SYNTHESIS OF GOLD-SILVER BIMETALLIC NANOPARTICLES (AU-AG NPs) AND STUDY ON ITS APPLICATION

Tin Moe Swe[1], Khin Aye May[2], Khin Than Yee[3]

Abstract

In this study, gold-silver bimetallic nanoparticles (Au-Ag NPs) were synthesized by reduction of different volumes of silver nitrate ($AgNO_3$) and chloroauric acid ($HAuCl_4$) using 1 % w/v chitosan solution as reducing agent. The existence of nanoparticles in colloidal solution was confirmed by Tyndall effect. From the UV-vis spectra, the maximum absorptions of Au-Ag NPs were observed at the wavelengths of 479-562 nm which confirms the characteristic of nanoparticles. The antimicrobial activities of nanoparticles against the organisms were evaluated by agar well diffusion method. Based on the results of UV-vis spectroscopy and antimicrobial activities, sample (Au-Ag NPs 2) was chosen and characterized by XRD, FT IR and EDXRF analyses. XRD spectral data indicated that the selected sample was crystalline nature with nanosizes. The corresponding functional groups and the relative abundance of elements in nanoparticles were observed from FT IR and EDXRF analyses. The application of selected sample was also studied in biomedical field especially burn wound healing compared with standard sofratu drug. It was found that bimetallic nanoparticles (Au-Ag NPs 2) was better than sofratu for healing of burn wound with well-developed sebaceous glands, sweat glands and hair follicles in epidermis layer of skin. This research therefore contributes to academics as well as biomedical application.

Keywords : Chitosan, gold-silver bimetallic nanoparticles, antimicrobial activity, burn-wound healing

Introduction

Alloying of metals is a way of developing new materials that have better technological usefulness than their starting substances. Bimetallic nanoparticles (BMNPs) have excelled monometallic nanocrystals owing to their improved electronic, optical and catalytic performances (Ramos *et al.,* 2010). Gold-silver alloy nanoparticles might have additional biomedical applications because of their distinct optical properties ranging from visible to the near- IR wavelength region. BMNPs often improve the selectivity of metal catalyzed reactions. They show better stability, selectivity and catalytic activity over monometallic nanoparticles (Shah *et al.,* 2012). Bimetallic nanoparticles as catalysts can carry out certain chemical conversions that were unexampled with monometallic nanoparticles as catalyst. This is because bimetallic nanoparticles have a certain combination of two metals wherein each performs a particular function to carry out the overall reaction mechanism (Mandal and Sastry, 2014).

Bimetallic nanoparticles have promising usage in field of nanomedicine. Tunable chitosan-capped spiky urchin like gold-silver bimetallic nanoparticles (Au-Ag NPs) created via single reactor synthesis process, when targeted against cancer in photothermal cancer therapy gave promising results in ablating cancer cells. Bimetallic can be effectively used in drug delivery as they have high surface area to volume ratio, hence can cross blood-brain barrier and epithelial cell junction to reach target site. These bimetallic nanoparticles, due to their excellent electrical and optical properties can be used as biosensors. Bimetallic nanoparticles are of great importance in restitution of environment (Matti *et al*., 2012).

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Bimetallic nanoparticles also serve as great antimicrobials. Bimetallic nanoparticles as antimicrobials can complement the role of antibiotics in combating with bacteria. These nanoparticles can interfere with bacterial growth either by disrupting their membrane or by producing ROS (reactive oxygen species) that cause destruction of DNA and also impede its protein functioning machinery. Au-Ag NPs show antibacterial activity against *Staphylococcus aureus* and *Klebsiella.* These bimetallic nanoparticles showing antibacterial activity can be used in the field of nanomedicine, to form nanodrugs against human pathogens in order to fill the gaps wherein antibiotics failto give positive results (Mazhar *et al*., 2017). Burns are classified as burns by flame, chemical (caused by acids or alkalis), electric high voltage (>1000 mV) and low voltage (< 200 mV), by scalding and by contact. Due to their depth they are divided in first degree that affect only epidermis (sunburn), second superficial degree that affect epidermis and papillary dermis, second deep degree that affect epidermis and reticular dermis and third degree or total thickness that affect the three layers of the skin and muscles. (Garcia-Espinoza *et al*., 2017). This study is aim to synthesize and characterize the gold-silver bimetallic 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 investigations, the recommended standard methods and techniques involving both conventional and modern methods were provided.

Synthesis of Gold-Silver Bimetallic Nanoparticles (Au-Ag NPs)

Gold-silver bimetallic nanoparticles (Au-Ag NPs) were prepared using chitosan solutions (1% w/v) as reducing agents as well as stabilizing agents. 50 mL of 1% (w/v) chitosan solution was heated to 80 ± 3 ºC. Five samples of bimetallic gold-silver nanoparticles were prepared by adding equal volumes of 0.01 M silver nitrate and 0.001 M chloroauric acid (2 mL, 10 mL, 20 mL, 25 mL and 50 mL) in 50 mL of 1 % w/v chitosan solution. These samples were assigned as Au-Ag NPs 1 to 5.

Characterization of Prepared Gold-silver Bimetallic Nanoparticles

The prepared gold-silver bimetallic nanoparticles (Au-Ag NPs 1-5) were characterized by Tyndall effect, UV-vis spectroscopy, XRD analysis, FT IR, EDXRF and investigated their antimicrobial properties. Biomedical application of gold-silver bimetallic nanoparticle (Au-Ag NPs 2) was carried out.

Application of Gold-Silver Bimetallic Nanoparticles on Burn Wound Healing

A total of 3 male Wistar rats strain were used for making the burn wounds.

The wounded animals of three Wistar rats were divided into three groups. The group A used as negative control (without any treatment), group B was dressed with sofratu (standard drug) and an elastic bandage. For the group C, the burns were dressed by Au-Ag NPs 2. The progress of burn wounds of each rat were recorded by taking photographs after 3, 7, 14 and 20 days. The skins of each rat at 20 days were taken to study the histopathological finding.

Results and Discussion

Tyndall Effect

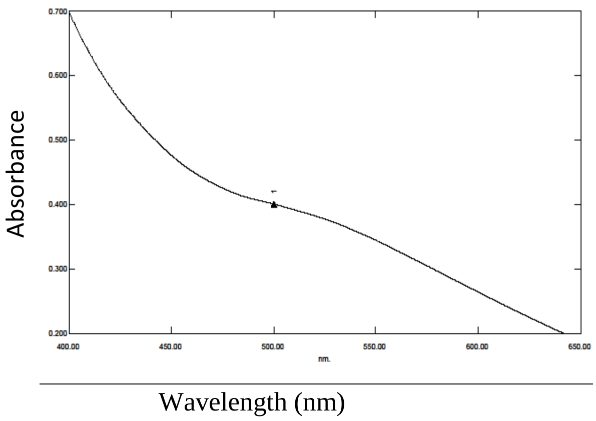
The reduction of silver nitrate and chloroauric acid to gold-silver bimetallic nanoparticles (Au-Ag NPs) were performed at 80 °C for 1 h with continuous stirring by using chitosan as reducing agent and stabilizing agent. The presence of nanoparticles in solutions was detected by using laser beam as Tyndall effect. The scattering of laser beam through solution indicated the presence of nanoparticles. Tyndall effects on gold-silver bimetallic nanoparticles are shown in Figure 1.

Au-Ag NPs 1 Au-Ag NPs 2 Au-Ag NPs 3 Au-Ag NPs 4 Au-Ag NPs 5

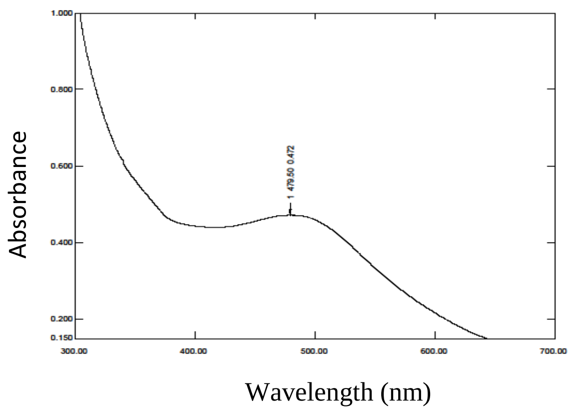
Figure 1 Tyndall effect on gold-silver bimetallic nanoparticles

Analysis of Gold-silver Bimetallic Nanoparticles by UV-vis Spectroscopy

The prepared Au-Ag NPs samples were subjected to UV-visible spectrophotometer and recorded the spectra. Figures 2 shows UV-visible spectra of Au-Ag NPs 1, 2, 3, 4 and 5. The maximum absorption peaks were observed at 479 nm to 562 nm for Au-Ag NPs which correspond to the wavelengths of surface plasmon resonance (SPR) of Au-Ag NPs. These results are in agreement with the various reports (Zhang *et al.*, 2007). The wavelengths of maximum absorption of prepared Au-Ag NPs are summarized in Table 1.



Au-Ag NPs 1



Au-Ag NPs 2

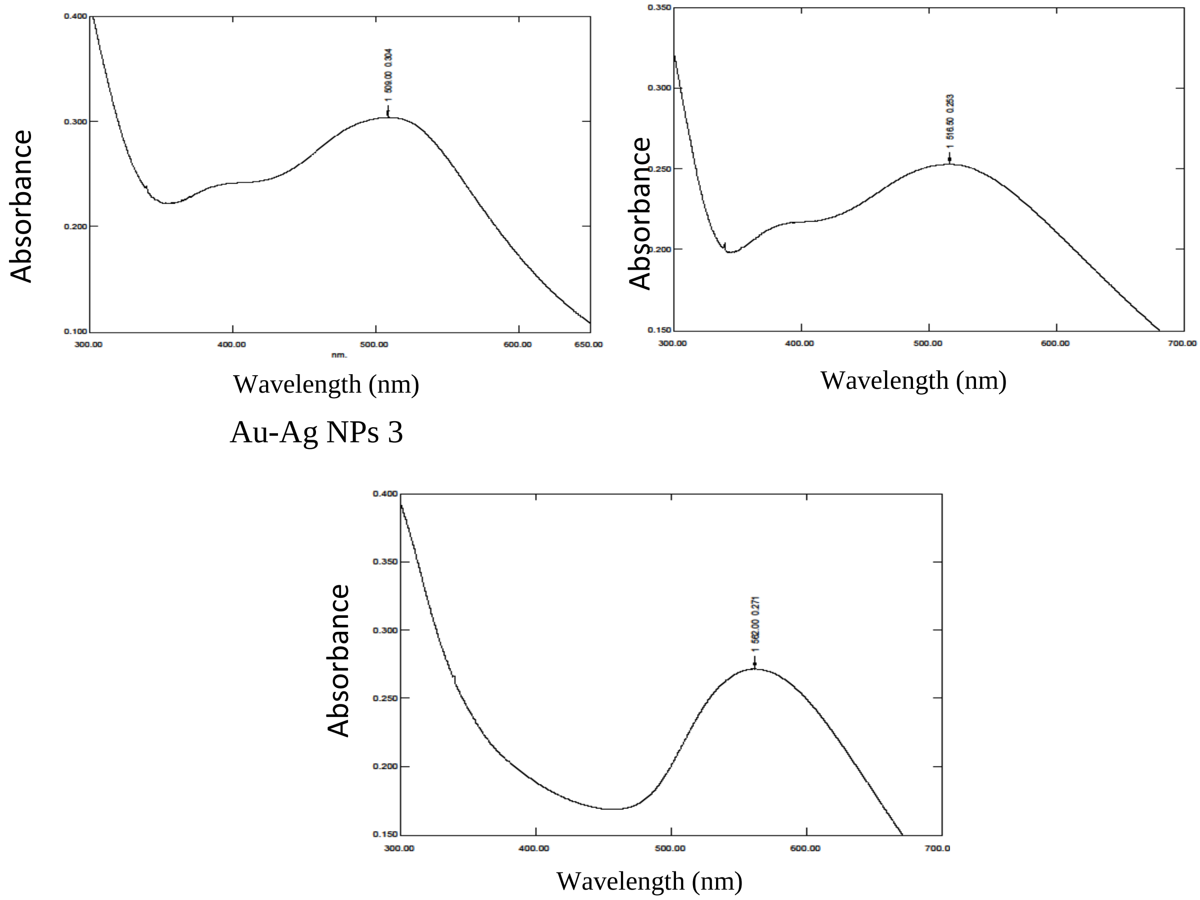


Figure 2 UV-vis spectrum of gold-silver nanoparticle in colloidal solution

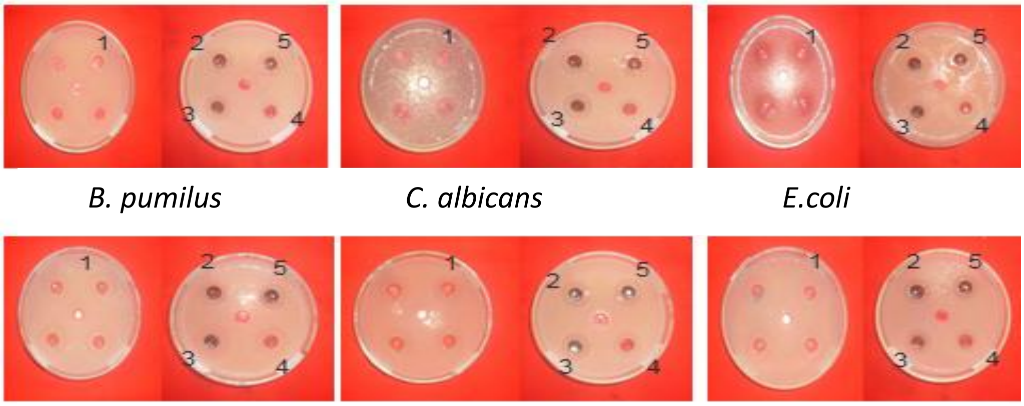
Table 1 Wavelengths of Maximum Absorption of the Prepared Gold-silver Bimetallic Nanoparticles (Au-Ag NPs)

Au-Ag NPs	Observed wavelengths of maximum absorption (nm)	Literature value (nm)
1	-	
2	**479**	
3	509	*396-524
4	516	
5	562	

* Zhang *et al.*, 2007

Antimicrobial Activities of Gold-silver Bimetallic Nanoparticles (Au-Ag NPs)

Screening of antimicrobial activities of Au-Ag NPs were evaluated against with different strains of microorganisms by agar well diffusion method. The antimicrobial activities of Au-Ag NPs nanoparticles are shown in Figure 3 and the results are summarized in Tables 2. According to the results, gold-silver bimetallic nanoparticles Au-Ag NPs 1 to 5 showed active to all tested microorganisms. Based on the results of UV-visible spectroscopy and antimicrobial tests, Au-Ag NPs 2 was chosen for the treatment of burn would healing because the maximum wavelength of Au-Ag NPs 2 is more similarly with the literature values.



B. pumilus *C. albicans* *E.coli*

B. subtilis *S. aureus* *P. aeruginosa*

Figure 3 Antimicrobial Activities of gold-silver bimetallic nanoparticles (Au-Ag NPs 1-5)

Table 2 Antimicrobial Activities of the Prepared Gold-silver Bimetallic Nanoparticles (Au-Ag NPs 1-5) against Six Microorganisms

Au-Ag NPs	Inhibition Zone Diameters (mm)					
	B. subtilis	***S. aureus***	***P. aeruginosa***	***B.pumilus***	***C.albicans***	***E. coli***
1	13 (+)	17 (++)	20 (+++)	13 (+)	11 (+)	14 (+)
2	18 (++)	18 (++)	17 (++)	18 (++)	18 (++)	17 (++)
3	18 (++)	18 (++)	16 (++)	18 (++)	18 (++)	18 (++)
4	12 (+)	15 (++)	17 (++)	17 (++)	17 (++)	17 (++)
5	17 (++)	17 (++)	20 (+++)	19 (++)	18 (++)	17 (++)

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

X-ray Diffraction Analysis

Structural characterization had been performed using XRD analysis and the XRD diffractogram of Au-Ag NPs 2 is illustrated in Figure 4. The lattice parameters of XRD patterns and calculated average crystallite sizes using Scherrer formula are described Table 3. X-ray diffraction pattern of Au-Ag NPs 2 showed the number of Bragg's reflection angles (2θ) 36.213, 44.385 and 64.783 corresponding to planes 111, 200 and 220 respectively, peats were matched with fcc structure and showed the crystalline nature. These results were compared with the literature and found to be matched. The mean crystallite size of Au-Ag NPs 2 was12.91 nm.

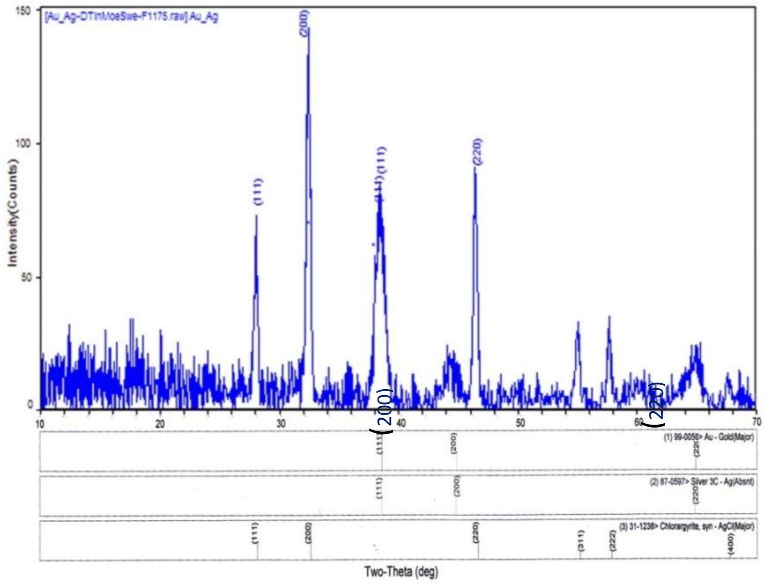


Figure 4 X-ray diffractogram of gold-silver bimetallic nanoparticles (Au-Ag NPs 2)

Table 3 Lattice Parameters of Gold-silver Bimetallic Nanoparticles (Au-Ag NPs 2)

2-Theta (°)	d (Å)	(h k l)	Peak area (%)	Phase ID	FWHM	Crystallite size (nm)
32.421	2.7592	(2 0 0)	80.2	AgCl	0.338	25.6
38.213	2.3533	(1 1 1)	90	Ag	0.775	10.9
38.409	2.3417	(1 1 1)	100	Au	0.714	11.9
44.385	2.0378	(2 0 0)	31.4	Ag	0.728	12.2
44.405	2.0384	(2 0 0)	31.5	Au	0.725	12.0
46.371	1.9565	(2 2 0)	68.5	AgCl	0.398	4.3
64.783	1.3845	(2 2 0)	29.2	Au	0.699	13.6
64.778	1.3853	(2 2 0)	29.1	Ag	0.689	12.8
Average Crystallite size						12.91

Fourier Transform Infra Red Spectrpscopy (FT IR)

Fourier transform infra-red spectroscopy of Au-Ag NPs 2 was performed to study the interaction of bimetallic gold-silver nanoparticles with that of chitosan. The respective spectrum is presented in Figure 5. The band assignments of this sample is expressed in Table 4. All spectra exhibited the functional groups of chitosan. The band appearing at 3446 cm^{-1} correspond to stretching vibration of NH. The bands nearly 1630 cm^{-1} were due to the stretching of C=O of amide group. This C=O functional group may be due to the presence of chitin left in the chitosan (incomplete the deacetylation step in synthesis of chitosan from chitin). The stretching of C-C group appears at 1540 cm^{-1}. The stretching of C-O bond observed at nearly 1400 cm^{-1}. The results indicated that chitosan may have the ability to perform reducing agent as well as capping agent.

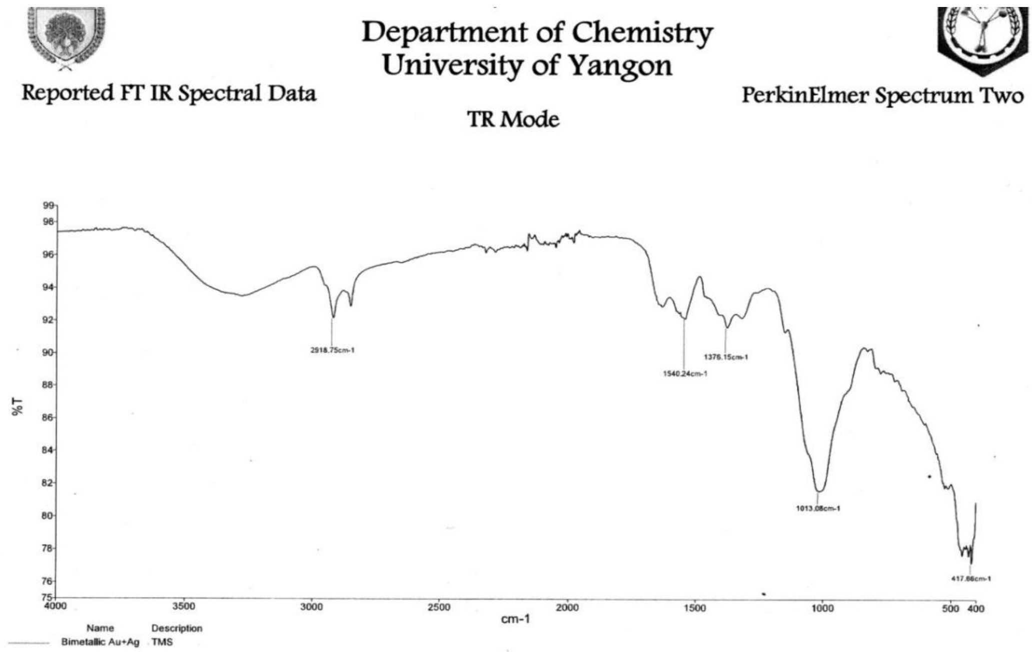


Figure 5 FT IR spectrum of gold-silver bimetallic nanoparticles (Au-Ag NPs 2)

Table 4 FT IR Spectral Assignment for Gold-silver Bimetallic Nanoparticles (Au-Ag NPs 2) using Chitosan Solution as Reducing Agent

Observed wave number (cm^{-1})	*Literature wave number (cm^{-1})	Band assignment
3446	3200-3600	-OH stretching and N-H stretching
2875	2800-2950	C-H stretching
1633	1560-1650	C=O stretching of amide
1540	1550-1600	C-C stretching
1384	1315-1441	C-O stretching

*(Silverstein and Basseler, 1967)

Energy Disperse X-ray Florescence (EDXRF)

EDXRF technique was used for the determination of relative abundance of elements present in samples. The film form of selected sample (Au-Ag NPs 2) was conducted with EDXRF analyses. Ag, Au elements (15.093 % Ag and 10.325 % Au in Au-Ag NPs 2) was detected (Figure 6). Calcium was attributed from commercial chitosan used which may be incomplete demineralization in chitosan production.

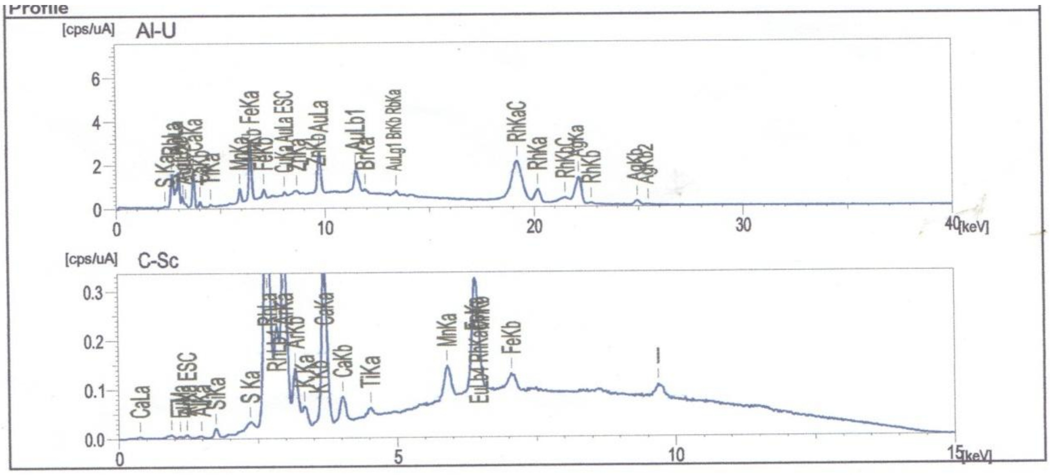


Figure 6 EDXRF spectrum of gold-silver bimetallic nanoparticles (Au-Ag NPs 2)

Biomedical Application of Gold-silver Bimetallic Nanoparticles

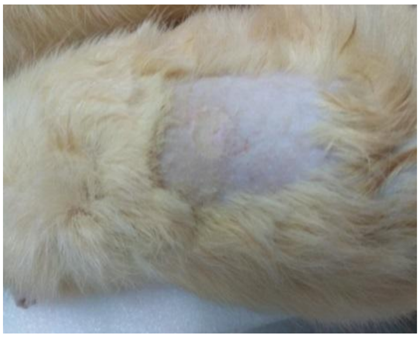
The biomedical application of prepared gold-silver bimetallic nanoparticles (Au-Ag NPs 2) was studied by animal experiment. The step by step making of burn wound are illustrated in Figure 7. The burn skins of rat were treated with gold-silver bimetallic nanoparticles and standard drug (Sofratu). The progress of these burned skins were recorded by photo at specific time intervals in DMR. The photograph of progression of burn skins treated with Sofratu and Au-Ag NPs 2 are shown in Figures 8 and 9.

(a) Injected the chloroform and ringer solution

(b) Fleeced the blade

(c) Heated the iron stick at 100˚C

(d) The iron stick tough the skin for 20s



(d) Burn-wound

Figure7 Making the burn wound on rat model

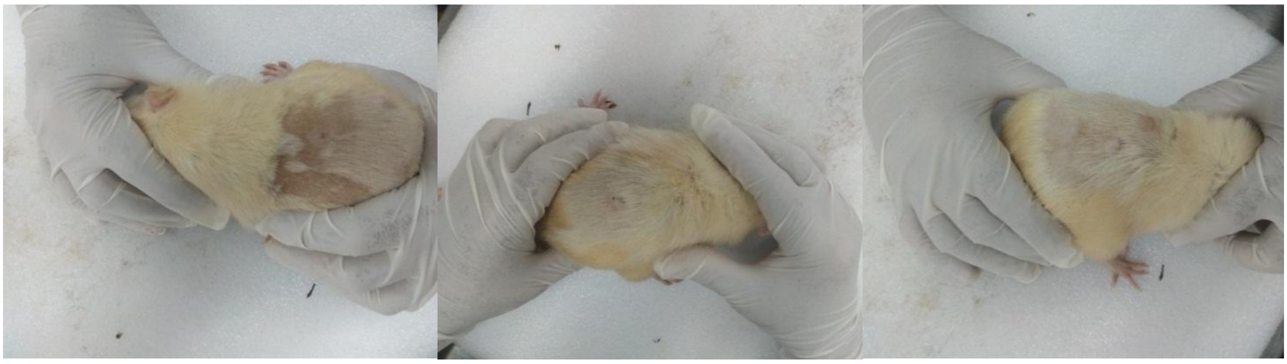
(a) Treated with standard drug (Sofratu) (b) Treated with Au-Ag NPs 2

Figure 8 The physical appearance of skin leaching of rats covered with standard sofratu and Au-Ag NPs 2

(a) Negative Control (b) Control Sofratu (c) Au-Ag NPs 2 (3-Days)

(a) Negative Control (b) Control Sofratu (c) Au-Ag NPs 2 (7-Days)

(a) Negative Control (b) Control Sofratu (c) Au-Ag NPs 2 (14-Days)

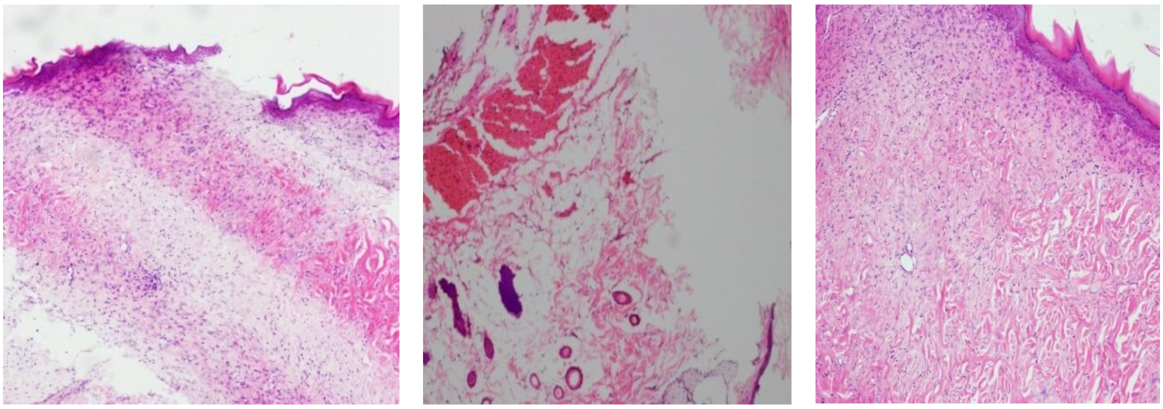


(a) Negative Control (b) Control Sofratu (c) Au-Ag NPs 2 (20-Days)

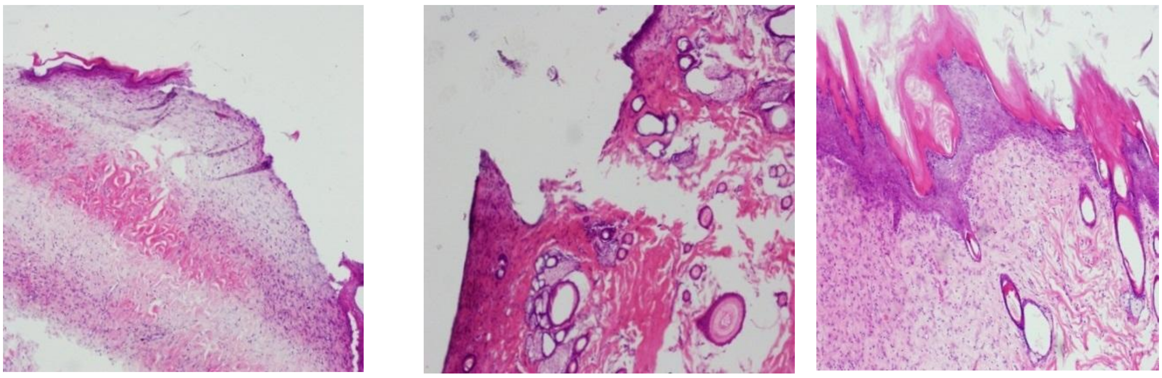
Figure 9 The physical appearences of burned skin after treatment by using standard sofratu and Au-Ag NPs 2 on 3, 7, 14 and 20 days

Histopathological finding of treated and control burned skin

Since the physical appearance changes of skin on visual is not enough to decide the efficiency of treated drugs, histopathological finding was carried out. Wound skin tissue samples treated with Sofratu and Au-Ag NPs 2 were taken on days 20 for histological observation. Histological finding of burned wound skins (epidermis and dermis) treated with Sofratu and Au-Ag NPs 2 are presented in Figure 10. This finding indicated that Au-Ag NPs 2 was better than Sofratu drug to use in burn wound healing because the skin treated with Au-Ag NPs 2 exhibited well developed granulation tissue in both epidermis and dermis layers.



Negative Control Control Sofratu Au-Ag NPs 2 (Epidermis)



Negative Control Control Sofratu Au-Ag NPs 2 (Dermis)

Figure 10 Hematoxyline and eosin-stained section of biopsies for the morphological evaluation of skin lesions of burn skin treated with Standard Sofratu and Au-Ag NPs 2 on days 20

Conclusion

From the overall assessment of the present work, purple colour of gold-silver bimetallic nanoparticles were successfully synthesized. By the Tyndall effect, the laser light scattering indicates the presence of nanoparticles. From the UV-visible spectrum, the maximum absorption wavelengths of prepared gold-silver bimetallic nanoparticles were found to be 479-562 nm. From the results of antimicrobial investigation, all samples of Au-Ag NPs are active to all tested microorganisms. Based on UV-vis spectra and antimicrobial activities, Au-Ag NPs 2 was chosen for treatment of burn wound healing. The X-ray diffractogram of Au-Ag NPs 2 showed the crystalline nature and the average crystallite size was 12.91 nm. The FT IR spectra of selected nanoparticles showed the absorption bands of functional groups of chitosan. It also confirmed that the reducing agents have ability to perform the capping agent. From the EDXRF results, the relative abundance of Ag and Au were found to be 15.09 % Ag and 10.32 % Au in Au-Ag NPs 2. The significant improvement in burn contraction by visually was observed when using Au-Ag NPs 2 on days 20 after burning. Histopathological finding under microscope also reported that the skin treated with Au-Ag NPs 2 exhibited good wound healing and well developed granulation tissue composed of sebaceous glands, sweat gland and follicles in epidermis and dermis layers. From the results, bimetallic nanoparticle can be used effectively in treatment of burned skin.

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