nanosilver content. 5µm filter coated with 100 ppm of nanosilver showed nearly 75% reduction in bacterial colonies in 1 hour in comparison with un-coated filter. When the AHU unit was in operation for 2 hours, 100% reduction in bacteria was observed in the clean-room.

Table 4: Bacterial reduction in Clean-Room after fixing with Coated NanoSilver 5µm Filter

S. N.	Sample	% cfu/ml reduction		
		10 ppm	50 ppm	100 ppm
1.	Nano Silver coated 5 µm Filter	37.5 %	50.0 %	75.0 %

Ointment containing different concentrations of nanosilver was prepared and tested for its antibacterial activity. Unlike room-spray application, this application demands for 100% antibacterial activity. As expected with the increase in concentration of silver, the antibacterial activity increases. Ointment with silver concentration of 0.5 % (NanoSil-2) showed more than 90 % of CFU reduction and that of 1 % showed 100 % reduction of bacterial strains (NanoSil-3).

Table 5: Antibacterial analysis of nanosilver ointment and comparison with silver sulphadiazine ointment

S.N.	Sample	Concentration of silver (%) and % cfu/mL reduction					
		NanoSil-1 (0.1%)		NanoSil-2 (0.5%)		NanoSil-3 (1.0%)	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
1.	NanoSil Ointment	65	50	90	95	100	100
2.	Silver sulphadiazine Ointment	--	--	--	--	100	100

The NanoSil-3 ointment was subjected to stability studies following conventional standard methods and found to be stable without discoloration. In the case of SSD- ointment, even though it showed 100 % antibacterial activity, Silver sulphadiazine being organic molecule, will be susceptible to degradation by sun-light, exposure to atmospheric air and high temperature. While, nanosilver being in metallic form will be stable for quite a long period of usage.

Another important application of nanosilver as antibacterial agent is for food storage. Nanosilver powder was incorporated in plastic cups and its efficiency towards bactericidal action was studied. The results are presented in Figure 7.

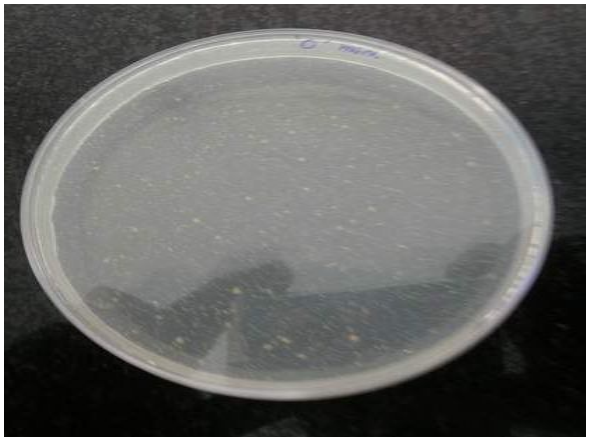


Figure 7-A: CFU/ml of blank plastic cup at 0 minutes (control)

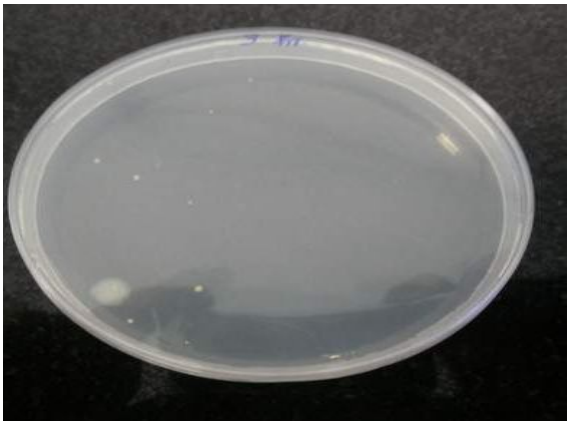


Figure 7-B: CFU/ml of nanosilver coated cup (100 ppm) after 3 hours.

It is evident from the Table 6 that, nanosilver cup coated with 100 ppm of nanosilver showed better activity when compared with 50 ppm. Plastic cups with 200 ppm of silver showed activity within 1 hour, but the intensity of the colour was high.

Table 6 : Antimicrobial reduction of Nano Silver in coated measuring cup

S.N	Sample	Contact time in minutes & cfu/mL reduction %			
		0 min	60 min	120 min	180 min
1.	Nanosilver Cup – 50 ppm	0	21%	52%	71%
2.	Nanosilver Cup – 100 ppm	0	66%	89%	91%
3.	Nanosilver Cup – 200 ppm	0	75%	93%	99%

CONCLUSION

Nanosilver colloid and nanosilver powder were prepared and characterized using XRD, TEM and chemical analysis. The average particle size of silver was around 10 nm. Nanosilver colloid aerosol spray was prepared using antibacterial room-spray and for coating on 5µm filter. Using nanosilver powder Ointment and plastic cups were prepared. All the above products were analyzed for its antibacterial property. The room-spray and 5.0 µm filter showed effective antibacterial property with 100 ppm of silver content. The nanosilver ointment with 1% of silver showed equivalent antibacterial property with that of silver sulphadiazine ointment and was found to be more stable. The plastic cups showed good antibacterial activity at 200 ppm.

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