

Research Article



Bioreduction Based Synthesis of Silver Nanocoats and their Application in Development of Nano Embedded Medical Fabrics

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ABSTRACT

The use of environmentally benign materials like plant leaf extracts for the synthesis of silver nanocoats offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols. Three main aspects were taken into consideration for the synthesis of silver nanocoats such as selection of solvent medium, selection of environmentally benign reducing agent, and selection of nontoxic substances for the Ag NPs stability. The aqueous extracts of ten different medicinal herbs were investigated for their antibacterial activity against gram negative and positive bacteria (*Staphylococcus aureus*, *Salmonella typhi*, *Escherichia Coli*). Among the selected herbs *polygala chinensis* extract showed better reduction on the synthesis of silver nanocoats. Surfactant (Polysorbate-80) aids in successful preparation of nanocoats with uniform distributions and long stability, given their tendency to rapidly agglomerate in aqueous solution. The silver nanocoats thus formed are analyzed through scanning electron microscope (SEM) analysis. The present investigation reveals that *polygala chinensis* extract of silver nanocoats have greater effectiveness as an antibacterial activity against microorganisms and used as nano embedded fabrics for wound healing.

Keywords: Antibacterial activity, Nanocoats, *Polygala chinensis*, Surfactant, SEM.

INTRODUCTION

Nano-coatings provides a cheap, safe alternative for drug delivery capsules and protective coatings. They are inexpensive, they are generally recognized as safe and their assembly is rapid. The fields of medicine and environmental science are increasingly interested in self-assembling nano-materials. Such materials can be used to make a wide array of things ranging from nano-capsules that deliver drugs to different parts of the body, to anti-corrosive coatings. The coatings form at particular pH and the researchers can control the thickness of the coating, which can be a few nanometers or more. One application of the thin film is to coat particles in solution, and then to remove the particle, leaving behind a hollow vessel made of tannic acid and iron. This seems to be an alternative strategy for delivering, for example, vaccines or therapeutics. The capsules can be designed to disassemble at different acidic pH, which means they could be used to deliver drugs to acidic environments, such as the gut.¹ Research has found that impregnating any materials with silver nanoparticles can exploit the antimicrobial activity of silver and is cost effective. The size allows them to easily interact with other particles and also intensifies the broad spectrum antimicrobial activity. Also silver possess far lower propensity to induce microbial resistance than antibiotics.² Nanoparticles constitute the smallest unit of the material and hence it exhibits the material's most distinguishing and natural properties. Ordinary materials such as carbon, silicon and certain metals exhibits novel extraordinary characteristics such as chemical reactivity, electrical conductivity, extra strength, etc in its nanofom;

which is not exhibited in its micro or bulk form.³ Silver nanoparticles are nanoparticles of silver, i.e. silver particles of size between 1 nm and 100 nm. Among all metal nanoparticles, silver nanoparticles received more attention because of their outstanding physicochemical properties. It is used in molecular labeling due to the Surface Plasmon Resonance and large effective scattering cross section of individual silver nanoparticle.⁴ One of the highly useful properties is its broad antimicrobial activity. Silver nanoparticles can be synthesized by several physical and chemical methods such as ion implantation, physical vapor deposition, electrochemical reduction, spark discharging, solution irradiation and cryochemical reaction. All these methods may not be eco-friendly. So biological synthesis has proven to be better eco-friendly method as it doesn't involve any toxic chemicals and is also cost effective. *Polygalachinensis* L. belongs to Polygalaceae family. It is commonly known as "Siriyanangai". Genus *Polygalais* annual, diffuse herb, 10-25cm tall. Flowers are papilionaceous, primary root orange, stems woody at base, branchesterate, crisped pubescent. Leaf blade green, obovate, ellipticorlanceolate, 2.6-10x1-1.5cm, papery, pubescent, inflorescence raceme, super-auxiliary, rarely auxiliary, shorter than leaves, densely few flowered. *Polygala* was traditionally used by Americans to treat snakebites⁵ and as an expectorant to treat cough and bronchitis. *Polygalais* considered as a powerful tonicherb⁶ that can help to develop the mind and aid in creative thinking. The current work involves (1) Selection and preparation of different plant samples, (2) Screening of plant samples for silver reduction properties, (3) Rapid Synthesis of Silver



nano particles using selected phyto-reductant, (4) Evaluation of antimicrobial properties of Silver nano particles, (5) Optimization of Silver nanoparticle synthesis, (6) Embedding of silver nano particles in cotton fabrics and analysis on antimicrobial nature of fabrics.

MATERIALS AND METHODS

Sample collection

Fresh leaves of ten different plants like; *Phyllanthusamarus*; *Calotropisgigantea*; *Vitexnegundo.L*; *Acalyphaindica*; *Cardiospermumhelicacabrim*; *Polygala chinensis*; *Cocosnucifera*; *Ponganiasp*; *Aeglemarmelos*; *Vincarosea*; were taken from different regions of Kanchipuram district near Tamilnadu.

Plant extracts preparation

The excised plant leaves were washed in running tap water to remove the dust and other impurities. The leaves were then washed with distilled water and dried using blotting paper. 2.5g of leaves were weighed and added to 10ml distilled water in a conical flask. The flasks were kept in boiling water bath for 45mins. The extract was then filtered using Whatman No1 filter paper and the filtrates were stored in clean vials in refrigerator.

Antibacterial activity

Well diffusion assay

Well diffusion assay is mainly considered as a qualitative assay and widely used to determine the anti-bacterial activity of crude extract. Nutrient agar prepared was poured in the Petri dish. 24 h growing cultures were swabbed on it. The wells (8mm diameter) were made using a cork borer. The plant extracts with various concentration and the standards were loaded into the wells. The plates were then incubated at 37°C for 24 h in the incubator. The inhibition diameter was then measured.⁷

Preparation of silver nitrate solution

1mM solution of Silver Nitrate (1ml of 1mM AgNO₃ in 99ml deionized water) was prepared and stored in clean amber bottle in dark.

Synthesis of silver nanocoats

20ml of Silver Nitrate working solution was boiled in water bath for 10mins. The solution was then kept on a magnetic stirrer cum hot plate and stirred uniformly. The temperature of the solution was maintained at 60°C. 1000µl of the leaf extract was added in drop wise at an interval of 10 sec. The development of pale yellow colour indicates the formation of silver nanoparticles. The same is repeated for the remaining plant extracts. Optical density of the silver nanoparticles was measured in visible spectrophotometer at 420nm.⁸

UV-Spectrophotometric characterization of Silver Nanocoats

Nanoparticles synthesized from the leaf extract were characterized by UV-Visible spectrophotometer at 420nm to confirm the presence of nanoparticles. *Polygalachinensis* silver nanoparticle was selected as the best because of its best antibacterial activity and absorbance at 420nm.

Optimization of synthesis of silver nanocoats by bio-reductant method

Effect of concentration of bio-reductants of green silver nanocoats

In this optimization experiment, different concentrations of the aqueous extract of *polygala chinensis* leaves (Bio-reductant) were tested to reduce 10 ml of 1 mM silver nitrate solution in order to identify the minimum reducing concentration. The varying volumes tested were 50 µl, 100 µl, 150 µl, 200 µl and 250 µl.⁹

Effect of pH on Bio-reductant of silver nanocoats

In this second part of optimization experiment, silver nitrate solution with different pH was tested with the aqueous extract of *Polygala chinensis* leaves (Bio-reductant) to identify the optimum pH conditions for the green synthesis of silver nanocoats. Throughout the experiment, 50 µl of the extract was kept constant for reducing 10 ml of silver nitrate solution.⁹

Effect of Temperature on Bio-reductant of silver nanocoats

In the third part of optimization experiment, the prepared silver nanocoats were incubated at various temperatures such as 24°C, 37°C, 45°C, 55°C.⁹

Stabilization of silver nanocoats using different polymer

Selection of different polymers

Polymers of different varieties such as Poly vinyl alcohol (alcohol), Starch (carbohydrate), Palmitic acid (lipids) and Polysorbate-80 (surfactants) were prepared at 1% concentration and stored in the test tubes for further use.⁹

Addition of polymer to the silver nanocoats

10 ml of the total complex were prepared by adding 5 ml of Ag nanocoats solution with 5 ml of polymer and vortexed for 20 minutes.⁹

Preparation of Nano embedded fabrics

Preparation of uncoated fabrics

The uncoated fabrics which act as control were prepared by using sterilized cotton which was further soaked in the double sterilized distilled water and kept for 24 hr.⁹

Preparation of coated fabrics with polymer

Pure cotton was used as fabrics. They were cut into small square shaped pieces and were soaked in beaker



containing silver nanocoats solution with the addition of polymer. It was then wrapped with brown paper and was stored in a dark place.⁹

Preparation of coated fabrics without polymer

Pure cotton was used as fabrics. They were cut into small square shaped pieces and were soaked in beaker containing silver nanoparticle solution without the addition of polymer. It was then wrapped with brown paper and stored in a dark place. Some pieces of cotton were placed in distilled water as control (C).⁹

Antibacterial analysis of nanocoats embedded fabrics

LB Agar plate of *Salmonella typhi* and *Staphylococcus aureus* was prepared and the fabrics were placed over it. The plates were incubated at 37°C for 24hrs and observed for zone formation.⁹

Coated fabrics with polymer

The fabrics those were soaked for about 24 hr in the mixture of silver nanocoats and polymer were placed on the two spread plates that contain the microorganism such as *Salmonella typhi* and *Staphylococcus aureus* respectively.⁹

Coated fabrics without polymer

The fabrics those were soaked for about 24 hr in the silver nanocoats alone were placed on the two spread plates that contain the microorganism such as *Salmonella typhi* and *Staphylococcus aureus* respectively.⁹

Coated fabrics with sterile distilled water

The fabrics those were soaked for about 24 hr in the sterile distilled water alone were placed on the two spread plates that contain the microorganism such as *Salmonella typhi* and *Staphylococcus aureus* respectively.⁹

Analysis of silver nanocoats by Scanning Electron Microscopy

Scanning Electron Microscopic (SEM) analysis of silver nanocoats was performed. Samples of silver nanoparticles were prepared on a carbon coated copper grid as a thin layer by using a small quantity of sample. Excess sample was removed and the grid was allowed to dry for 5 min and analyzed.¹⁰

Statistical Analysis

The experimental results were given as mean \pm SD of three parallel measurements. The experimental values were evaluated by using one-way analyses of variance (ANOVA). P values < 0.05 were regarded as "significant". The SPSS 16.0 (Statistical Program for Social Sciences) was used for statistical analysis.

RESULTS

The current research work presents the screening of plants for reduction of silver nanocoats, Spectrophotometric analysis, Antibacterial activity and SEM analysis for nanocoats embedded fabrics.

Screening of plants for silver nanoparticles reduction

Preliminary screening investigation was carried out to identify the better plant which shows higher antibacterial activity and better absorbance. The aqueous extract of *Polygala chinensis* was found to have better antibacterial activity as presented in Table 1.

From the screening studies *Polygala chinensis* indicated a zone of inhibition of about 24 mm for the first day, 24 mm for the fifth day and 23 mm for the tenth day of pour plate against the *E. Coli*.

Eight wild plant species namely *Tragiainvolucrata* L., *Cleistanthuscollinus* (Roxb.) Benth. Ex Hook.f., *Sphaeranthusindicus* L., *Vicoa indica* (L.) Dc., *Allmanianodiflora* (L.) R.Br. ex wight., *Habenariaelliptica* Wight., *Eriocaulonthwaitesii* Koern. and *Evolvulusalsinoides* L. were used for phytochemical extraction with four different solvents. Antibacterial activity of these plants was studied against *Escherichia coli* NCIM 2065 using Kirby Bauer agar disc diffusion assay. Effective antibacterial activity was shown by *T. involucrata* acetone extract (27.3 mm), compared to standard medicinal drug amoxicillin (28.3 mm).¹¹

Table 1: Screening of plants for reduction of Silver nanocoats

Plant Species	Zone of Inhibition (mm)*		
	1 st day	5 th day	10 th day
<i>Phyllanthusamarus</i>	25 \pm 1.1	24 \pm 1.3	22 \pm 1.1
<i>Calotropispigigantean</i>	20 \pm 1.2	26 \pm 1.4	21 \pm 1.3
<i>Vitexnegundo</i> L.	21 \pm 0.9	24 \pm 1.1	23 \pm 1.3
<i>Acalypha indica</i>	21 \pm 1.8	24 \pm 1.5	20 \pm 1.4
<i>C. helicacabrim</i>	19 \pm 1.4	22 \pm 1.4	22 \pm 1.5
<i>Polygala chinensis</i>	24 \pm 1.1	24 \pm 1.7	23 \pm 1.7
<i>Cocosnucifera</i>	18 \pm 1.7	25 \pm 1.9	18 \pm 1.7
<i>Ponganiasp</i>	19 \pm 0.3	27 \pm 1.1	19 \pm 1.8
<i>Aeglemarmelos</i>	18 \pm 1.6	24 \pm 1.2	18 \pm 0.8
<i>Vincarosea</i>	20 \pm 1.7	22 \pm 1.2	22 \pm 1.9

*values are represented as mean \pm SD

Synthesis of silver nanocoats from *Polygalachinensis*



Figure 1: Synthesized silver nanocoats solution from *Polygalachinensis*

The Erlenmeyer flask with the mixture of Bioreductant and silver nitrate were incubated at 37 °C under agitation (200 rpm) for 24–48 h. The solution turned from dark green to dark brown as shown in figure 1. Further it was centrifuged and stored for further studies.

Effect of concentration of Bioreductant on silver nanocoats

In this part of optimization study, the parameter included is the concentration of Bioreductant. The concentrations of Bioreductant (*Polygala chinensis*) analyzed, revealed that 200 µl for 10 ml of 1 mM Silver nitrate solution (1:50 ratio) was optimum for the synthesis of silver nanocoats (table 2).

Table 2: Effect of concentrations of *Polygala chinensis* extract on silver nanocoats

Concentration of 1mM AgNO ₃ solution (ml)	Concentration of Bioreductant (µl)	Absorbance at 420 nm
10	50	0.270
10	100	0.690
10	150	0.940
10	200	1.07
10	250	0.6

Effect of pH on silver nanocoats

In this part of optimization study, the parameter included is the pH. The silver nanocoats were synthesized under 5 different pH conditions, such as 3, 5, 7, 9 and 11, of which pH 7 was identified to be the optimum which is shown in table 3.

Table 3: Effect of pH of *Polygala chinensis* extract on silver nanocoats

pH	Concentration of 1 mM AgNO ₃ solution (ml)	Concentration of Bioreductant (µl)	Absorbance at 420 nm
3	10	50	0.35
5	10	50	0.92
7	10	50	0.78
9	10	50	0.97
11	10	50	1.97

Effect of Temperature on silver nanocoats

In this part of optimization study, the parameter included is the temperature. The silver nanocoats were synthesized under 5 various temperature conditions, such as 24°C, 37°C, 45°C and 55°C of which temperature at 45°C was identified to be the optimum (table 4).

Similar parameters were optimized to synthesize nanoparticles from the phyto pathogenic fungus rapidly which were environmentally friendly. Different concentration exhibited different effects on the nanoparticles.¹²

Table 4: Effect of Temperature of *Polygala chinensis* extract on silver nanocoats

Temperature At °C	Concentration of 1 mM silver nitrate solution (ml)	Concentration of Bioreductant (µl)	Absorbance at 420 nm
24	10	50	0.96
37	10	50	0.7
45	10	50	1.02
55	10	50	0.95

Effect of coated and uncoated fabrics

The coated and uncoated fabrics are kept in the dark room for 5 days and then the fabrics are plated (spread plate technique) against *Staphylococcus aureus* and *Salmonella typhi*.

Coated and uncoated fabrics against *Staphylococcus aureus* and *Salmonella typhi* by using spread plate method

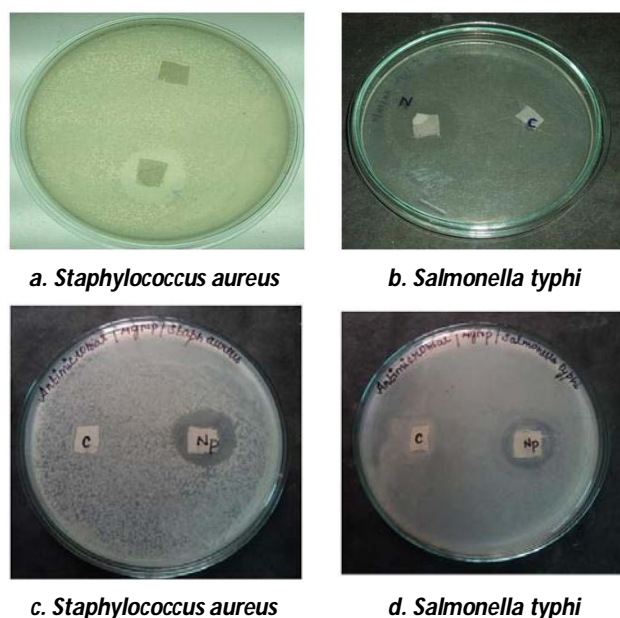


Figure 2: (a) & (b) Uncoated fabrics (without polymer); (c) & (d) Coated fabrics (with polymer)

Table 5: Determination of zone of inhibition for the Coated and uncoated fabrics

Microorganism	Zone of inhibition (mm)*			
	Uncoated fabrics		Coated Fabrics	
	Control	Nanocoats	Control	Nanocoats
<i>Staphylococcus aureus</i>	Nil	18 ± 0.1	Nil	20 ± 0.1
<i>Salmonella typhi</i>	Nil	20 ± 0.2	Nil	22 ± 0.05

*Values are expressed as mean ± SD

Among the fabrics tested against the two organisms *Staphylococcus aureus* and *Salmonella typhi* effective zone was observed for the coated fabric against

Salmonella typhi figure 2(a) & (b) and table 5. Similarly a silver-silica nano composite material with a novel structure and composition was investigated to determine its antimicrobial properties. The material exhibited very good antimicrobial activity against a wide range of microorganisms. The inhibition of microbial growth due to surface contact with the silver-silica nano composite-containing polystyrene demonstrated that materials functionalized with the silver nano composite have excellent antimicrobial properties.¹³

SEM analysis of nanofabrics for identifying the structure of silver nanocoats:

Silver nanocoats were seen in the embedded fabrics from the extract of *polygala chinensis*. This was achieved by Scanning electron microscope (SEM) analysis. The SEM analysis of the nano coated fabric showed that the nanocoats synthesized adhered better which is evident in figure 3(a) & (b).

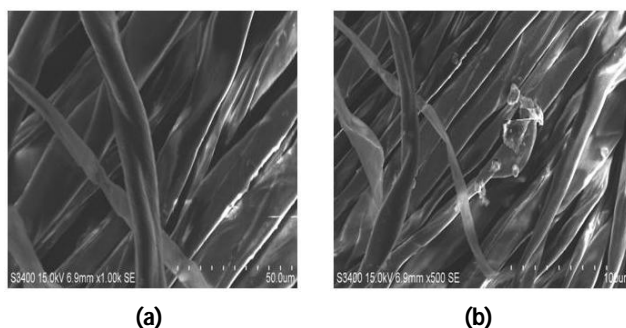


Figure 3: (a) SEM image of Control fabrics; (b) SEM image of Silver nanoparticles embedded fabrics

Similarly SEM analysis shows high-density AgNPs synthesized by cannonball leaf extract. It was shown that relatively spherical and uniform AgNPs were formed with diameter of 13 to 61 nm. The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements.¹⁴

DISCUSSION

The research work involves the efficiency of phyto chemical extracts obtained from different plant sources to reduce silver metal to nano sized particles. Many plants were selected for screening the best Bioreductant. Among these, *Polygala chinensis* constitutes for better bio reduction. The interpretation was based on the optical density values of Silver Nanocoats at 420 nm which was measured using UV Spectrophotometer. The selected plant extract were further investigated for the antibacterial efficacy of the Silver nanocoats and the following parameters were optimized which includes effect of concentration of Bioreductant on silver

nanocoats, effect of pH on silver nanocoats, effect of Temperature on silver nanocoats. The silver nanocoats material thus synthesized was further coated with polymeric fatty acids namely Polysorbate (Tween 80). The coated Silver Nanoparticle was then impregnated on to cotton fabrics for the development of bacterial resistant medical cloths. Further in this study the antibacterial efficiency of silver nanocoats was tested against two different pathogens namely *Salmonella typhi* and *Staphylococcus aureus* which exhibited fairly a good inhibition diameter.

CONCLUSION

Development of nanotechnology leads to improvisation of many science sectors especially the health care. Nosocomial infections are most common in the hospitalized conditions and are mainly due to the outbreak of potential pathogens in such environment. The worse conditions involves trauma and the other surgery related were wounds are prone to severe pathogenic infections. The bandage cloths covering the wounds must be a restricting factor for resisting there microbial attack. In this research work such a microbial resistant nano based medical fabric was developed using polymer coated silver nanocoats synthesized using stable Bioreductant based methodology. The fabrics exhibited antibacterial potency against pathogens and further research and upscale activities with this work will bring the most applicable nano medical products to the humanity.

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