SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SILVER-POLYVINYL ALCOHOL NANOCOMPOSITE FILMS

Aye Mya Nwe[1], Mya Kay Thi Aung[2], Khin Than Yee[3]

Abstract

In this research work, silver nanoparticles were synthesized by using chemical synthesis. In chemical synthesis, trisodium citrate was used as reducing agent. Silver nanoparticles (SNP) were prepared by mixing different volume ratios of 1 % trisodium citrate solution and 1 mM $AgNO_3$ solution (1:10, 2:10 and 3:10 v/v). The existence of SNP in colloidal solutions was confirmed by Tyndall effect and UV-visible spectroscopy. The UV-visible spectrum was revealed the formation of silver nanoparticles by exhibiting the typical surface plasmon absorption maxima at 415 nm. The silver nanoparticles were characterized by modern techniques such as XRD, FT IR, SEM and EDXRF analyses. In XRD analysis, it was found that average crystallite size of SNP powders are in the range of 36.5 nm to 41.7 nm and all the SNP-TSC had the crystalline nature. According to the XRD spectra of all prepared SNP-TSC, there was impurity peaks in the SNP-TSC 2 and SNP-TSC 3 but no impurity peaks in the SNP-TSC 1. From the FT IR spectra of all prepared SNP-TSC, it was observed that a strong symmetrical stretch was observed in the range of 1400 cm^{-1} to 1200 cm^{-1} where major peaks at 1390-1380 cm^{-1} which showed the C-H stretching. SEM micrographs of all prepared SNP-TSC showed agglomeration and therefore larger particle size distribution. From EDXRF analyses, the main constituent element in the SNP-TSC1 is Ag (87.177 %). The different types of polyvinyl alcohol (PVA) film were prepared by using different concentrations (1 - 5 % w/v) of PVA to distilled water. The obtained PVA films were designated as PVA-1, PVA-2, PVA-3, PVA-4 and PVA-5. According to the mechanical properties, PVA-3 film was chosen as selected film. The selected PVA-3 film was characterized by SEM, FT IR and TG-DTA analyses. The PVA-SNP composite films were prepared by varying the volume ratios of 3 % (w/v) PVA solution and colloidal SNP-TSC 1 solution. According to the mechanical properties, PVA-SNP-3 was selected and characterized by SEM, FT IR, TG-DTA and EDXRF analyses. The antimicrobial activity of prepared PVA-SNP composite films were investigated using agar well diffusion method.

Keywords; trisodium citrate, silver nanoparticles, chemical synthesis, PVA- SNP compositfilms

Introduction

Nanoparticles are now being developed for various biological applications such as medicines, antimicrobial agents, wound dressing, drug targeting and deliveries, transfection vectors, bioimaging, and labeling agents etc. Colloidal particles are increasingly receiving attention as an important starting point for the generation of micro and nanostructures. Nanoparticles are under active research because they posses interesting physical properties differing considerably from that of the bulk phase. It comes from small sizes and high surface/volume ratio. The extremely small size of nanoparticles means they exhibit enhanced or different properties when compared with the bulk material. Silver nanoparticles thus allows them to easily interact with other particles and increases their antibacterial efficiency. This effect can be so great that one gram of silver nanoparticles is all that is required to give antibacterial properties to hundreds of square meters of substrate material (Basavaraj, 2012). Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and is capable of killing about 650 type of diseases causing microorganisms.

[1] Assistant Lecturer, Department of Chemistry, Pyay University
PhD Candidate, Department of Chemistry, University of Yangon
[2] Associate Professor, Department of Chemistry, Technological University (Myeik)
[3] Lecturer, Department of Chemistry, Myeik University

Silver has been described as being oligodynamic because of its ability to exert a bactericidal effect at minute concentrations. It has a significant potential for a wide range of biological applications such as antifungal agent, antibacterial agents for antibiotic resistant bacteria, preventing infections, healing wounds and anti-inflammatory. Silver ions (Ag^+) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells. Therefore, silver ions, being antibacterial component, are employed in formulation of dental resin composites, bone cement, ion exchange fibers and coatings for medical devices (Prema, 2011).

The synthesis of silver nanoparticles by chemical methods is popular as they require little instrumental and are relatively inexpensive. The most common way to synthesize silver nanoparticles is via chemical reduction (Zaheer, 2011), where the reduction of silver ions take place in controlled conditions, which can limit the nucleation of reduced (neutral) silver atoms (Angela *etal*., 2018). The most popular preparation of SNP colloids is chemical reduction of silver salts by trisodium citrate. This preparation is simple, but the great care must be exercised to make stable and reproducible colloid (Basavaraj, 2012). Among the methods, chemical reduction was widely studied, due to its advantages of yielding nanoparticles without aggregation, high yield and low preparation cost and simplicity (Prema, 2011).

Mechanism of reaction could be expressed as follows:

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

Trisodium citrate has the chemical formula of $Na_3C_6H_5O_7.2H_2O$.Trisodium citrate anhydrous occurs as white, granular crystals or as white, crystalline powder. It is freely soluble in water and practically insoluble in ethanol (96 %). It is a non-toxic, neutral salt with low reactivity. It is chemically stable if stored at ambient temperatures. Trisodium citrate anhydrous is fully biodegradable and can be disposed of with regular waste or sewage. Trisodium citrate is a tribasic salt of citric acid. It is widely used in food, beverages and various technical applications mainly as buffering, stabiliser or emulsifying agent.

Polyvinyl alcohol (PVA) is a bio- friendly polymer as it is water soluble and has extremely low cytotoxicity. PVA belongs to the group of polymers which can be used in combination with silver nitrate. PVA is one of the synthetic, biodegradable, biocompatible polymer utilized in medical applications such as wound dressing, artificial skin, coatings, transdermal patches, cardiovascular devices and drug delivery systems (Sayed, 2014). In this research work, antimicrobial activities PVA-SNP composite films are investigated against strains of different bacteria.

Materials and Methods

Chemicals

Silver nitrate ($AgNO_3$, 99.8 %), trisodium citrate ($C_6H_5Na_3O_7.2H_2O$, 99 %) and polyvinyl alcohol (Molecular weight 14,000, degree of hydrolysis 98 %) were purchased from the British Drug House (BDH) Chemical Ltd., England.

Preparation of Silver Nanoparticles (SNPs)

Silver nanoparticles were prepared by using chemical reduction method. All solutions of reacting materials were prepared in deionized water. Firstly, 50 mL of 1mM $AgNO_3$ was heated and stirred on magnetic stirrer to boiling. To this solution 5mL of 1 % (w/v) trisodium citrate was added drop by drop. Silver nanoparticles (SNP) were prepared by mixing different volume ratios of 1 % trisodium citrate solution and 1 mM $AgNO_3$ solution (1:10, 2:10 and 3:10 v/v). During this process solution was mixed vigorously and heated until colour change was evident (yellowish brown). Then it was removed from heating element and stirred until cool to room temperature. This solution was placed on ultrasonic bath for 30 min. Sonication was

carried out to reduce size and purify the silver nanoparticles in the colloidal solution. The solution containing silver nanoparticles were centrifuged at 7000 rpm for 20 min. The purified particles were dried by using a hot air oven up to 70 ºC for one and half hours. And then solid silver nanoparticles were obtained. The prepared silver nanoparticles were denoted as SNP-TSC 1 for 1:10, SNP-TSC 2 for 2:10 and SNP-TSC 3 for 3:10 v/v ratios respectively.

Confirmation for the Existence of Silver Nanoparticles in Solution by Tyndall Effect

The laser pointer was placed to the edge of the bottle containing SNP colloidal solution and the light was passed through the solution.

Characterization of Silver Nanoparticles

UV-visible spectrophotometer (SHIMADZU UVmini-1240, JAPAN) by using UV spectra of the silver colloid in the range 330 nm - 460 nm were measured. UV absorption spectra have proved to be quite sensitive to the formation of silver colloids because silver nanoparticles exhibit an intense absorption peak due to the surface plasmon excitation. The absorption band in visible light region (350 nm- 550 nm) is typical for silver nanoparticles.

The phase identification of the silver nanoparticles was carried out by X-ray diffraction method. The solid sample was grounded using a motar and pestle into powder. X-ray powder diffraction measurement was carried out by using (Rigaku, Miniflex-600) powder diffractometer with long fine focus Cu anode.

FT IR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The samples were measured by using Perkin Elmer GX System, FT IR spectrophotometer.

Morphology of the silver nanoparticles were observed on JSM 5610 LV Scanning Electron Microscopy, JEOL-Ltd., Japan Elemental compositions in the prepared silver nanoparticles were determined by EDXRF using EDX-8000 spectrometer (Shimadzu Co.Ltd., Japan).

Preparation of Pure Polyvinyl Alcohol Films (PVA)

Polyvinyl alcohol (PVA) films were prepared by solution casting method. Different concentrations of PVA (molecular weight 14,000, degree of hydrolysis 98 %) (1, 2, 3, 4 and 5 % w/v) solution were prepared with distilled water by stirring and heating at 50 ºC. The PVA solutions were placed in an autoclave at 0.1 MPa and 121 ºC for 20 min. Each polymer solution was casted on melamine plate and dried in air. The series of PVA films were obtained. The prepared PVA films were designated as PVA-1, PVA-2, PVA-3, PVA-4 and PVA-5 according to the percent of PVA.

Preparation of Polyvinyl Alcohol- Silver Nanoparticles Composite Films (PVA-SNP)

Polyvinyl alcohol- silver nanoparticles composite films were prepared by mixing different volume ratios of 3 % (w/v) of PVA solution and the prepared SNP-TSC1 solution (95:5, 90:10, 85:15, 80:20, 75:25, 70:30 v/v) to make up 100 mL. The mixed solutions were stirred by using a magnetic stirrer at 80 rpm for 20 min. Then polymer solutions were kept for sufficient time to remove any bubble formation. Each polymer solution was placed on melamine plate and dried in air. The melamine plates containing the composite solutions were left about 7 days to obtain PVA-SNP composite films. The composite films after drying were removed easily from the melamine plates. The obtained composite films were designated as PVA-SNP-1, PVA-SNP-2, PVA-SNP-3, PVA-SNP-4, PVA-SNP-5 and PVA-SNP-6 respectively.

Determination of the Antimicrobial Activity by Agar Well Diffusion Method

The PVA-SNP composite films were tested with (a) *Bacillus subtilis* (b) *Staphylococcus aureus* (c) *Pseudomonas aeruginosa* (d) *Bacillus pumilus* (e) *Candida albicans* (f) *Escherichia coli* species to investigate the nature of antimicrobial activity.

Results And Discussion

Synthesis of Silver Nanoparticles by using Trisodium Citrate as Reducing agent

Different volumes of 1% w/v trisodium citrate were mixed with 1mM $AgNO_3$ solution in three different ratios of 1:10, 2:10 and 3:10 v/v without varying the other conditions to obtain SNP-TSC 1, SNP-TSC 2 and SNP-TSC 3 respectively. The formation of silver nanoparticles occurs after the reduction of aqueous silver salts with trisodium citrate(1 %) within duration of 10-20 minutes, colour change appear after the completion of reaction, it is well known that, the silver nanoparticles exhibit pale yellow colour. This is due to the excitation of surface plasmon vibration in silver nanoparticles.

Tyndall Effect

Tyndall effect on silver nanoparticles (SNPs) is shown in Figure 1. It was found that the laser light passes through the solution due to the presence of nanoparticles.

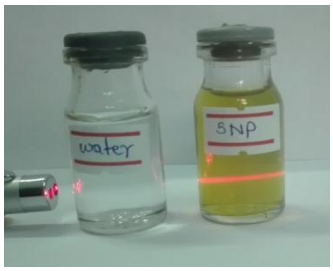


Figure 1 Tyndall effect on the prepared SNPs by chemical synthesis

Characterization of Silver Nanoparticles

UV-visible Studies

The sample when treated with complete reaction conditions, changed in colour of colloids solution from pale yellow to yellowish brown. This colour change preliminary showed the presence of silver nanoparticles or reduction of Ag^+ of $AgNO_3$ to Ag^0. The maximum absorbance was observed at 415 nm due to surface resonance of silver nanoparticles (Figure 2).

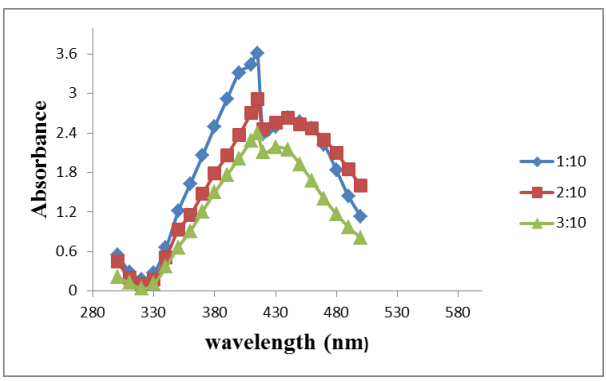
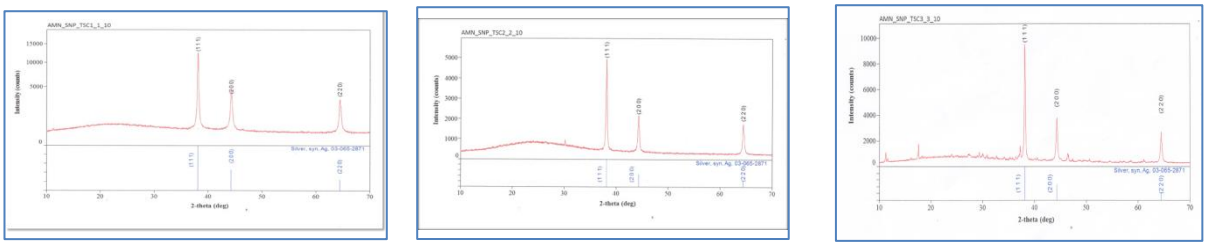


Figure 2 Wavelength of maximum absorption of the prepared silver nanoparticles (SNP-TSC) by chemical method

X Ray Diffraction Studies

The XRD data were obtained in the 2θ range from 10º to 70º in step scan mode with 2θ step of 0.02º. The diffraction pattern indicated that the sample is the silver nanoparticles. The XRD pattern of SNPs is shown in the Figure 3 (a, b, c). The intensive diffraction peak at a 2θ value of 38.08º from the (111) lattice plane of face centered cubic(fcc) silver unequivocally indicated that the particles are made of pure silver. Two additional broad bands were observed at 44.27º (2θ) and 64.41º (2θ) corresponding to the (200) and (220) planes of silver respectively. However, SNP-TSC1 samples showed only single phase of Ag and no impurity peaks. Therefore, SNP-TSC1 was chosen for the further experiment. The crystallite sizes of all of the prepared SNP powders were calculated by Debye-Scherrer equation (Table 1). According to the Table, the average crystallite size of the prepared SNP powders are SNP-TSC1 (40.6 nm), SNPTSC2 (36.5 nm) and SNP-TSC 3 (41.7 nm).



(a) (b) (c)

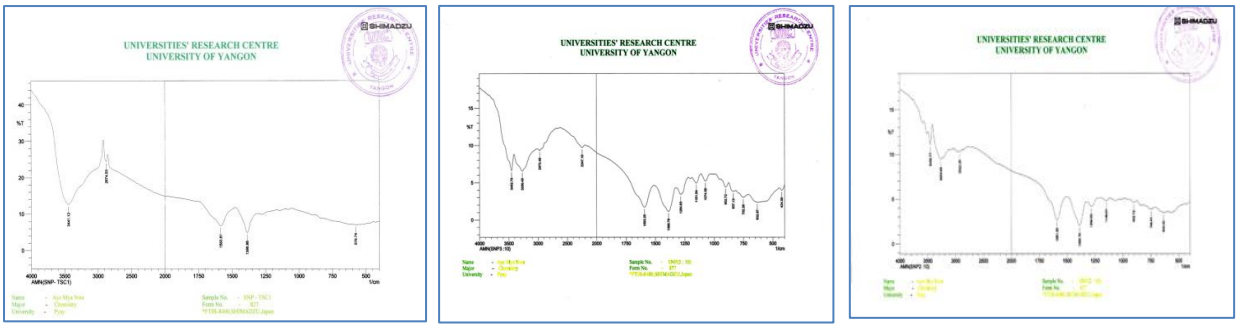
Figure 3 XRD diffractograms of silver nanoparticles by chemical synthesis
(a) SNP-TSC 1, (b) SNP-TSC 2 and (c) SNP-TSC 3

Table 1 Crystallite Size of Silver Nanoparticles by XRD Analysis

Sample	2θ (degree)	FWHM (degree)	(hkl)	Crystallite size(nm)	Average crystallite size(nm)
SNP-TSC 1	38.089	0.196	111	44.7	
	44.269	0.266	200	33.7	40.6
	64.409	0.226	220	43.4	
SNP-TSC 2	38.116	0.242	111	36.2	
	44.282	0.294	200	30.5	36.5
	64.421	0.229	220	42.8	
SNP-TSC 3	38.128	0.197	111	44.6	
	44.307	0.249	200	36.0	41.7
	64.438	0.219	220	44.7	

FT IR Analysis

Figure 4 shows the FT IR spectra of all of the prepared SNP-TSC samples. The characteristic absorption bands at 3441, 2874, 1583, 1386 and 576 cm^{-1} were observed. These peaks correspond to groups present in the sample and are indicated to O-H stretching, C-H stretching, C=C stretching, O-H bending and C-H out of plane bending. These bands are attributed from tri-sodium citrate a capping agent. FT IR spectral peaks of SNP-TSC1, SNP-TSC2 and SNP-TSC3 are shown in Table 2.



(a) (b) (c)

Figure 4 FT IR spectra of the prepared (a) SNP-TSC1, (b) SNP-TSC2 and (c) SNP-TSC3

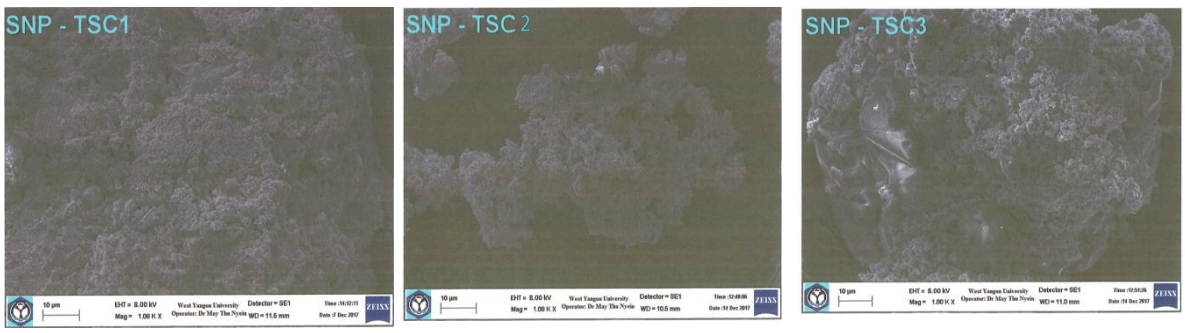
Table 2 FT IR Spectral Assignments of the Prepared Silver Nanoparticles

Observed Frequency (cm^{-1})			*Literature Frequency (cm^{-1})	Band Assignments
SNP-TSC1	SNP-TSC2	SNP-TSC3		
3441	3450	3452	3600-3200	-OH stretching vibration
2874	2922	2970	2980-2850	C-H stretching vibration of sp^3 hydrocarbons
1583	1591	1593	1610-1560	C=C ring skeletal stretching vibration
1386	1388	1388	1425-1380	-OH bending vibration
576	748,636	837,752,632	830-500	C-H out of plane bending vibration

*Silverstein and Webster, 1998

SEM Analysis

The scanning electron micrographs of the prepared silver nanoparticles are shown in Figure 5. The present investigation of nanoparticle using SEM micrograph clearly illustrates the spherical shaped or roughly spherical shaped and some irregular shaped nanoparticles having the size range of 40 nm. It can be concluded that SNPs are initially monodispersed but drying process lead to agglomeration of many particles resulted into larger size particles.



(a) (b) (c)

Figure 5 Scanning electron micrographs of the silver nanoparticles by chemical synthesis (a) SNP-TSC1, (b) SNP-TSC 2 and (c) SNP-TSC3

EDXRF Analysis

Figure 6 shows EDXRF spectrum of SNP-TSC1. According to the EDXRF spectrum of the prepared SNP-TSC1, silver was the major constituent (87.177 %) and other were trace constituents. Table 3 shows the relative abundance of elements in the prepared SNP-TSC1 by EDXRF.

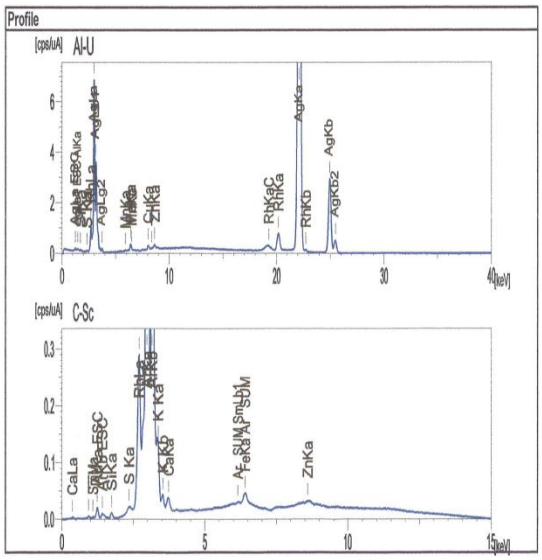


Figure 6 EDXRF spectrum of SNP-TSC1

Table 3 Relative Abundance of Elements in Prepared SNP-TSC1 by EDXRF

No.	Elements	Relative Abundance (%)
1	Silver	87.177
2	Aluminum	7.166
3	Silicon	2
4	Potassium	1.997
5	Calcium	0.465
6	Iron	0.43
7	Sulphur	0.357
8	Copper	0.15
9	Zinc	0.146
10	Manganese	0.112

Aspect of the Preparation of Pure PVA Film

Pure PVA films were prepared using various percents of PVA (1 % to 5 % w/v) in distilled water by solution casting method. The prepared pure PVA films appeared to be homogeneous, transparent and colourless. According to the mechanical properties (tensile strength, elongation at break and tear strength) of prepared PVA films, PVA-3 was chosen for the optimum films (Figure 7 and Table 4).

Figure 7 The photographs of (a) PVA-1 (b) PVA-2 (c) PVA-3 (d) PVA-4 (e) PVA-5 films

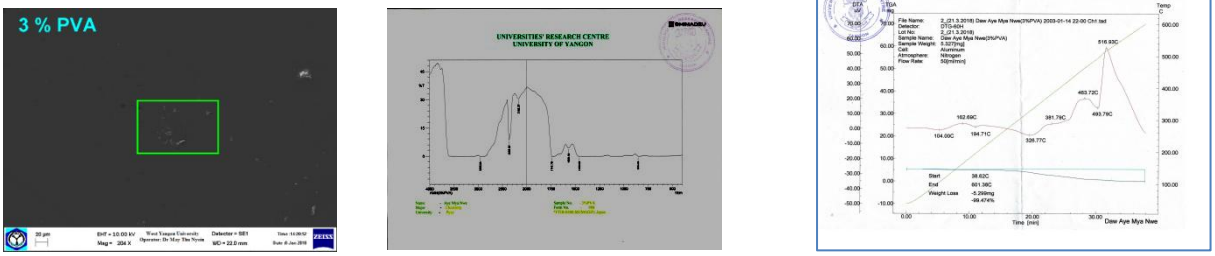
Table 4 Mechanical Properties of the Prepared Polyvinyl Alcohol Films

Prepared Films	PVA(%) w/v	Tensile Strength (MPa)	Elongation at Break (%)	Tear Strength (kNm^{-1})
PVA-1	1	26.0	128	96.3
PVA-2	2	29.7	202	114.0
PVA-3	3	31.7	241	155.8
PVA-4	4	27.1	216	87.9
PVA-5	5	33.0	282	101.0

Thickness = ~ 0.57 mm

Characterization of the Prepared PVA Film

The selected prepared PVA-3 film was characterized by modern techniques such as SEM, FT IR and TG-DTA as shown in Figure 8 (a, b, c). The SEM micrograph of the prepared PVA-3 membrane has smooth surface and homogeneous film. The FT IR spectrum of pure PVA film exhibits a major peaks associated with PVA. The major peaks were observed at 3600 cm^{-1}, 2955 cm^{-1}, 1568 cm^{-1} and 1458 cm^{-1} corresponding to O-H stretching, C-H stretching, C=C stretching and O-H bending. As seen in Figure 8(c), the thermogram of PVA-3 film possesses three stages of distinct weight loss between 38 ºC to 600 ºC. The first stage ranged between 38 ºC and 120 ºC with 11.04 % of weight loss and this was due to the evaporation of loosely bound water. The second stage ranged between 120 ºC and 350 ºC was due to the scission of functional group of polymer chain. The third stage of weight loss indicated the degradation of polymer backbone and progressive rupture of chain, combustion and formation of residue (Table 5)



(a) (b) (c)

Figure 8 (a) SEM micrograph, (b) FT IR spectrum and (c) TG-DTA thermogram of the prepared polyvinyl alcohol PVA-3 film

Table 5 Thermal Analysis Data of the Prepared Polyvinyl Alcohol PVA-3 Film

TG Thermogram			Nature of Peak DTA	Remark
Temperature Range (°C)	**Total Weight Loss (%)**	**Break in Temperature (°C)**		
38-120	11.04	104	endothermic	-due to the evaporation of loosely bound water
120-350	33.13	326	endothermic	due to the scission of functional group of polymer chain
350-600	55.30	463 516	exothermic	Due to the degradation of polymer backbone and progressive rupture of the chain, combustion and formation of residue

Aspect of Preparation of PVA-SNP Films

The PVA-SNP composite films were prepared by solution casting method from solutions of PVA-3 and SNP–TSC1 in deionized water at various compositional ratios. The basic method for the synthesis of NPs in PVA is to disperse metal ion solution in the polymer and reduce to zero valent states. The PVA-SNP composite films were prepared by using different volume ratios of PVA-3 solution and SNP-TSC1 colloidal solutions (95:5, 90:10, 85:15, 80:20, 75:25 and 70:30). Solutions of PVA-SNP appeared to be homogeneous and transparent. The colour of the solution varied from colourless of pure PVA solution to greenish yellow colour with increasing SNP content. The distinctive colours of nanosilver are due to the phenomenon known as plasmon absorbance. With an increase in reaction time, particle size and aggregation of silver nanocrystal gradually increased together. The prepared PVA-SNP composite films are shown in Figure 9.

(a) (b) (c) (d) (e) (f)

Figure 9 The photographs of PVA-SNP composite films (a) PVA-SNP-1, (b) PVA-SNP-2, (c) PVA-SNP-3, (d) PVA-SNP-4, (e) PVA-SNP-5 and (f) PVA-SNP-6

Aspect of Mechanical Properties of Polyvinyl Alcohol- Silver Nanoparticles Composite Films

The mechanical properties of polyvinyl alcohol-silver nanoparticles composite films are shown in Table 6. From the resulting data, PVA-SNP-3 composite film was found that tensile strength of 37.7 MPa, elongation at break of 262 % and tear strength of 139 kNm^{-1}. Therefore, PVA-SNP-3 was chosen for optimum film due to its highest elongation at break.

Table 6 Mechanical Properties of Polyvinyl Alcohol- Silver Nanoparticles composite Films

No.	Parameters	PVA-SNP Composite Films					
		PVA-SNP-1	**PVA-SNP-2**	**PVA-SNP-3**	**PVA-SNP-4**	**PVA-SNP-5**	**PVA-SNP-6**
1	Tensile strength (MPa)	39.4	35.1	37.7	29.3	27.6	32.9
2	Elongation at Break (%)	256	219	262	158	235	168
3	Tear Strength (kNm^{-1})	146.7	129.2	139.0	125.5	156.0	172.7

Thickness = ~ 0.43

Characterization of the Prepared Optimum PVA-SNP-3 Film

The selected prepared PVA-SNP-3 film was characterized by modern techniques such as SEM, FT IR and TG-DTA analysis (Figures 10 and 11). The surface morphology of PVA-SNP-3 film was observed using SEM micrograph as shown in Figure 10(a). The PVA-SNP-3 film exhibits a smooth and compact surface with spherical in shape. This spherical shape is due to the distribution of silver nanoparticles through the PVA matrix. To determine the functional group on PVA-SNP-3 film, FT IR analysis was performed. The band intensities in different regions of the spectrum for PVA-SNP-3 film is shown in Figure 10(b). The band intense absorption peak around 3292 cm^{-1} was due to the O-H stretching vibration. The peak located at around 2938 cm^{-1} was attributed to the C-H stretching. The peak at 1714 cm^{-1} indicated the C=O stretching vibration. The peak at 836 cm^{-1} was due to C-H bending vibration. According to TG-DTA thermogram, the initial weight loss (around 100 ºC) observed was due to the loss of moisture present in the PVA-SNP-3 film. The subsequent steps of degradation were varied depending on the type of film. The second decomposition step of PVA-SNP-3 film was observed approximately 140-370 ºC, which was described to the decomposition of volatile materials. The main stage of weight loss or the
maximum thermal decomposition exhibited around 400 ºC (Figure 11 and Table 7).

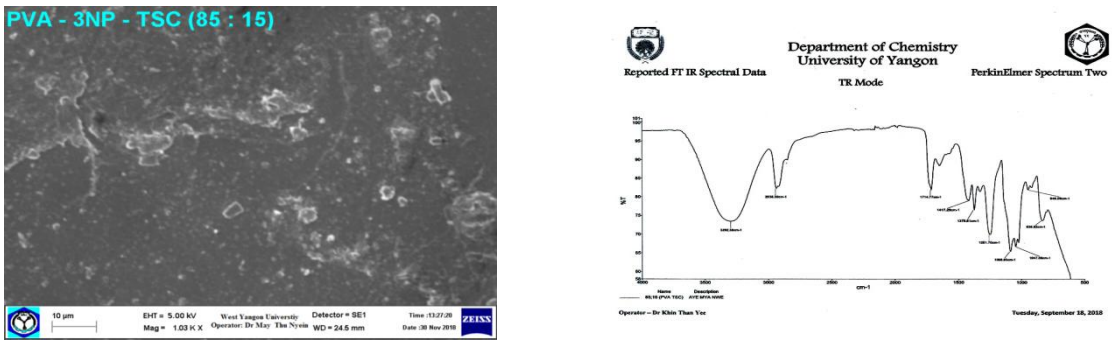


Figure 10 (a) SEM micrograph and (b) FT IR spectrum of PVA-SNP- 3 film

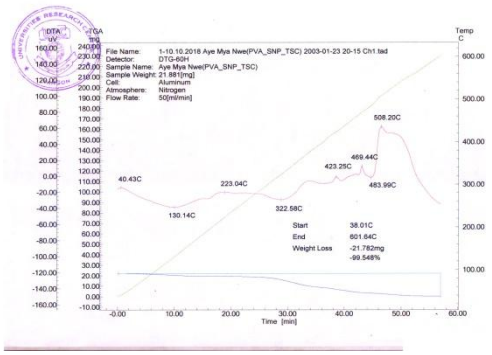


Figure 11 TG-DTA thermogram of PVA-SNP-3 film

Table 7 Thermal Stability of PVA-SNP-3 Film

Temperature Range (°C)	Weight Loss (%)	Peak's Temperature (°C)	Nature of Peak	TG and DTA Remarks
38 -140	7.03	130	endothermic	due to the removal adsorbed water and moisture due to the decomposition of volatile materials
140 -370	42.17	322	endothermic	
370 – 600	50.33	508	exothermic	due to the degradation and decomposition of polymers

EDXRF Analysis

Figure 12 shows the EDXRF spectrum of PVA-SNP-3 film. According to the EDXRF spectrum of the prepared PVA-SNP-3 film, carbon and hydrogen were major constituent (99.951 %) and silver was trace constituent (0.002 %) (Table 8).

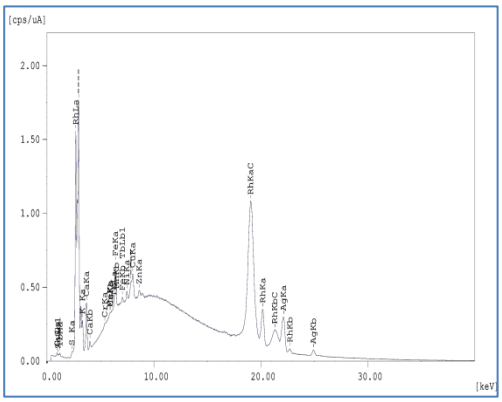


Figure 12 EDXRF spectrum of PVA-SNP-3 film

Table 8 Relative Aboundance of Element in PVA-SNP-3 Film

Elements	Relative Abundance (%)
Potassium	0.019
Calcium	0.012
Sulphur	0.011
Silver	0.002
Iron	0.001
Copper	0.001
Terbium	0.001
CH	99.951

Antimicrobial Activity of PVA-SNP Composite Films

Silver is known for its antimicrobial properties and has been used for many years in the medical field for antimicrobial applications. Additionally, silver has been used in water and air filtration to eliminate microorganisms. Inhibition zone diameters were obtained from the synthesized PVA and PVA-SNP composites tested against six microorganisms: (a) *Bacillus subtilis* (b) *Staphylococcus aureus* (c) *Pseudomonas aeruginosa* (d) *Bacillus pumilus* (e) *Candida albicans* (f) *Escherichia coli*. Antimicrobial activity of PVA-SNP composite films was investigated by agar well diffusion method as shown in Figure 13 and Table 9. It was

observed that the prepared PVA-3 film did not show antimicrobial activity, however PVA-SNP composite films showed the antimicrobial activity. According to the antimicrobial screening, PVA-SNP-3 film possesses higher antimicrobial activity than other PVA-SNP films.

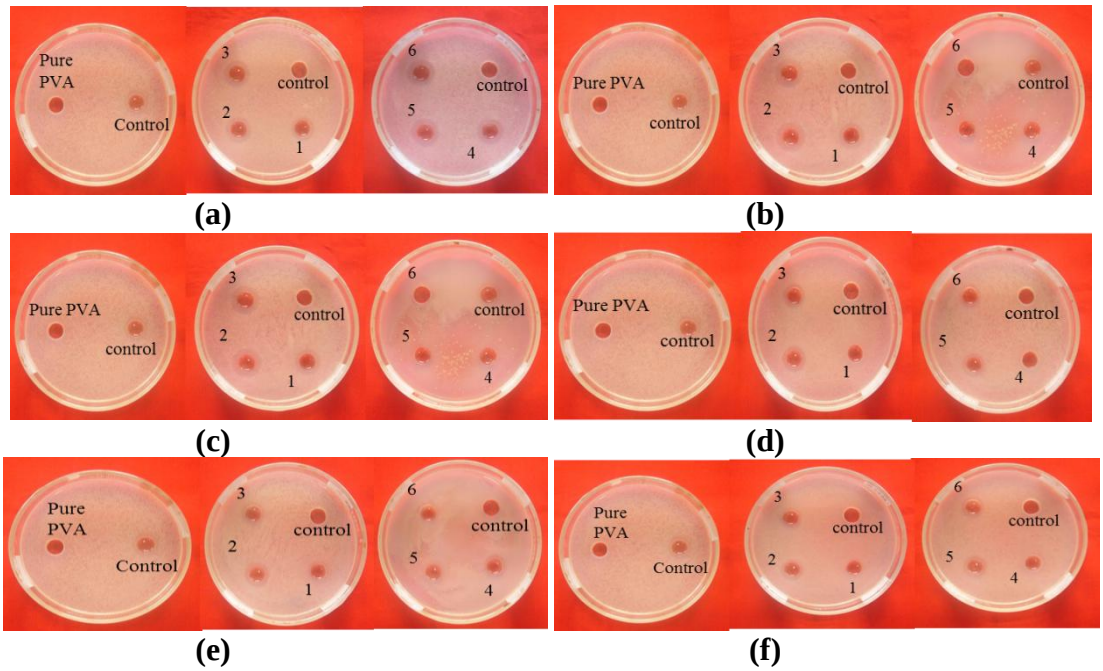


Figure 13 Antimicrobial activity of the prepared (1) PVA-SNP-1, (2) PVA-SNP-2, (3) PVA-SNP-3, (4) PVA-SNP-4, (5) PVA-SNP-5 and (6) PVA-SNP-6 composite films
(a) *Bacillus subtilis* (b) *Staphylococcus aureus*
(c) *Pseudomonas aeruginosa* (d) *Bacillus pumilus* (e) *Candida albicans*
(f) *Escherichia coli*

Table 9 Antimicrobial Activity of the Prepared Polyvinyl Alcohol-Silver Nanoparticles Composite Films by Agar Well Diffusion Method

Sample Films	Inhibition zone diameters of the samples against six microorganisms (mm)					
	Bacillus subtilis	***Staphylococus aureus***	***Pseud-omonas aeruginosa***	***Bacillus pumilus***	***Candida albicans***	***Escher-ichia coli***
Pure PVA	-	-	-	-	-	-
PVA-SNP-1	-	-	12 (+)	12 (+)	-	11 (+)
PVA-SNP-2	14 (+)	14 (+)	13 (+)	15 (++)	13 (+)	14 (+)
PVA-SNP-3	16 (++)	15 (++)	18 (++)	17 (++)	17 (++)	16(++)
PVA-SNP-4	15 (++)	13 (+)	17 (++)	16 (++)	15 (++)	14 (+)
PVA-SNP-5	13 (+)	13 (+)	12 (+)	13 (+)	13 (+)	13 (+)
PVA-SNP-6	16 (++)	16 (++)	12 (+)	17 (++)	17 (++)	16(++)

Agar Well 10 mm (-), 10 mm ~14 mm (+), 15 mm ~19 mm (++), 20 mm ~above (+++)

Conclusion

In this research work, the silver nanoparticles were synthesized by using trisodium citrate as reducing agent by chemical synthesis. In this synthesis, the volume ratio of silver nitrate (1 mM) and 1% w/v trisodium citrate solution was changed at optimum pH. The synthesized silver nanoparticles were characterized by UV-Visible spectroscopy, XRD, FT IR, SEM and EDXRF analyses. By the determination of UV-Visible spectrum, the maximum absorption peak of colloidal silver nanoparticles were appeared at 415 nm. According to the XRD spectra of all of the prepared SNP-TSC, there was no impurity peaks in the SNP-TSC1 and the crystallite size of SNP-TSC1 is 40.6 nm. In the FT IR spectrum of prepared SNP-TSC1, the characteristic absorption bands at 3441, 2874, 1583,1386 and 576 cm^{-1} were observed. From the SEM analysis, all prepared SNP-TSC illustrate the spherical shape and roughly spherical shape. According to SEM micrographs, the individual SNP were agglomerated to form either clusters or large nanoparticles. From the EDXRF spectrum of prepared SNP-TSC1, silver was major constituent (87.177 %) and other were trace constituents. The polyvinyl alcohol- silver nanoparticles (PVA-SNP) composite films were prepared using SNP and the optimum concentration of PVA solution (3 % w/v) solvent evaporating method. According to the mechanical properties of the prepared PVA-SNP composite films, PVA-SNP-3 has optimum tensile strength (37.7 MPa), elongation at break (262 %) and tear strength (139 kNm^{-1}) respectively. The characterization by modern techniques such as FT IR, SEM, TG-DTA and EDXRF analyses were able to reveal the characteristic functional group, morphological texture, thermal stability and relative abundance of the constituent elements. Antimicrobial activity of the prepared composite films (PVA-SNP) were investigated by Agar well diffusion method. According to the antimicrobial activity, pure PVA-3 film did not show antimicrobial activity but all prepared PVA-SNP composite films were observed to exhibit the antimicrobial activity against all of the tested microorganism. According to the antimicrobial screening, PVA-SNP-3 film possesses higher antimicrobial activity than other PVA-SNP films.

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