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Synthesis and characterization of silver nanoparticles and its immobilization on alginate coated sutures for the prevention of surgical wound infections and the *in vitro* release studies

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ABSTRACT

The Silver nanoparticles are well-known for their antimicrobial, anti-inflammatory wound healing abilities. We synthesized Silver nanoparticles using chemical reduction method and the formation of the silver nanoparticles was characterized by UV-Vis absorption spectroscopy, FT-IR and SEM techniques. The minimum inhibitory concentration of the synthesized nanoparticles was determined for both gram negative and gram positive bacteria. Then these nanoparticles were coated over an absorbable surgical gut suture, after the immobilization of them in Sodium alginate, by slurry dipping technique. The controlled release of the nanoparticles checked *in vitro* in Simulated Body Fluid. Antimicrobial activity also evaluated by Disc diffusion method on the culture plates of *E. coli* and *S. aureus*. The successful release of silver nanoparticles under physiological PH depicts the applicability of this novel suture in surgery for the prevention of surgical wound infection and to enhance wound healing.

Keywords: *Silver nanoparticles; Antimicrobial activity; Alginate; Suture; Drug delivery.*

INTRODUCTION

A considerable amount of research has been conducted on drug delivery by biodegradable polymeric devices, following the entry of bioresorbable surgical sutures in the market about two decades ago [1]. Nowadays the researchers are trying to develop biodegradable sutures with antimicrobial and wound healing properties. There are many polymeric materials which have been implemented to hold the drugs on the surface of sutures. The alginate has been studied due to the low toxicity, favorable mechanical properties and capacity for bioresorption of the constituent materials [2].

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Alginates are water soluble linear polysaccharide extracted from brown seaweed and are the anionic block copolymers of a (1-4)-L-guluronic and β(1-4)-D-mannuronic acid and available as the sodium salt [3,4]. The alginates are known to effectively promote wound healing by maintaining a moist environment over the wound [5]. Alginate gel polymers can be made by incorporation of sodium or potassium alginate solution into an aqueous solution of calcium ions typically made from calcium chloride ($CaCl_2$). Since the sodium alginate and its composites have been used in many biomedical applications including drug delivery and wound dressings, it can also be effectively used for the immobilization of silver nanoparticles.

The antimicrobial properties of silver ions have been known since ancient times and silver ions are widely used as bactericide in surgical prostheses and dental implants [6].

Many methods have been used for the synthesis of nanoparticles including microbial, chemical and physical methods. The most popular among them is the chemical reduction of silver salts by sodium citrate. Silver nanoparticles show optical properties, which are not observed neither in molecules nor in bulk metals. The formation of the silver nanoparticles can be monitored using UV-Vis absorption spectroscopy since it exhibits the typical surface plasmon absorption maxima at 418-420 nm from the UV–Vis spectrum [7].

Surgical gut sutures are widely used sutures made from beef serosa or sheep submucosa. It usually has strength retention of 7-10 days and takes about 70 days to be completely absorbed by the body. In the present study, the combination of the alginate having wound healing and tissue adherence abilities, Silver nanoparticles having antimicrobial, anti inflammatory and wound healing abilities and the surgical gut suture having biodegradability, and good tissue adherence were tried to design a suture material with all these qualities.

EXPERIMENTAL

Materials

Sodium alginate was supplied in powder form by spectrum, USA. Silver nitrate ($AgNO_3$) was purchased from Merck. Trisodium citrate, NaCl, KCl, Na_2HPO_4, K_2HPO_4 were purchased from S.D fine-Chemical Ltd, Mumbai, India. Surgical gut plain suture was obtained commercially from Ethicon Inc. Luria Bertani, Nutrient and Mueller Hinton Broth media were used here and supplied by Hi-Media Laboratories, India.

Synthesis of Silver nanoparticles

The silver colloid was prepared by using chemical reduction method according to the description of Lee and Meisel [8]. For the synthesis of silver nanoparticles, silver nitrate solution was used as a metal salt precursor. Tri-sodium citrate solution (1.0mM to 2.0mM) was used as a reducing agent. Tri-sodium citrate was also used as stabilizing agent at room temperature. All solutions of reacting materials were prepared in distilled water. In typical experiment 50 ml of 1mM $AgNO_3$ was heated to boiling. To this solution 5 ml of 1% trisodium citrate was added drop by drop. During the process solution was mixed vigorously. Solution was heated until color's change became evident (pale yellow). Then it was removed from the heating element and stirred until cooled to room temperature. The transparent colorless solution was converted to the characteristic pale yellow and then to brownish-red color. The occurrence of color indicated the formation of silver nanoparticles. The silver nanoparticles were purified by centrifugation. To remove excess silver ions, the silver colloids were washed at least three times with deionized water. A dried powder of the nanosized silver was obtained by freeze-drying.

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow$$
$$\rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2\uparrow$$

Characterization

For the preliminary determination of silver nanoparticles, Ultraviolet–visible Spectroscopy (UV-Vis) was performed in a Perkin-Elmer Lambda 2 Spectrophotometer. The FT-IR (Perkin-Elmer Spectrum RX1) analysis was used for the characterization of the suspension and the resulting nanoparticles. FTIR absorption spectra before and after reduction of Ag ions were taken. The studies of size, morphology and composition of the nanoparticles were performed by means of scanning electron microscopy (HITACHI, S-

3000H). Histograms of size distribution were calculated from the SEM images by measuring the diameters of at least 50 particles.

Determination of Minimum Inhibitory Concentration (MIC)

Serial dilution technique was used to find out the minimum inhibitory concentration of the synthesized nanoparticles. The bacterial strains *E. coli* **(**ATCC 12228) and *Staphylococcus aureus* **(**ATCC6538-P) were used as representatives of gram negative and gram positive bacteria respectively. Microbial inoculums were prepared by subculturing microorganisms into Muller Hinton Broth (MHB) at 37°C for 18 h and were diluted to approximately 105 to 106 of organisms/ml in twofold MHB. To each of a series of stored test tubes, a standard volume of medium which will support the growth of the test organism was added. A stock solution of silver nanoparticles of a concentration of 10mg/100ml was prepared in distilled water and used for the assay**.** A control tube containing no antimicrobial agent was included. The inoculums were added equally to all test tubes. The tubes were incubated at 37°C for 18 hours and examined for turbidity. The tube with high test dilution showing no visible turbidity was its MIC. For both *E.coli* and *S. aureus* two separate sets of experiments were performed in triplicates and the mean value was taken. The MIC obtained was used for the immobilization on sutures.

Preparation of sodium alginate- Silver nanoparticle colloid

Sodium alginate 3.6% was prepared and added in 0.1% of sodium chloride solution with constant stirring in a magnetic stirrer. Then this slurry was kept for 6-8 hours at room temperature. The characterized silver nanoparticles (12.5µg/ml) were added to the above mixture and mixed thoroughly by continuous agitation in a magnetic stirrer (at 150 rpm) to prevent particle sedimentation.

Coating of sodium alginate- Silver nanoparticle colloid over the suture

The sodium alginate- silver nanoparticle colloid coat was made over the surgical gut suture by slurry dipping technique. Sutures were cut to 5cm long and immersed in the sodium alginate-silver nanoparticle colloid for 30 minutes. Then the sutures were taken out and rubbed gently with sterile cotton cloth to remove excess slurry over the suture. Then these sutures were suddenly dipped into 4% calcium chloride solution, kept undisturbed for 30 minutes to ensure the deposition of calcium and then washed in deionized water.

Invitro release studies

The invitro bioactivity of calcium alginate-silver nanoparticle coated suture was determined by immersion studies in Simulated Body Fluid (SBF) solution of pH 7.4. The method formulated by Kokubo *et al*. adopted for the preparation of SBF [9]. Coated sutures were immersed in 50 ml of SBF in clean conical flasks, which had been previously rinsed with hydrochloric acid and deionized water. The flasks were tightly made airtight using cotton plug to prevent contamination. The flasks containing the specimens were placed in a shaker which maintained at temperature of 37°C and rotated at 175 rpm. The sutures were left in immersion in SBF for time period of 96 hours and Ultraviolet-visible Spectroscopy (UV-Vis) was performed to determine the presence of silver nanoparticles in the SBF solution at every 12hrs time period.

Antibacterial assay

The antibacterial assays were done on human pathogenic *Escherichia coli* and *Staphylococcus aureus* by disc diffusion method. Briefly Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculum (100µl) of each culture were spread on to LB agar plates. The alginate-silver nanoparticle colloid coated sutures were cut into 3cm length and were placed in each plate. In each plate uncoated sutures with similar length were placed as control.

RESULTS AND DISCUSSION

The transparent colorless solution was changed into the characteristic pale yellow and finally to a dark red color (Figure 1). This change in color is an indication of the formation of silver nanoparticles. The color change was due to the change of plasmon resonance of silver nitrate as a result of reduction process. Mirror like illumination on the walls of the Erlenmeyer flask clearly

indicated the formation of silver nanoparticles in the reaction mixture.

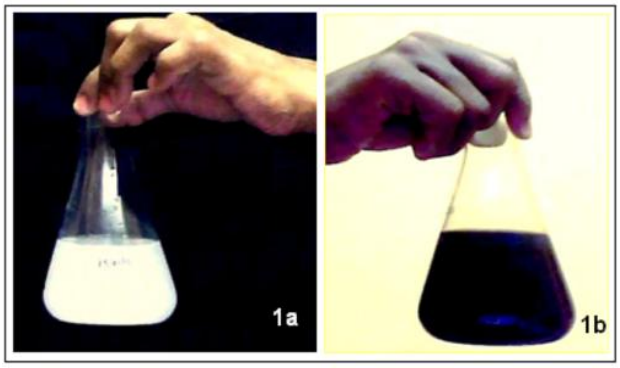

Fig.1. Visible color change of the reaction mixture before the formation nanoparticles (1a) and after the formation of nanoparticles (1b).

The prepared silver nanoparticles showed an absorption band at 411 nm as shown in Figure 2, which is a typical absorption band of spherical Ag nanoparticles due to their surface plasmon [8]. The absorption band in visible light region (350 nm – 550 nm, plasmon peak at 411 nm) is typical for silver nanoparticles.

The FTIR spectra of pure silver nitrate and reduced silver nitrate show considerable variation in the peaks of spectra (Figure 3). Regarding the silver nitrate about 23 peaks were found where as in the purified silver nanoparticles only 19 peaks found. The reduction of certain peaks is the clear indication of the loss of certain groups. A sharp peak at 1654.23 which is present in the spectrum of $AgNO_3$ is not found in the spectrum of Ag-Nanoparticles. It is due to the loss of nitrate group from the silver species. Molecules containing NO_2 groups, such as nitro compounds, nitrates, and nitramines, commonly exhibit asymmetric and symmetric stretching vibrations of the NO_2 group at 1660 to 1500 and 1390 to 1260 cm^{-1} region. The band of carboxyl or carbonyl groups also comes under the same region. This may be the reason for the reduction of the transmittance at this region in the case of spectrum of nanoparticles since the NO_2 group lost for it. The shift of the band from 1654.23 to 1583.04 indicates the formation of metal carbonyl groups. It is due to the stabilization of Ag nanoparticles by the –coo- group of trisodium citrate. This asymmetric shift can be comparable with the data presented by previous works (Anupam Giri et al, 2010). According to them, when the citrate ligand bound to magnetite nanoparticles surfaces the antisymmetric stretching of COO- at 1595 cm^{-1} almost remains the same but the symmetric COO- stretching mode of citrate becomes redshifted and appears sharply at 1398 cm^{-1} [10].

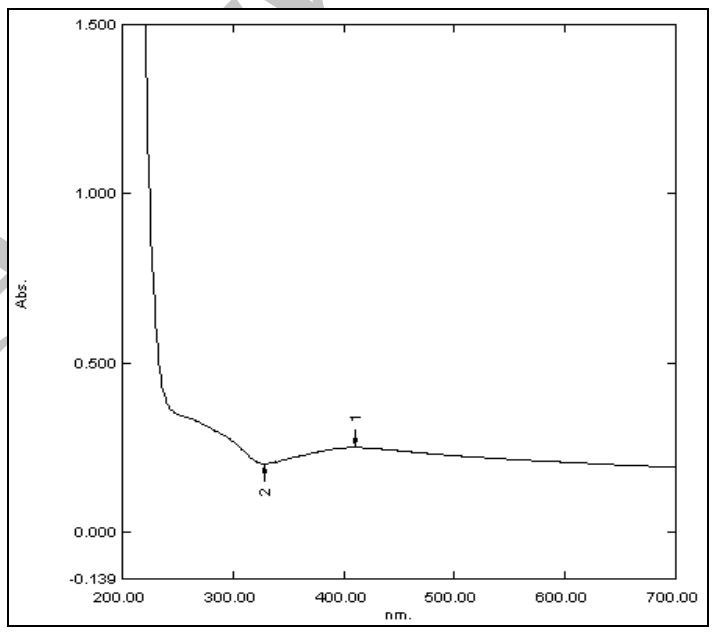

Fig. 2. Ultraviolet-visible Spectrum (UV-Vis) of silver nanoparticles. Peak 1 at 411.00nm with absorption at 0.249 indicates the presence of nanoparticles.

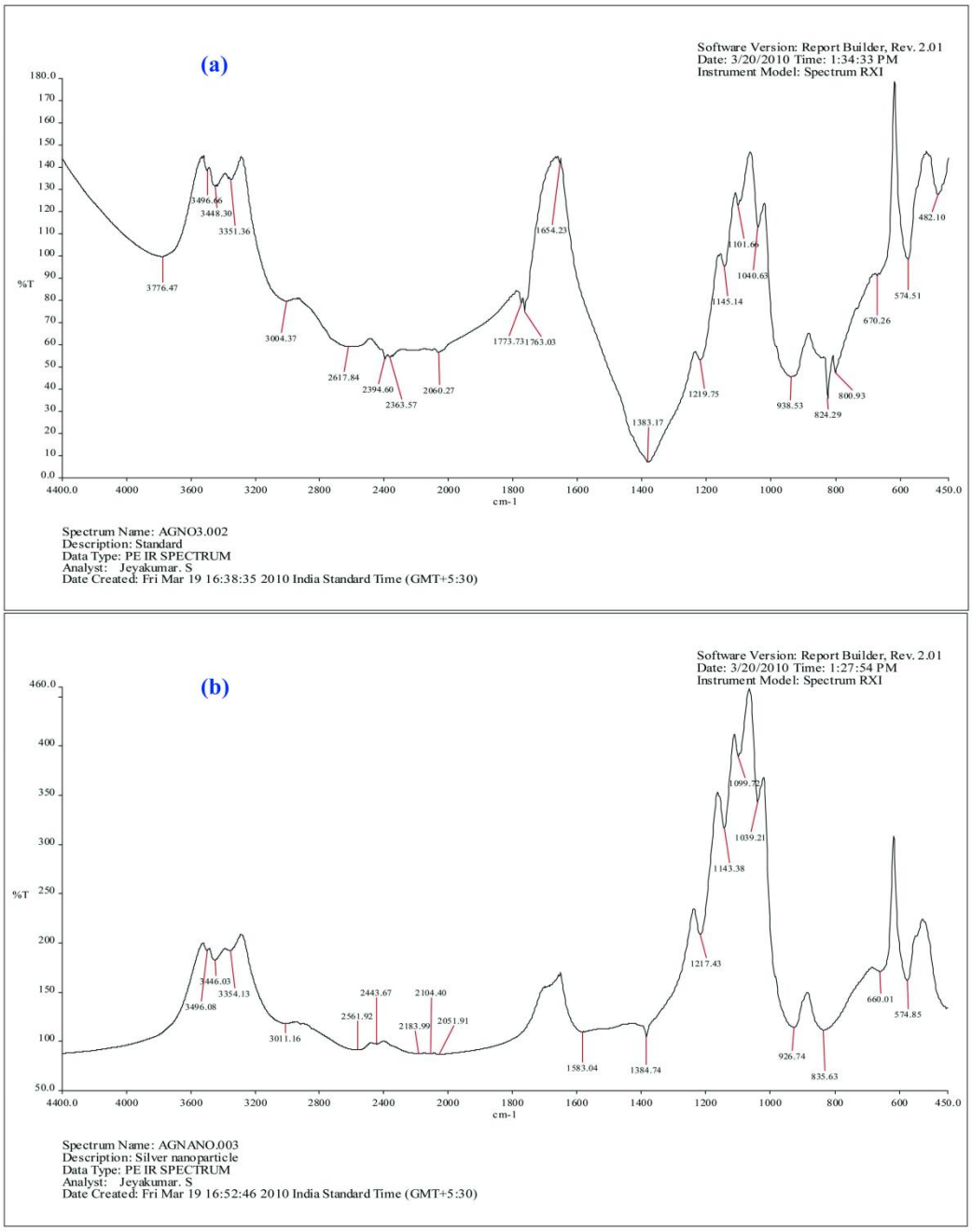

Fig. 3. FT-IR spectrum of unreduced $AgNO_3$ (a) and after the formation of nanoparticles by reduction (b).

From the SEM image (Figure 4) for the synthesized silver nanoparticles, it is clear that the particles are of almost uniform size and shape. All the particles are spherical and have an approximate size of 20nm.

The results obtained from the MIC indicate the synthesized silver nanoparticles are very efficient against both gram positive and gram negative bacteria (Table 1). Regarding the *E. coli* (ATCC 12228) and *S. aureus* (ATCC6538-P) the MIC is 10µg/ml and 12.5µg/ml respectively. The results are supportive to the data obtained by Ansari et al, 2011 [11]. From these results the optimum amount of nanoparticles needed for the immobilization is taken as 12.5µg/ml.

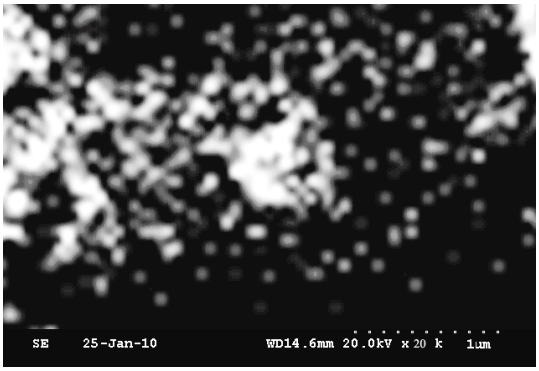

Fig.4. SEM image of the synthesized silver nanoparticles.

Table 1. Minimum Inhibitory Concentration (MIC) of the synthesized nanoparticles against *E.coli* (ATCC 12228) and *S.aureus* (ATCC6538-P).

Minimum Inhibitory Concentration (MIC)	
Bacteria	**MIC**
***E. coli* (ATCC 12228)**	10µg/ml
***S. aureus* (ATCC6538-P)**	12.5µg/ml

The UV-Visible spectrum taken at an interval of each 12 hours clearly depicts release of silver nanoparticles from the calcium alginate into the Simulated Body Fluid (Figure 5). In each spectrum the plasmon peaks at about 415nm indicates the presence of silver nanoparticles. The increase in absorbance along with the time period is due to the increasing concentration of silver nanoparticles in SBF.

As depicted in the graph of release profile (Figure 6) it is clear that the nanoparticles are continuously released into the SBF up to the 72 hours of the incubation. After this point, there is a decline in the release profile which may be due to the complete release of nanoparticles or may be due to the increase in the ionic concentration of SBF.

The invitro antibacterial activity of the designed suture is evident from the results of disc diffusion method (Figure 7). There is a clear zone of inhibition in each plate around the alginate-nano colloid coated sutures where as no zone of inhibition can be seen around the uncoated sutures. As evident from the plates, the zone of inhibition is less in the case of *S. aureus* (Plate-A) in comparison with *E. coli* (Plate-B). This is because the amount of the nanoparticles immobilized in both cases is the same (12.5µg/ml) but the MIC for *E. coli* is only 10µg/ml.

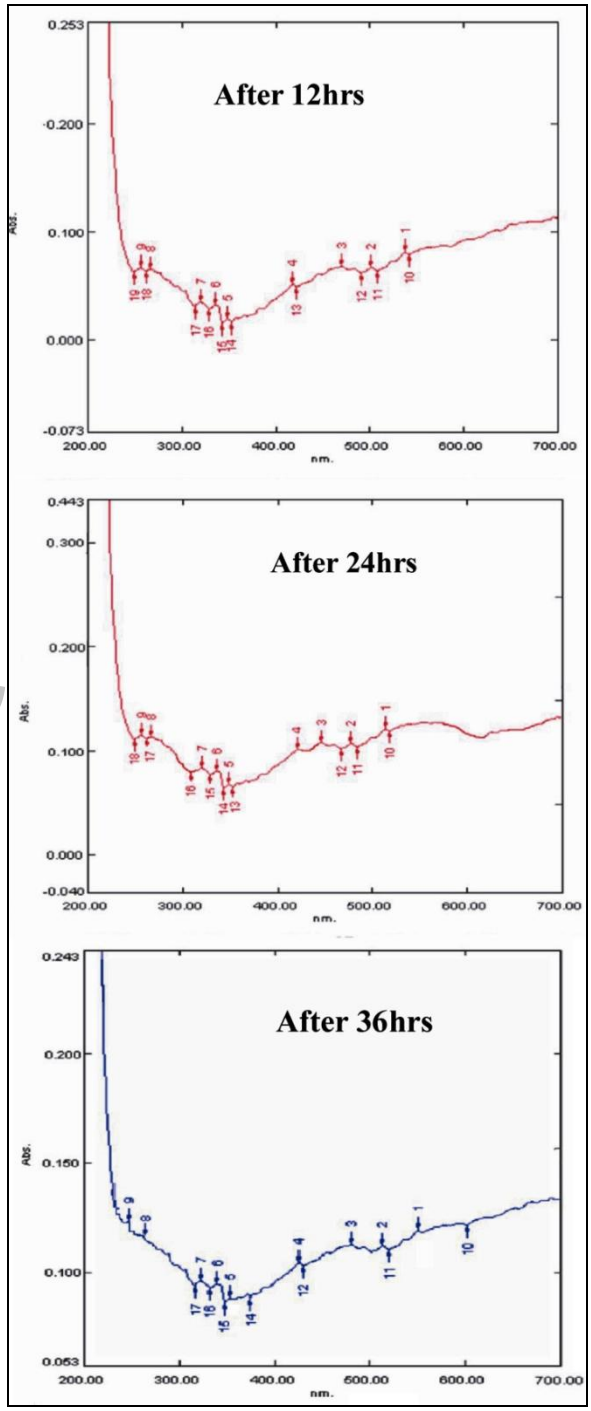

Fig. 5. UV-Visible spectra of SBF after 12 hrs, 24 hrs and 36 hrs of the immersion of alginate nanoparticles colloid coated suture.

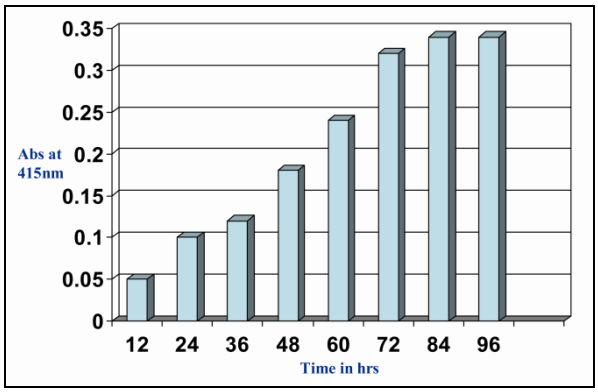

Fig. 6. Invitro release of nanoparticles into SBF from alginate nanoparticles colloid coating.

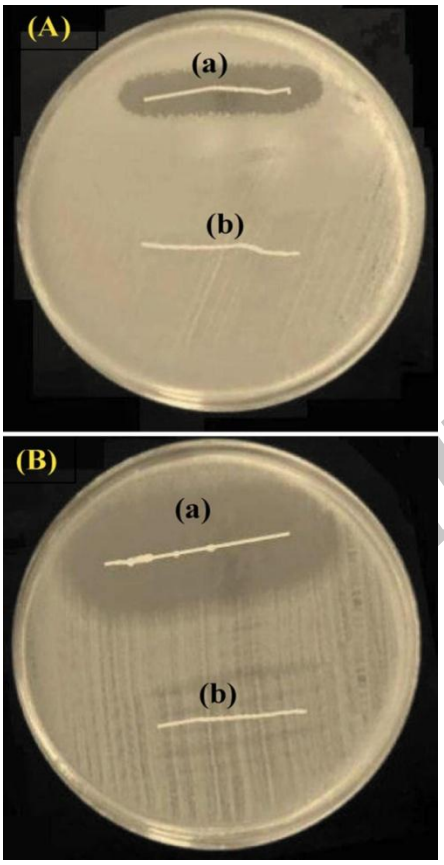

Fig. 7. Plates showing the *in vitro* antimicrobial activity of the suture. Plate (A): On *S. aureus* culture, (a) Ag nano-alginate coated suture, (b) control without coating. Plate (B): On *E. coli* culture, (a) Ag nano-alginate coated suture, (b) control without coating.

CONCLUSION

In summary, silver nanoparticles with approximate diameters of 20nm were synthesized by the chemical reduction of silver nitrate solution by using trisodium citrate as a reducing agent. The nanoparticles were characterized by UV/Vis, FT-IR and SEM. UV/Vis spectra shows the characteristic plasmon absorption peak for the silver nanoparticles ranging from 405 to 418 nm. The FT-IR of the nanoparticles particles confirmed the presence elemental silver signal. The peak 1583.04 indicates the formation of metal carbonyl groups due to the stabilization of Ag nanoparticles by the –coo- group of trisodium citrate. SEM imaging at 20kv and at a magnification of 20KX revealed the size and shape of the synthesized nano particles. The shape is almost spherical and the size is about 20nm. Additionally, the antibacterial activity of the nanoparticles was measured by finding out the Minimum Inhibitory Concentration. The results of this study clearly demonstrated that the colloidal silver nanoparticles inhibited the growth and multiplication of the tested bacteria, *Staphylococcus aureus* and *Escherichia coli*. Since the former is a gram positive bacteria and the latter is a gram negative bacteria, the synthesized silver nanoparticles are effective against both groups. Such high antibacterial activity was observed at very low concentrations of silver nanoparticles and was about 12.5µg/mL. Further the nanoparticles immobilized on surgical gut sutures by the use of alginate as cross linking agent. As a result of the formation of calcium alginate coating over the sutures it can facilitate moist environment that promotes wound healing along with the microbial inhibition contributed by the silver nanoparticles. The invitro release studies in Simulated Body Fluid shows the potential of the designed suture to deliver nanoparticles, the active principles on the wounds for a required period of time. The disc diffusion assay carried out on nutrient agar plates indicates the ability of the designed suture to inhibit the growth of both gram positive and gram negative bacteria successfully.

As a future direction animal experiments, cytotoxicity assays and clinical trials should be carried out to evaluate the tissue adherence, wound healing potential, biocompatibility and clinical usability. Finally a marketable form of new suture material may be released with wound healing ability, antimicrobial property and good tissue holding capacity.

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