SYNTHESIS OF GOLD NANOPARTICLES (Au NANOPARTICLES) USING CHITOSAN AS REDUCING AGENT AND ITS BIOMEDICAL APPLICATION

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Abstract

In this study, the synthesis of gold nanoparticles from chloroauric acid ($HAuCl_4$) by using chitosan solution as reducing agent. Gold nanoparticles were prepared by adding different volumes (1 mL, 2 mL, 3 mL, 4 mL) of 0.001M $HAuCl_4$ solution to various concentrations of chitosan solution (1 % and 0.5 % w/v). The existence of nanoparticles in colloidal solution was confirmed by Tyndall effect. The characteristic maximum absorption peak at 520 nm in UV spectrum showed the existence of gold nanoparticles. The antimicrobial activity of prepared gold nanoparticles were carried out. Among them the sample prepared from 1 % w/v chitosan and 4 mL 0.001M $HAuCl_4$ was found to be active agent to all tested microorganisms. The synthesized gold nanoparticles was characterized by XRD, SEM, FT IR and EDXRF. XRD analysis of gold nanoparticles in colloidal solution showed the crystalline nature. From the SEM micrograph, the prepared gold nanoparticles were spherical shape. The relative abundance of gold in the prepared sample was investigated by EDXRF. The prepared gold nanoparticles was applied for the treatment of burn wound healing.

Keywords: Chitosan, gold nanoparticles, antimicrobial activity, Tyndall effect, burn wound healing

Introduction

Innovations and discoveries are constantly being made in the medical field. Nanoparticles have contributed to many of these advances. Nanoparticles have important properties, including size, shape, biocompatibility, and selectively. Metal nanoparticles, due to their special properties and also small dimensions, find important applications in optical, magnetic, thermal, electronic and sensor devices, SERS (surface enhanced Raman scattering), catalysis etc. Almost all properties of nanoparticles are due to their small sizes (Collado-Gonzalez *et al*., 2015). Over the past few decades, inorganic nanoparticles, whose structures exhibit significantly novel and improved physical, chemical, and biological properties, phenomena, and

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functionality due to their nanoscale size, have elicited much interest. Nano-phasic and nanostructure materials are attracting a great deal of attention because of their potential for achieving specific process and selectivity, especially in biological and pharmaceutical applications (Khan *et al.*, 2014).

Nanoparticles are being used in medical field to create different therapies and treatment for different diseases. Nanoparticles are being used in many branches of medicine, one attribute these particles have in common is that they need to be biocompatible. Medicine still has many unsolved problems and nanomedicine may hold the key to some of the problems. In general the size and materials of the nanoparticles are very important and specific (Link *et al*., 1999). Specificity allows nanoparticle to be used to treat specific problems in the medical field. The use of nanoparticles in the field of medicine could revolutionize the way we detect and treat damage to the human body and disease in the future and many techniques only imagined a few years ago are making remarkable progress towards becoming realities (Shah *et al*., 2010).

Nanoparticles is very important, and it has recently drawn the attention of numerous research groups, making this area constantly develop. Nanoparticles used in biomedical applications include liposomes, polymeric micelles, block ionomer complexes, dendrimers, inorganic and polymeric nanoparticles, nanorods and quantum dots (Mandal and Sastry, 2014). All have been tested pre-clinically or clinically for targeted drug and gene delivery and as agents to enhance diagnostic imaging output like in MRI. Nanoparticles like water-soluble synthetic polymers (dendrimers) were tested in pre-clinical models for the delivery of drugs, genes, and as imaging agents showing a rich versatility for tailoring their binding properties to several requirements, among them facilitation of cellular uptake of drugs (e.g. cancer drugs) (Ramos *et al.*, 2012).

Gold nanoparticles are of considerable interest for use as a radiosensitizer, because of their biocompatibility and their ability to increase dose deposited because of their high mass energy absorption coefficient (Mc Mahon *et al*., 2011). Chitosan is generally recognized as being non-toxic, biocompatible and biodegradable, and it has many other interesting properties, especially valuable for the prospective biomedical applications. In this paper,

gold nanoparticles were synthesized by chitosan that acts not only as a stabilizer, but also as reducing agents of gold salt (Parida *et al*., 2011). The present study is aimed to synthesize and characterize the gold nanoparticles and apply on burn wound healing.

Materials and Methods

Materials

Commercial chitosan sample from shrimp shell was purchased from Asian Technology Groups Co., Ltd., Local Industry, Yangon, Myanmar. All other chemicals used were of analytical reagent grade. In all experiments, the recommended standard methods and techniques involving both conventional and modern methods were used.

Preparation of Chitosan Solutions (1 % w/v and 0.5 % w/v)

Chitosan (1g) was dissolved in 100 mL of 1 % (v/v) acetic acid solution. It was stirred until the chitosan was completely dissolved. Then the solution (1 % w/v) was filtered and stored for further use and analyses. The above solution was diluted with acetic acid solution to form 0.5 % (w/v) chitosan solution.

Synthesis of Gold Nanoparticles (Au Nanoparticles)

Gold nanoparticles (Au nanoparticles) were prepared using chitosan solutions (1 % and 0.5 % w/v) as reducing agents as well as stabilizing agents. 100 mL of 1 % and 0.5 % (w/v) chitosan solution was individually heated to 80 ± 3 ºC. The collagen when heated at 80 ± 3 ºC denatures chitosan which acts as reducing/stabilizing reagent. 1, 2, 3, 4 mL each of the $HAuCl_4$ solution (0.001 M) was added rapidly at a stirring rate of 3000 rpm. A colour change to wine red colour was observed due to the complex formation between chitosan and Au^{3+} ion. The reaction was carried out under dark conditions and the contents were subjected to vigorous stirring at 80 ± 3 ºC to ensure the complete formation of chitosan capped gold nanoparticles. The prepared Au nanoparticles were denoted as Samples 1, 2, 3, 4 when using 1, 2, 3, 4 mL of 0.001M $HAuCl_4$ solution respectively in 1 % (w/v) chitosan solution. Samples 5, 6, 7 and 8 were prepared by addition of 1, 2, 3, 4 mL each of 0.001M $HAuCl_4$ solution to 0.5 % w/v of chitosan solution.

Characterization of the Prepared Gold Nanoparticles

The prepared Au nanoparticles (Samples 1-8) were characterized by Tyndall effect, UV-vis spectroscopy, XRD, SEM, FT IR and EDXRF analysis. Their antimicrobial activities were also determined. For biomedical application of the selected gold nanoparticle was carried out.

Antimicrobial Activities of Prepared Gold Nanoparticles

The antimicrobial activities of prepared gold nanoparticles were tested with six types of microorganisms-*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilu s, Candida albicans* and *E. coli*.

Biomedical Application of Selected Gold Nanoparticle

From the results of characterizations, the selected gold nanoparticle was utilized in the treatment of burn wound skin of rats. A male Wister rat strain was used, from DMR-LM at the time of experiments. Procedure of this done was carried out by the Institutional Animal Care and Use Committee of Laboratory and were conducted in accordance with established guidelines.

A rat was anaesthetized in chloroform cotton swab bottle with screw cap for 30-45 sec and induced the ringer solution to resistance the heat effect. The hair from dorsal side of albino Wister rat was shaved. A 100 g cylindrical stainless-steel rod (1 cm diameter) was heated to 100 ˚C in boiling water with an insulated rubber handle was used for the infliction of burn. Temperature was monitored using a thermocouple. Burn infliction was limited to the loin. The skin was pulled upward, away from the underlying visera, creating a flat surface. The rod rested on its own weight for 20 seconds at different site on each rat. Average wound size was 1 cm^3. This is a simple method for creating burn wound in a rat burn model.

The burn area was disinfected using methylated alcohol. After surgery, animal was kept in polycarbonated plastic cage and was fed with DMR pellet food and water *ad libitum*. Animal showed good general health condition throughout the study. The animal was sacrifices after 0, 7, 14 and 20 days.

Results and Discussion

Synthesis of Gold Nanoparticles

Confirmation Test for the Presence of Nanoparticles in Solution

Tyndall effects on gold nanoparticles are shown in Figures 1. It was found that the laser light passes through the solutions due to the presence of nanoparticles.

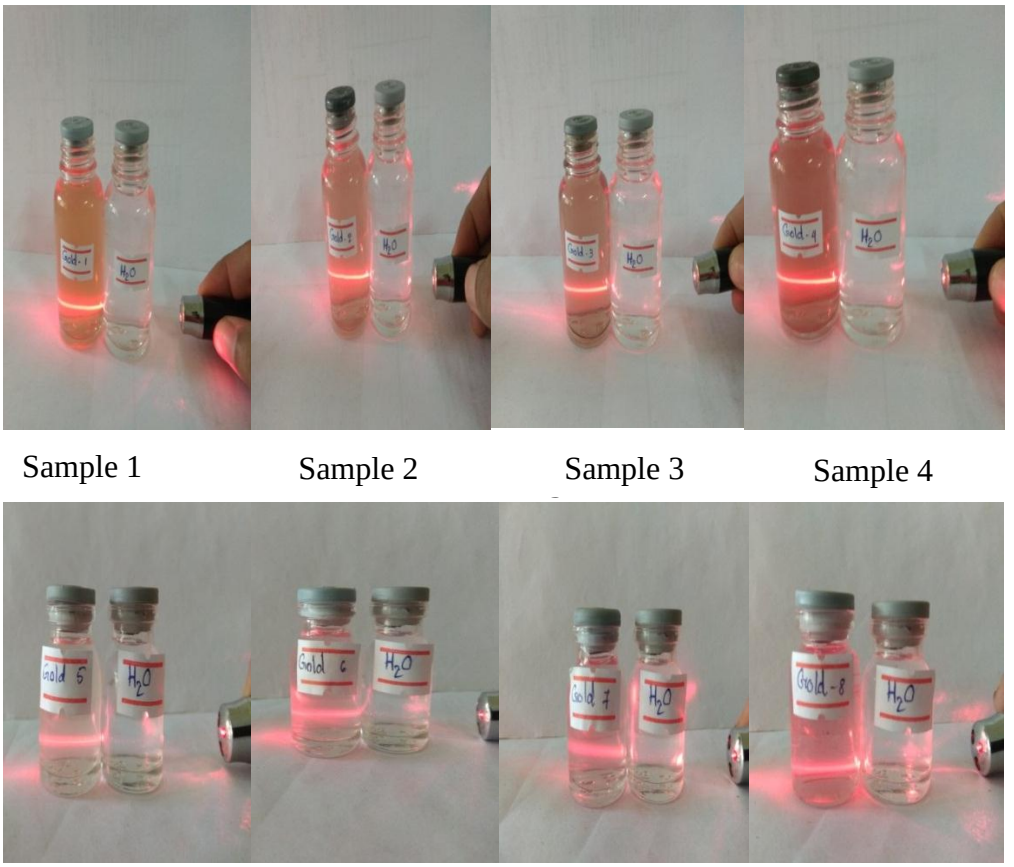
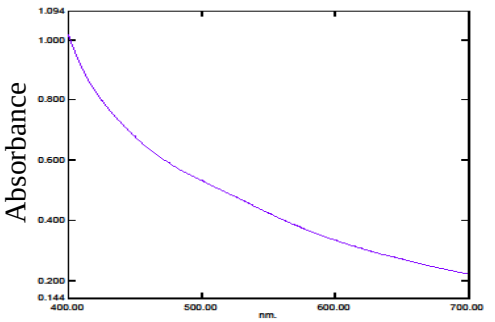


Figure 1: Tyndall effect on gold nanoparticles (Samples 1, 2, 3, 4, 5, 6, 7, 8)

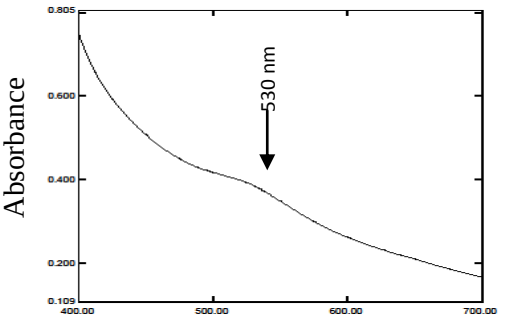
Analysis of Gold Nanoparticles by UV-vis Spectroscopy

The synthesized nanoparticles were confirmed using UV-vis spectroscopy as depicted in Figure 2. The wavelengths of maximum absorption of all Au nanoparticles except sample 1 and 5 were found to be near 530 nm (Table 1) which confirmed that the presence of nanoparticles in colloidal solutions were Au nanoparticles. UV-vis inactive of sample 1 and 5 may be due to the size of nanoparticles (aggregate of nanoparticles). In the literature, the maximum wavelength range of gold nanoparticles is 514-550 nm which depend on their sizes (Martinez *et al.*, 2012). Therefore the wavelengths of maximum absorption of all of the prepared gold nanoparticles except sample 1 and 5 are accordance with the literature value.



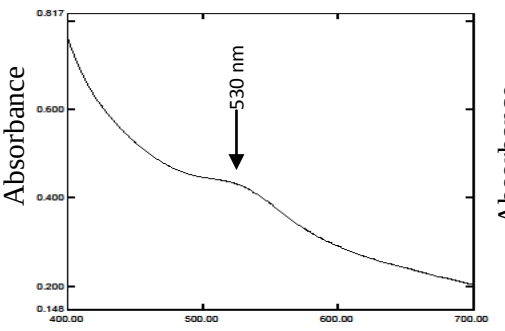
Wavelength (nm)

Sample 1



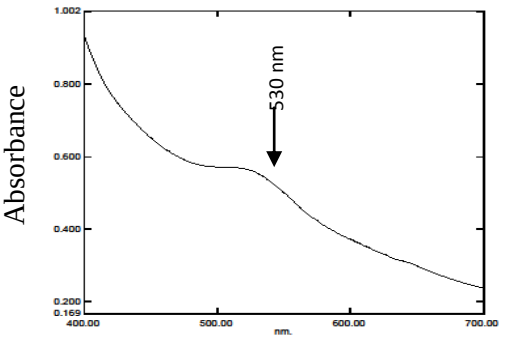
Wavelength (nm)

Sample 2



Wavelength (nm)

Sample 3



Wavelength (nm)

Sample 4

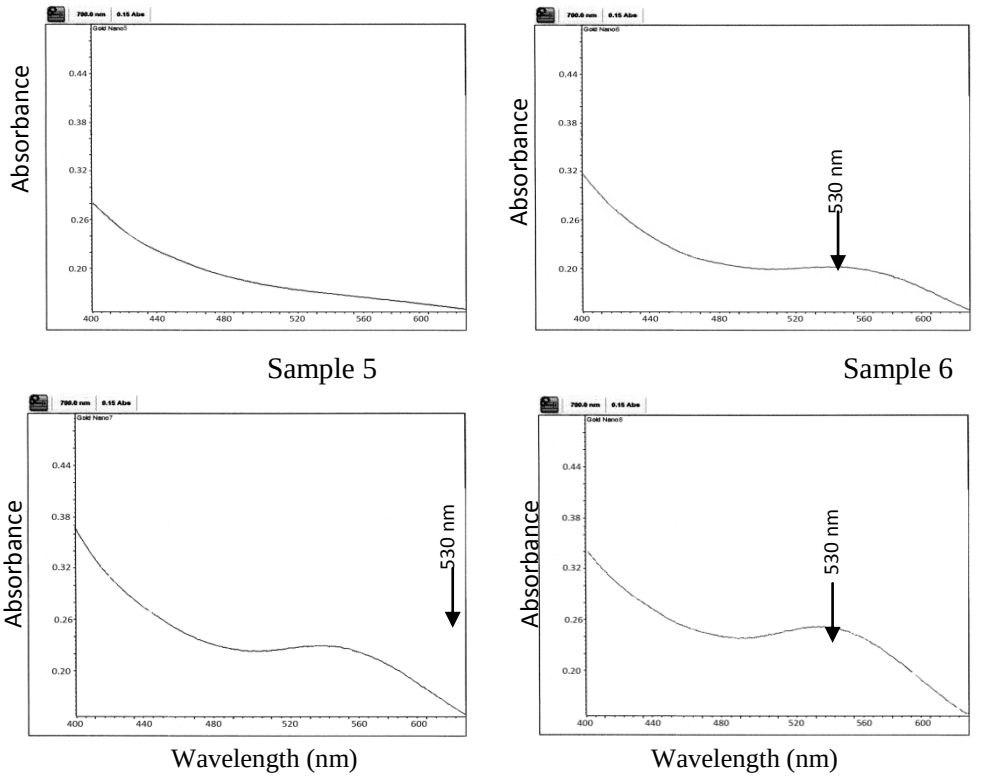


Figure 2: Visible spectra of prepared gold nanoparticle (Sample 1, 2, 3, 4, 5, 6, 7 and 8)

Table 1:The Wavelength of Maximum Absorption of Gold Nanoparticles

Au nanoparticles (Sample)	Observed wavelengths of maximum absorption (nm)
1	-
2	530
3	530
4	530
5	-
6	530
7	530
8	530

*(Martinez *et al*., 2012)

Antimicrobial Activities of Gold Nanoparticles (Au Nanoparticles)

The antimicrobial activities of Au nanoparticles tested against 6 microorganisms which are shown in Figures 3 and 4 and Tables 2 and 3. According to the results, chitosan did not show active to tested microorganisms. The gold nanoparticles, samples 1 and 4, were active to all microorganisms. The gold nanoparticles in samples 5 to 8 showed more active to *S. aureus* and *B. pumilus* than sample 1 to 4 but not active to other microorganisms. Among these samples 1 to 8, sample 4 was chosen for the treatment of burn wound healing.

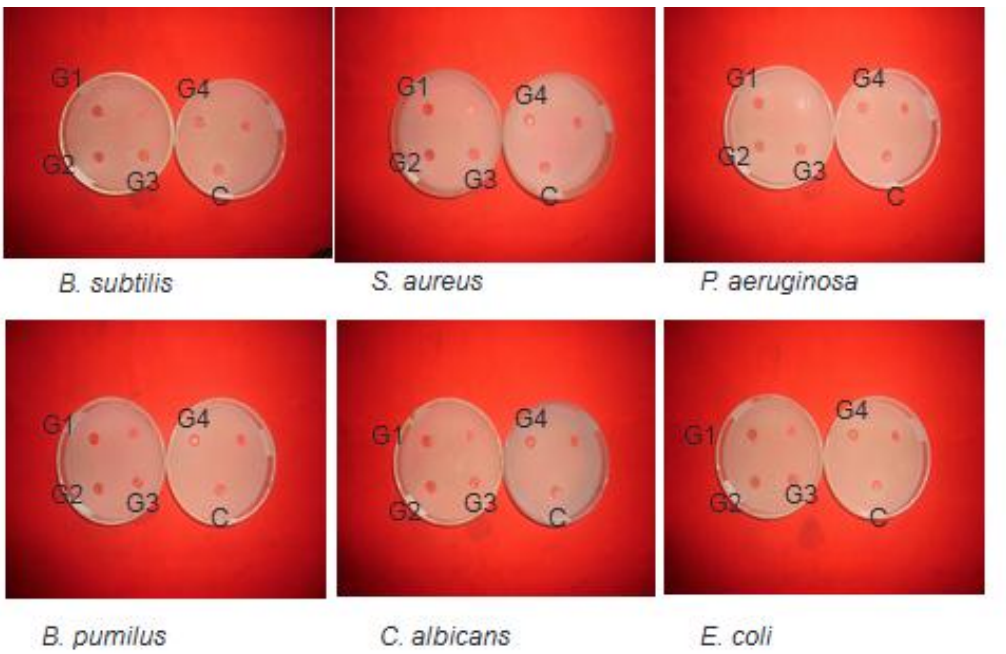


Figure 3: Antimicrobial activities of gold nanoparticles (Samples 1, 2, 3, 4)

Table 2: Antimicrobial Activities of the Prepared Gold Nanoparticles (Sample 1-4) against Six Microorganisms

Sample	Inhibition Zone Diameter (mm)					
	B. subtilis	*S. aureus*	*P. aeruginosa*	*B. pumilus*	*C. albicans*	*E. coli*
1	11 (+)	14 (+)	11 (+)	14 (+)	14 (+)	11 (+)
2	-	13 (+)	11 (+)	14 (+)	12 (+)	11 (+)
3	-	12 (+)	11 (+)	13 (+)	13 (+)	13 (+)
4	15 (++)	11 (+)	12 (+)	13 (+)	13 (+)	12 (+)
1 % Chitosan	-	-	-	-	-	-

Agar well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

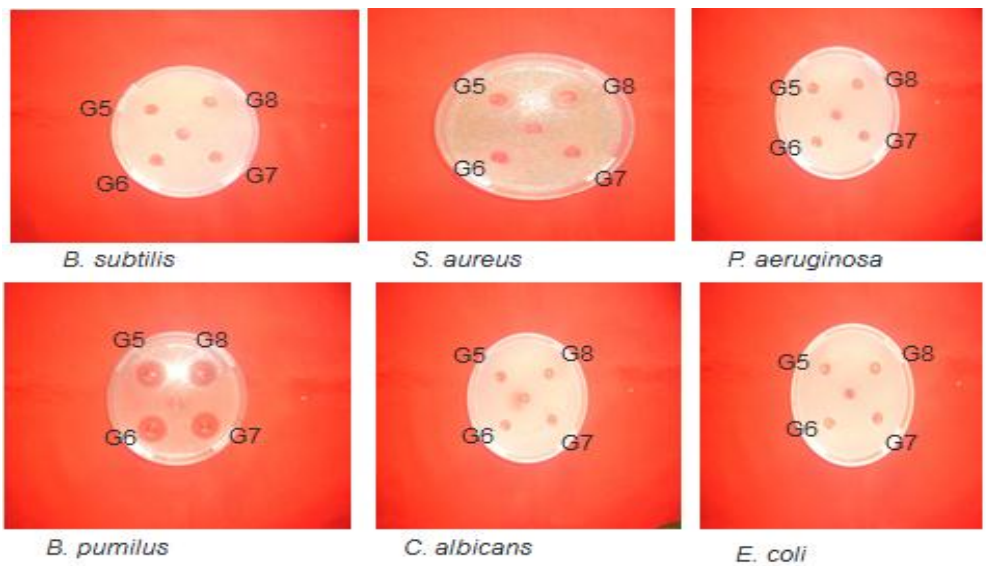


Figure 4: Antimicrobial activities of gold nanoparticles (Samples 5, 6, 7, 8)

Table 3: Antimicrobial Activities of the Prepared Gold Nanoparticles (Sample 5-8) against Six Microorganisms

Sample	Inhibition Zone Diameter (mm)					
	B. subtilis	***S. aureus***	***P. aeruginosa***	***B. pumilus***	***C. albican***	***E. coli***
5	-	20 (+++)	-	26 (+++)	-	-
6	-	20 (+++)	-	25 (+++)	-	-
7	-	20 (+++)	-	25 (+++)	-	-
8	-	20 (+++)	-	29 (+++)	-	-
0.5 % Chitosan	-	-	-	-	-	-

Agar well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

X-ray Diffraction Analysis

X-ray diffractogram of synthesized gold nanoparticle (Sample 4) is shown in Figure 5. Table 4 describes the average crystallite size with respect to their relating parameters of 2θ, d (interplanar spacing) and FWHM (the full-width at half maximum). The Bragg reflections corresponding to the (111), (200) and (220) sets of lattice planes were observed from the patterns. The XRD spectrum of prepared gold nanoparticles show crystalline nature and average crystallite size is 8.56 nm.

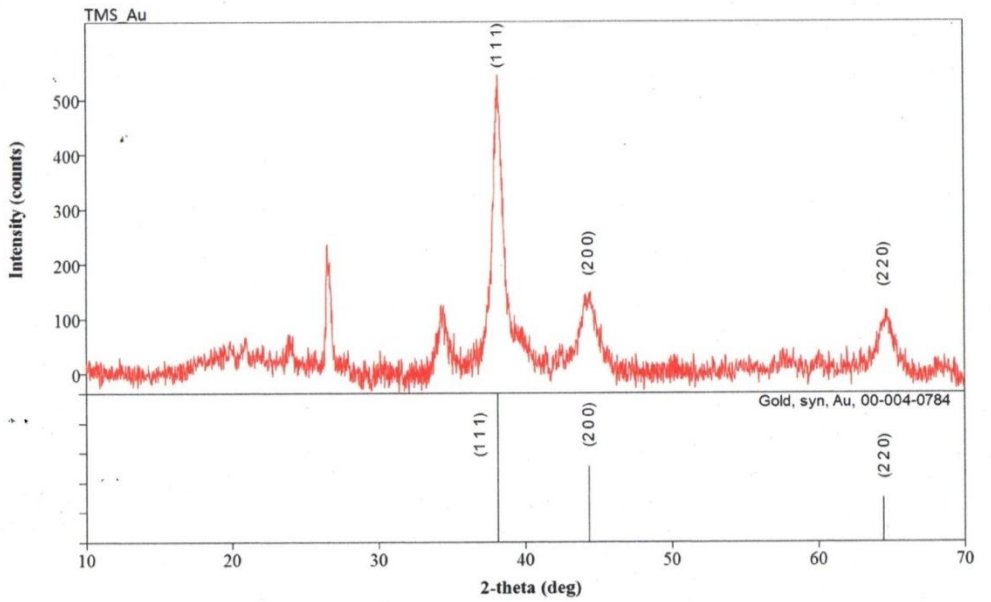


Figure 5: X-ray diffractogram of gold nanoparticles (Sample 4)

Table 4: Lattice Parameters of the Prepared Gold Nanoparticles

2-Theta (°)	d(Å)	(h k l)	Peak area (%)	Phase ID	FWHM	XS (nm)
38.119	2.3589	(1 1 1)	100	Au	0.765	11.5
44.250	2.0453	(2 0 0)	43.03	Au	1.40	6.4
64.50	1.4435	(2 2 0)	27.51	Au	1.25	7.8
				Average Crystallite Size		**8.56**

Scanning Electron Microscopic (SEM) Analysis

Structural and surface morphology of gold nanoparticles (Sample 4) was analyzed by SEM analysis. The micrograph is presented in Figure 6. The various spherical shape of gold nanoparticles were observed.

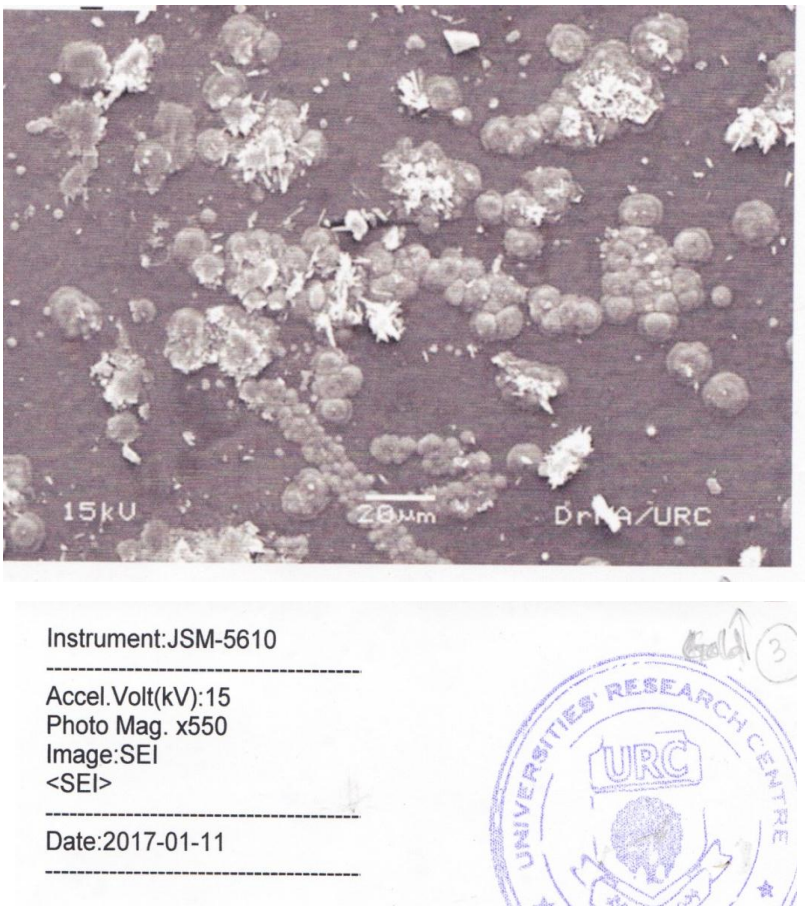


Figure 6: SEM micrograph of gold nanoparticles (Sample 4)

Fourier Transform Infra Red Spectrpscopy (FT IR)

The synthesized gold nanoparticles (Sample 4) was characterized by FT IR spectroscopy. The FT IR spectrum of gold nanoparticles is shown in Figure 7 and the band assignments are shown in Table 5. Infrared study was carried out in order to ascertain the purity and nature of the metal nanoparticles. Metals-oxygen bond generally give absorption band 450 cm^{-1} arising from inter-atomic vibrations. The peak observed at 3458 cm^{-1} are due to O-H strecting and N-H stretching. The peaks at 2825 cm^{-1}, 1639 cm^{-1}, 1560 cm^{-1} and 1413 cm^{-1} are corresponding to the presence of C-H stretching, N-H deformation, C-O stretching and C-N stretching in amine group

respectively. The observed wavenumbers of prepared Au nanoparticles (Sample 4) were in accordance with the literature values (Kumar and Rani, 2013).

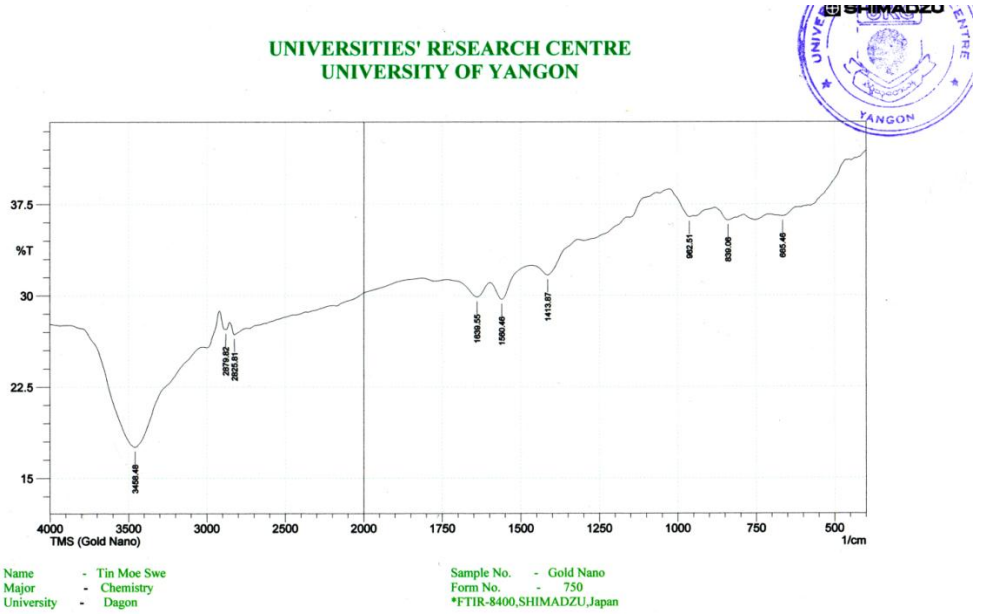


Figure 7: FT IR spectrum of gold nanoparticles (Sample 4)

Table 5: FT IR Spectral Assignment for Gold Nanoparticle (Sample 4)

Observed wavenumber (cm^{-1})	***Literature wavenumber (cm^{-1})**	**Band assignment**
3458	3200-3600	-OH stretching and N-H stretching
2825	2800-2950	C-H stretching
1639	1560-1650	N-H deformation
1560	1550-1600	C-O stretching
1413	1315-1441	C-N stretching in amine group

*(Kumar and Rani , 2013)

Energy Disperse X-ray Florescence (EDXRF)

The synthesized gold nanoparticles (Sample 4) was characterized by Energy Disperse X-ray Florescence (EDXRF). EDXRF spectrum confirms the presence of gold (%) in Sample 4 which is shown in Figure 8. In the spectrum, the presence of calcium may be due to the chitosan which is used as the reducing and stabilizing agent in the preparation of gold nanoparticles.

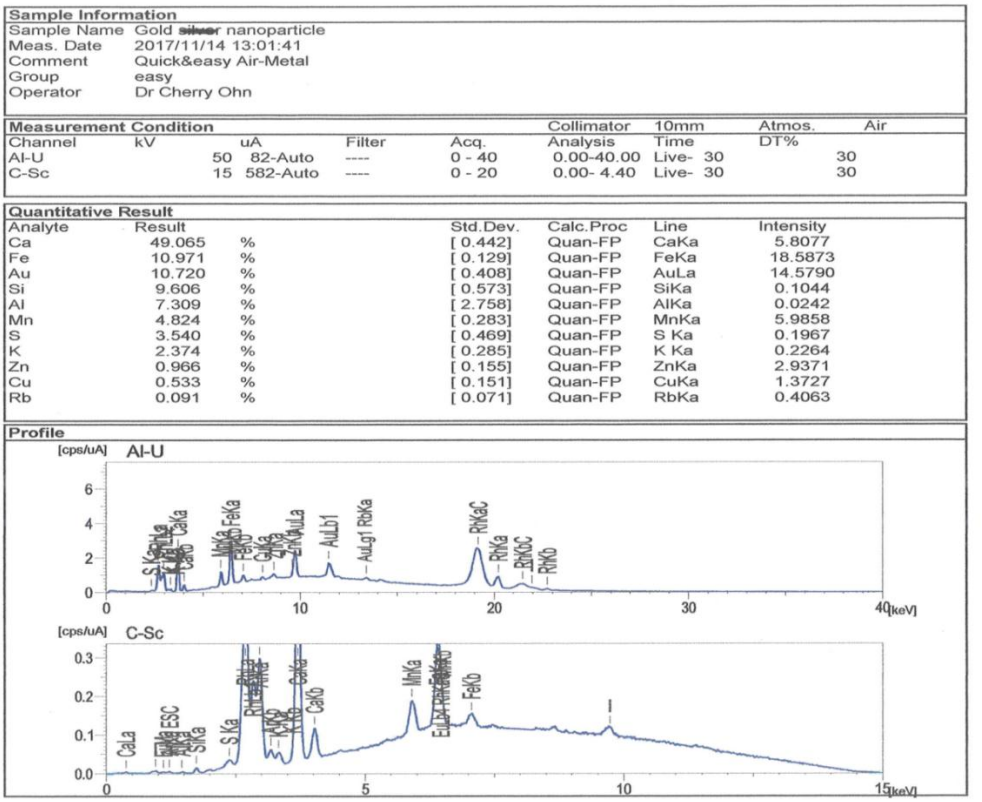


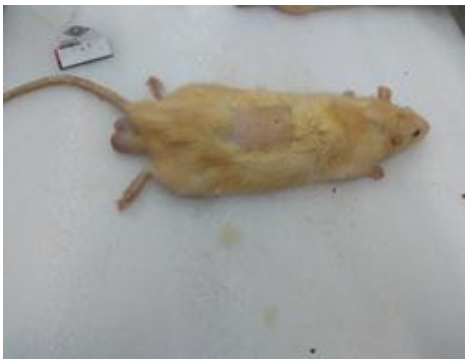
Figure 8: EDXRF spectrum of gold nanoparticles (Sample 4)

Biomedical Application of Gold Nanoparticles

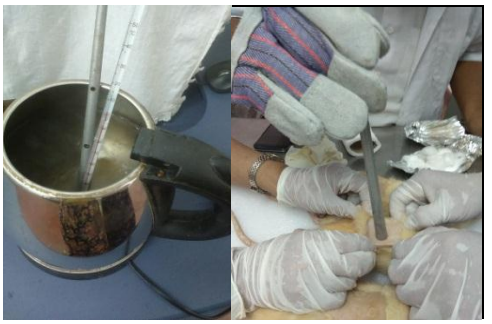
The biomedical application of prepared gold nanoparticles (Sample 4) was studied by animal experiment. The burn skins of rat was treated with gold nanoparticles. The progress of these burn skin was recorded by photo at

specific time interval in Animal Department, Department of Medical Research (DMR). The photographs of progression of burn skin treating with gold nanoparticles (Sample 4) are shown in Figure 9. Treatment with gold nanoparticle after 14th days, significant improvement of burn skin was observed. At 20th days, no scar on burn skin and recover as the original skin.

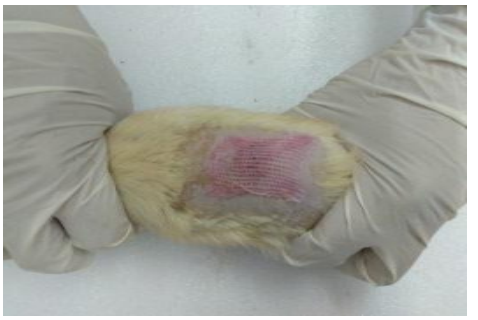
(a) Injected the chloroform



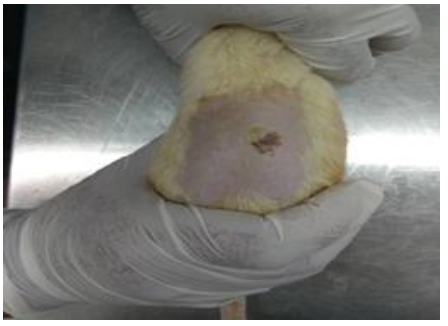
(b) Fleeced the blade



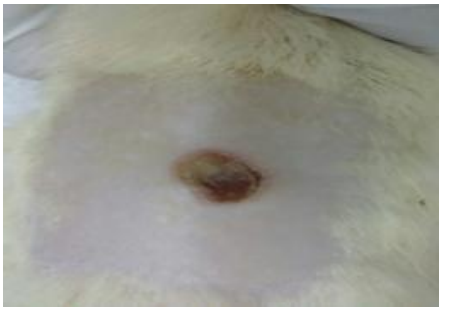
(c) Made burn wound



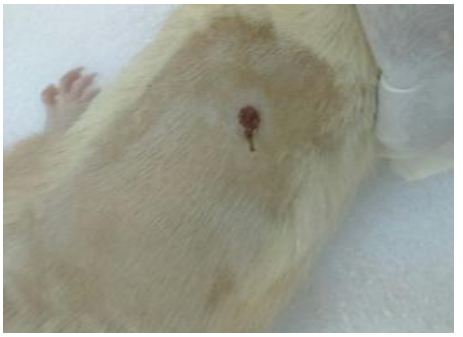
(d) Treated with Gold



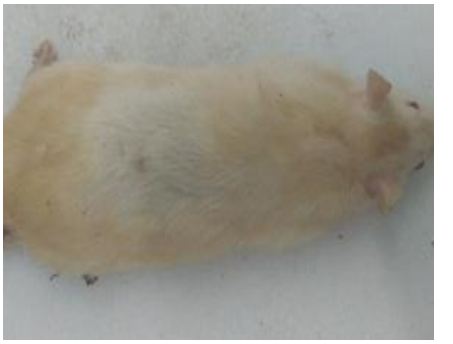
(e) 0- Day



(f) 7- Days



(g) 14- Days



(h) 20- Days

Figure 9: The physical appearences of skin leaching of rats before and after treated with gold nanoparticles

Conclusion

From the overall assessment of the present work, the following inferences can be deduced. Wine red colour of gold nanoparticles was successfully synthesized. The prepared particles which were in the nano range was confirmed by Tyndall scattering. The laser light passes through the solution indicate the presence of nanoparticle. The characteristic absorption peak of prepared particles were confirmed by UV-visible spectroscopy. The maximum absorption wavelengths of gold nanoparticles were found near 530 nm which confirmed that the prepared nanoparticles were gold nanoparticles. According to the results of antimicrobial activities of all gold nanoparticles using 1% w/v chitosan solution as reducing agent showed the activities for all tested microorganisms. Based on UV-vis and antimicrobial activities of samples 1 to 8, the maximum mixing ratio of 4 mL and 0.001 M $HAuCl_4$ in 1% w/v chitosan (Sample 4) was chosen for treatment of burn wound healing. The X-ray diffractogram of selected gold nanoparticles showed the crystalline nature with the crystallite size of 8.56 nm. In the SEM micrograph of sample 4, the prepared gold nanoparticles had spherical shape with various sizes. According to the FT IR spectrum of sample 4, three obvious infrared bands were observed at 3458 cm^{-1} which are due to O-H stretching and N-H stretching. The peaks at 2825 cm^{-1}, 1639 cm^{-1}, 1560 cm^{-1} and 1413 cm^{-1} are corresponding to the presence of C-H stretching, N-H deformation, C-O stretching and C-N stretching of amine group respectively.

The EDXRF results of Sample 4 was found to be 10.72 % of Au. In the biological applications, the burn area of the rat skin was treated with gold nanoparticles (Sample 4) for 20 days. The significant improvement in terms of burn contraction area was after 14-days. At 20^{th} days, no scar on burn skin and recover as the original skin. Therefore, gold nanoparticle (Sample 4) is the effective for the burn healing.

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