

## Phytogenic silver nanoparticles (*Alstonia scholaris*) incorporated with epoxy coating on PVC materials and their biofilm degradation studies

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**Abstract.** The advantages of nano-scale materials (size 1-99 nm in at least in one dimension) could be realized with their potential applications in diversified avenues. Herein, we report for the first time on the successful synthesis of homogeneous epoxy coatings containing phytogenic silver nanoparticles (Ag) on PVC and glass substrates by room-temperature curing of fully mixed epoxy slurry diluted by acetone. *Alstonia scholaris* bark extract was used to reduce and stabilize the silver ions. The surface morphology and mechanical properties of these coatings were characterized using the techniques like, UV-Vis (UV-Visible) spectrophotometry, X-ray diffraction (XRD), Fourier transform infrared spectrophotometry (FT-IR), Epi-fluorescence microscopy and scanning electron microscopy (SEM). The effect of incorporating Ag nanoparticles on the biofilm (scale) resistant epoxy-coated PVC was investigated by total viable counts (CFU/cm<sup>2</sup>) from epoxy coating from (Initial) 1<sup>st</sup> day to 25<sup>th</sup> days. The phytogenic Ag nanoparticles were found to be significantly improving the microstructure of the coating matrix and thus enhanced the anti-biofilm performance of the epoxy coating. In addition, the antimicrobial mechanism of Ag nanoparticles played an important role in improving the anti-biofilm performance of these epoxy coatings.

**Keywords:** *Alstonia scholaris*; Ag nanoparticles; epoxy coating; biofilm resistance; PVC coupons

### 1. Introduction

The term 'epoxy resin' refers to both the pre polymer and its cured resin/hardener system. The former is a low molecular weight oligomer that contains one or more epoxy groups per molecule (more than one unit per molecule is required if the resultant material is to be cross linked) (Azeez, Rhee *et al.* 2012). The characteristic group, a three membered ring known as the epoxy, epoxide, oxirane, glycidyl or ethoxyline group as shown in Fig. 1 is highly strained and therefore very reactive. Epoxy resins can be cross-linked through a polymerization reaction with a hardener at room temperature or at elevated temperatures (latent reaction). Curing agents used for room temperature cure are usually aliphatic amines, and for high temperature, aromatic amines and acid anhydrides are used. Poly functional amines, polybasic carboxylic acids, mercaptans and inorganic hardeners are also used as specialized curing agents. In general, the high temperature cured resin

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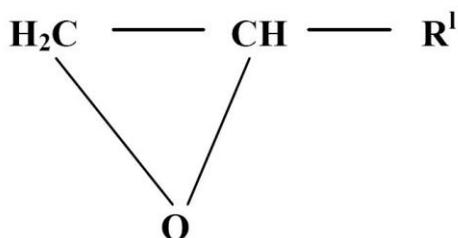


Fig. 1 Schematic representation of typical Epoxy group

systems have improved properties, such as higher glass transition temperature, strength and stiffness, compared to those cured at room temperature (Skiest 1978). Among the thermo set materials, epoxy resins shows special chemical characteristics such as absence of byproducts or volatiles during curing reactions, low shrinkage up on curing, curing over a wide temperature range and the control of degree of cross-linking. Depending on the chemical structure of the curing agents and curing conditions, the properties of cured epoxy resins will vary. Epoxy resins are versatile with excellent chemical and heat resistance, high adhesive strength, good impact resistance, high strength and hardness, and high electrical insulation (Ellis 1993)

Epoxy resins are characterized with outstanding performances such as toughness, rigidity, chemical resistance, adhesive properties and high thermal stability. Hence they are widely used as matrix resin for composites. However the highly cured resin cannot absorb energy under stress and hence is of a brittle nature. The most successful and the best established method so far of increasing the toughness of the epoxy resins is to incorporate a second phase of dispersed rubbery particles into the cross linked polymers (Deng, Zhou *et al.* 2013). However, the addition of rubbery particles is effective in increasing only the fracture toughness of epoxy resin while the glass transition temperature and its solvent stability are lowered. To retain the thermal stability of the thermoset, thermoplastics were used to modify the epoxy resin and to enhance its fracture toughness (Padma, Balasubramanya *et al.* 2014). However it has been shown that the thermoplastic modified epoxies exhibit various types of morphology, depending crucially on the backbone structure, molecular weight and the end group chemistry of the thermoplastic. Since the ultimate properties of the blend is dependent on the morphology of the cured resin, the difficulty of controlling morphology limits any further improvement of toughness using the thermoplastics.

Epoxy resins are widely used in protective coatings, adhesives, sealant, fiber reinforced composites and electronic industry due to their outstanding surface properties like low shrinkage, ease of cure and possessing good moisture, solvent and chemical resistance, and excellent adhesion performances (Ren, Sun *et al.* 2007). They lack fracture resistance, impact strength, low thermal stability, low pigment holding ability, flexibility and poor hydrophobicity, which restrict their wide application in the field of coatings and paints. To improve these properties the component like rubber, polyurethane silicone are added as modifier to the epoxy resins.

Epoxy has been widely used as a coating material to protect the steel reinforcement in concrete structures because of its outstanding process ability, excellent chemical resistance, good electrical insulating properties, and strong adhesion/affinity to heterogeneous materials (Joseph Rathish, Dorothy *et al.* 2013). Epoxy coatings generally reduce the corrosion of a metallic substrate subject to an electrolyte in two ways. First, they act as a physical barrier layer to control the ingress of deleterious species. Second, they can serve as a reservoir for corrosion inhibitors to aid the steel surface in resisting attack by aggressive species such as chloride anions. Nonetheless, the

successful application of epoxy coatings is often hampered by their susceptibility to damage by surface abrasion and wear (Wetzel, Hauptert *et al.* 2003). They also show poor resistance to the initiation and propagation of cracks. Such processes introduce localized defects in the coating and impair their appearance and mechanical strength. The defects can also act as pathways accelerating the ingress of water, oxygen and aggressive species onto the metallic substrate, resulting in its localized corrosion. Furthermore, being hydrophilic in nature, epoxy coatings experience large volume shrinkage upon curing and can absorb water from surroundings (Perreux and Suri 1997). The pores in the cured epoxy coating can assist in the migration of absorbed water and other species to the epoxy-metal interface, leading to the initiation of corrosion of the metallic substrate and to the delamination of the coating. The barrier performance of epoxy coatings can be enhanced by the incorporation of a second phase that is miscible with the epoxy polymer, by decreasing the porosity and the altered diffusion path for deleterious species. For instance, inorganic filler particles at nanometer scale can be dispersed within the epoxy resin matrix to form an epoxy nanocomposite. The incorporation of nanoparticles into epoxy resins offers environmentally benign solutions to enhancing the integrity and durability of coatings, since the fine particles dispersed in coatings can fill cavities (Lam and Lau 2006) and cause crack bridging, crack deflection and crack bowing.

Nanoscale materials have emerged as novel antimicrobial agents owing to their high surface area-to-volume ratio and the unique chemical and physical properties, which increase their contact with microbes and their ability to permeate cells. Further, nanotechnology has amplified the effectiveness of silver nanoparticles as antimicrobial agents (Jancy Mary and Inbathamizh 2012). Silver is also the only material whose plasmon resonance can be tuned to any wavelength in the visible spectrum. Fundamental studies carried out in the last three decades indicated that silver nanoparticles exhibit a rare combination of valuable properties, namely, unique optical properties associated with the surface plasmon resonance (SPR), well-developed surfaces, catalytic activity, high electrical double-layer capacitance, etc. Nanosilver has been used extensively as an antibacterial agent in the health industry, food storage, textile coatings, and a number of environmental applications (Lamsal, Kim *et al.* 2011).

Nanobiotechnology, a branch of Nano science has been playing a decisive role in 21<sup>st</sup> century in deciphering diverse tribulations particularly in the field of farming, medication and electronics (Anna Pratima Nikalje 2015). Nanoscience poses a basic scientific challenge as it requires a control over the connections between atoms. The possibility of utilizing biological materials for nanoparticles synthesis has appeared as the most efficient and greener approach. The present decade has witnessed the rapid shift in synthesis strategies from physicochemical methods to biological agents such as bacteria, fungi and plants for nanoparticles synthesis is an extension of the well established fact that the various organisms such as diatoms, magnetotactic and S-layer bacteria are capable to synthesize Nanoscale materials (Hussain, Javorina *et al.* 2006). Nanoparticles can also prevent epoxy disaggregation during curing, resulting in a more homogenous coating. Nanoparticles tend to occupy small hole defects formed from local shrinkage during curing of the epoxy resin and act as a bridge interconnecting more molecules. This results in a reduced total free volume as well as an increase in the cross-linking density (Judy, Prasad *et al.* 2012). In addition, epoxy coatings containing nanoparticles offer significant barrier properties for corrosion protection (Lamaka, Montemor *et al.* 2008) and reduce the trend for the coating to blister or delaminate.

The present study reveals the influence of phytogenic silver nanoparticles, which are incorporated with the epoxy coating on the surface morphology, anti-scale behavior of PVC

materials. It is expected to shed more light on the fundamental mechanisms through which nanoparticles interact with the epoxy matrix and thus provide guidance for the design of high-performance epoxy coatings used for anti scale protection of PVC pipelines from biofilm formation.

## 2. Materials and measurements methods

### 2.1 Materials

The epoxy resin and its hardener used in this research were obtained from Phoenix Resins Inc. (Hi-media), commercially known as MAS epoxies-FLAGTM. The liquid epoxy resin was a blend of multifunctional low molecular weight diluents and the diglycedal ether of Bis-phenol-A, whereas the hardener was based on adduction reaction chemistry of aliphatic amines. The weight ratio of the epoxy resin to the hardener was 2:1. Ag nanoparticles with a mean diameter of 50 nm, The PVC coupons were prepared from CECRI, Karaikudi.

### 2.2 Nanoparticles preparation (*Alstonia scholaris* bark)

Powdered plant material (bark 10 g) were mixed with 100ml of distilled water then the solution heated up to 80°C for 1hour, after that 90 ml aqueous solution of  $1.0 \times 10^{-3}$  M silver nitrate was mixed with a 10 ml of 5% aqueous solution of *Alstonia scholaris* bark extract was added and left at room temperature for 2days, formation of AgNPs were seen the initial color of the solution was color less and after formation of nanoparticles the color changes to dark brown then the AgNPs solution were centrifuged at 6000rpm for 20min to remove the supernatant and collection of the pellet indicates the presence of NPs, these solid powdered NPs were dried at room temperature for 24hours (Prabha, Supraja *et al.* 2014).

### 2.3 Epoxy coating nano composite preparation

Epoxy resin composites (40%) are usually prepared by dispersing nanoparticles (5%) into the epoxy matrix either with a solvent or through a heating process. However, the latter process is prone to clustering or agglomeration of nanoparticles, resulting in poor dispersion. These epoxy resin is dissolved in 100 ml of the solvent (Methy ethyl ketone and xylene) in 1:1 ratio and heated up to 110°C for 30 min after cooling epoxy resin 5% of biogenic silver nanoparticles (*Alstonia scholaris*) was added and stirred up to 1000 rpm (Model 14-503, Fisher Scientific, Inc.) and sonication (Model 50T, VWR, West Chester, PA) for 10 min. After that, the hardener 40ml of (Phenalkamine, acts as a curing agent) which dissolved in 50 ml of solvent was added to the mixture, followed again by stirring and sonication for 10 min. After that in order to remove bubbles in the solution and to prevent the settlement of particles, low speed (200 rpm) stirring was done at 50°C for 1hour (Cooled down naturally at room temperature). The PVC coupon about 2.5×2.5 cm was dipped into the finally obtained mixture for one time and then kept in a dry place at room temperature for 7 days to allow full curing, which led to the formation of a uniform coating for the anti-biofilm degradation and surface indentation tests in this work.

### 2.4 Characterization of epoxy coating with Ag nanoparticles

#### 2.4.1 UV-visible spectrum for synthesized nanoparticles

The bio-reductant nanoparticles were monitored by UV- visible (UV-Vis) spectrum at various time intervals. The UV-Vis spectra of this solution were recorded in spectra 2450, SHIMADZU Spectrophotometer, from 400 to 800 nm.

#### 2.4.2 Scanning electron microscopy (Morphological study of coatings)

The surface morphology and thickness of the obtained nanocomposite coatings were studied using Scanning Electron Microscopy (SEM). The films were removed from the PVC coupon (2.5×2.5 cm) surface, and then sputter-coated with a very thin Iridium layer (approximately 1-2 nm) to avoid the charging effect caused by the nonconductive nature of epoxy coatings and to get high resolution with this virtually grain-free coating material. The surface morphologies and the cross-section were analyzed, which offer an ultra-high resolution at a relatively low voltage.

#### 2.4.3 FT-IR (Fourier Transformance Infrared Spectroscopic) Analysis

The epoxy coated Ag nanoparticles was harvested and characterized by FT-IR. The FT-IR spectrum was taken in the mid IR region of 400-4000  $\text{cm}^{-1}$ . The spectrum was recorded using ATR (attenuated total reflectance) technique. The sample was directly placed in the KBr crystal and the spectrum was recorded in the transmittance mode.

#### 2.4.4 X-ray diffraction analysis for synthesized nanoparticles

The epoxy coated Ag nanoparticles was harvested and characterized by XRD. The XRD pattern was recorded using computer controlled XRD-system, JEOL, and Model: JPX-8030 with  $\text{CuK}\alpha$  radiation (Ni filtered= $13418 \text{ \AA}^{\circ}$ ) at the range of 40 kV, 20A. The 'peak search' and 'search match' program built in software (sync master 7935) was used to identify the peak table and ultimately for the identification of XRD peak.

#### 2.4.5 Dynammic light scattering (Zeta potential)

The aqueous suspension of the synthesized nanoparticles was filtered through a 0.22  $\mu\text{m}$  syringe driven filter unit and the size of the distributed nanoparticles were measured by using the principle of dynamic light scattering (DLS) technique using nanopartica (HORIBA,SZ-100) compact scattering spectrometer.

### 2.5 Anti biofilm activity through epoxy coating with Ag nanoparticles (Epi-fluorescence microscopy study)

PVC coupons were taken and sterilized by keeping the coupon in ultra sonication for 30mins and the coupon immersed in water test sample for 20 days and after completion of incubation period the biofilm was formed on PVC coupon. Now these coupons were tested by epoxy coated with *Alstonia scholaris* silver nanoparticles and kept for incubation period up to 27days and checked the total viable count for every 5days. Coupons were stained with 0.01% acridine orange solution for 2 min, and finally rinsed with sterile distilled water and subsequent quantification by Epi-fluorescence microscopy at 1,000-fold magnification. Epi-fluorescence images were taken for every 3days to identify the anti biofilm degradation. Total count of bacteria was enumerated by the spread plate method. Samples and their 10-fold dilutions were cultured on nutrient medium (Difco) and incubated at 37°C.

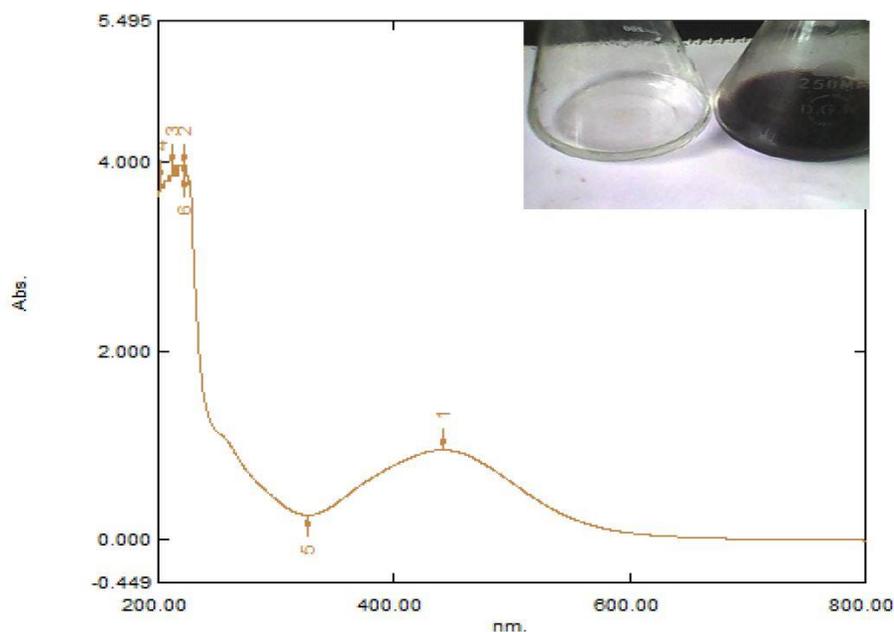


Fig. 2 UV-visible spectroscopic micrograph showing the localized surface plasmon resonance (LSPR) of Ag nanoparticles (420 nm) synthesized using *A. scholaris* bark extract

### 3. Results and discussion

#### 3.1 UV-Vis spectral analysis

It is well known that silver nanoparticles exhibit brown color, which arises due to excitation of localized surface plasmon vibrations. After addition of 90 ml of 1mM silver nitrate solution to the 10 ml of bark extract, the color of the composition has been changed to dark brown color and UV-Vis spectroscopy was used to record the localized surface plasmon resonance of the silver nanoparticles. The maximum absorbance peak was observed at 420 nm which is the characteristic UV-Vis absorbance of nanoscale silver Fig. 2.

#### 3.2 SEM (Scanning electron microscopic) analysis

SEM images of the control coating (plain epoxy, containing no nanoparticles) and the nanocomposite coatings are shown in Fig. 3. We used freshly prepared epoxy-acetone solutions and followed identical sample preparation procedures to prepare all the coating samples (with temperature, epoxy concentration, and pulling speed of the PVC coupon out of coating solution stayed the same). It was observed that the plain epoxy coating and the epoxy coatings modified by nanoparticles of Ag had a similar thickness of approximately 1-10  $\mu\text{m}$ . The epoxy coating modified by Ag nanoparticles with a high specific surface area of 340  $\text{m}^2/\text{g}$  was observed to be much denser than the plain epoxy coating.

#### 3.3 FT-IR (Fourier Transformance Infrared Spectroscopic) analysis

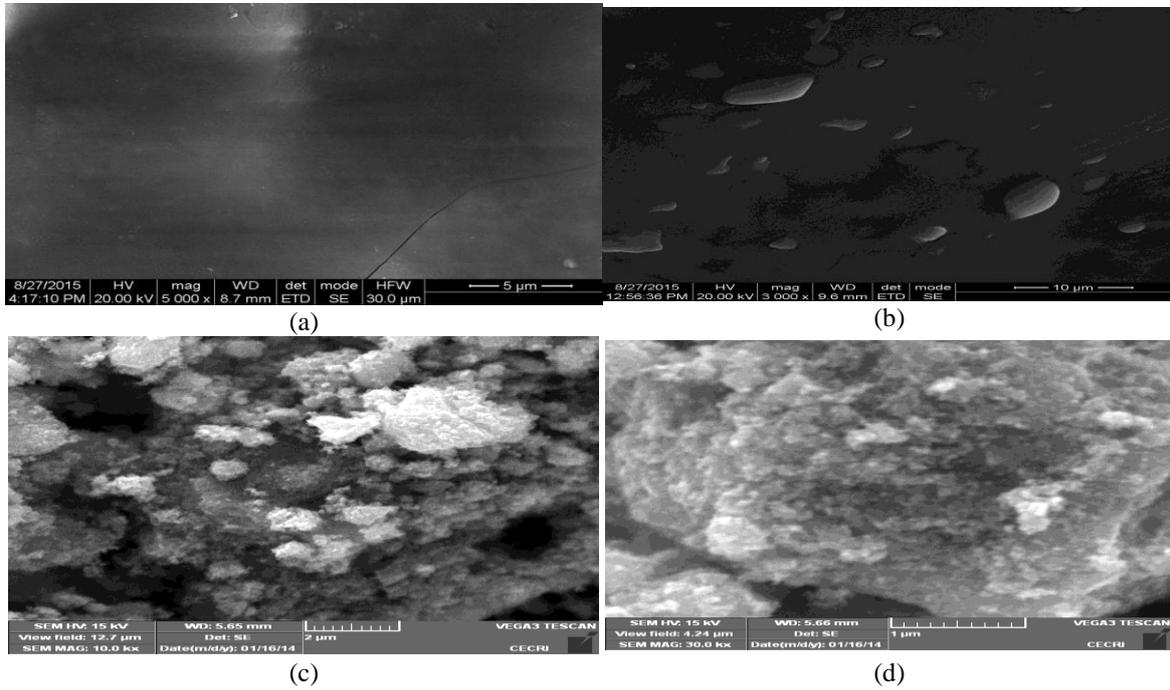


Fig. 3 SEM micrographs showing (a) PVC coupon, (b) epoxy coated PVC coupon and (c), (d) epoxy coated with silver nanoparticles

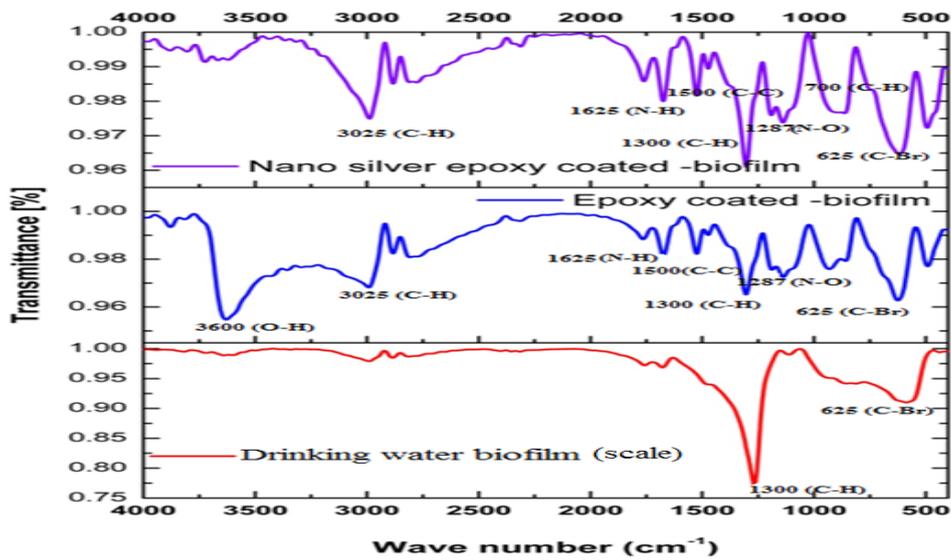


Fig. 4 FT-IR spectra of drinking water biofilm treated with Epoxy coating and Epoxy coated with silver nanoparticles

The FT-IR spectrum of drinking water biofilm Fig. 4 represents several peaks pertaining to different functional groups. The peak present at  $1300\text{ cm}^{-1}$  indicates C-H stretching vibration of

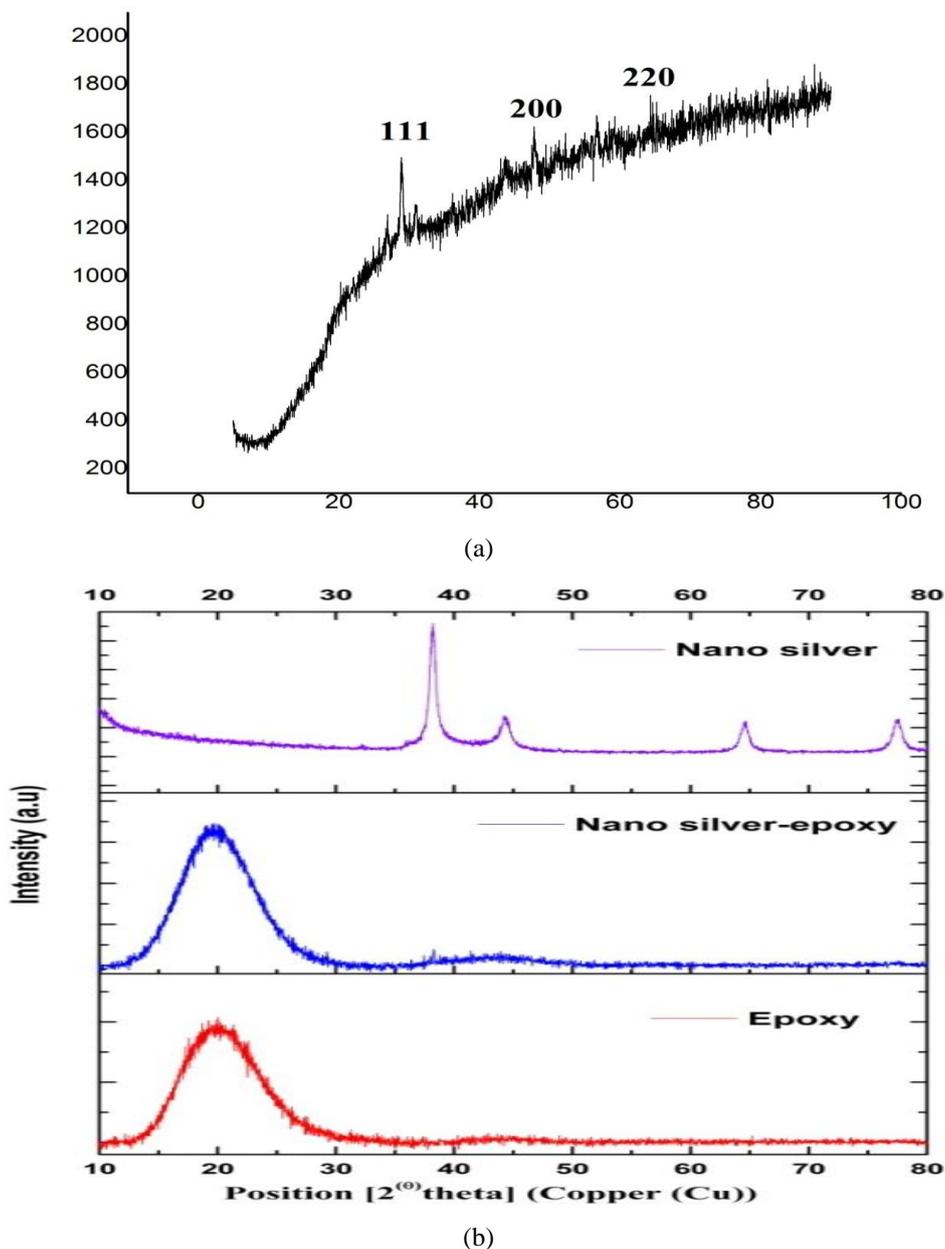


Fig. 5 XRD Patterns of (a) Nano silver, showing the typical face centered cubic crystal structure of the silver with predominant (111) face (b) Nano silver - epoxy

alkyl halides, the peaks present at  $3600\text{ cm}^{-1}$  indicates O-H stretching vibration of alcohols. In nano silver epoxy coated biofilm, the peaks present at  $700\text{ cm}^{-1}$  indicates C-H stretching vibration of alkynes, the peaks are common in epoxy coating and silver mediated epoxy coating nanoparticles are the peak present at  $3025\text{ cm}^{-1}$  indicates C-H stretching vibration of aromatics, the peak present at  $1625\text{ cm}^{-1}$  indicates N-H stretching vibration of primary amines, the peak present at  $1500\text{ cm}^{-1}$

indicates C-C stretching vibration of aromatics, the peak present at  $1287\text{ cm}^{-1}$  indicates N=O stretching vibration of nitro compounds and the peak present at  $625\text{ cm}^{-1}$  indicates C=Br stretching vibration of alkyl halides (Supraja, Prasad *et al.* 2015, Kayal Vizhi, Supraja *et al.* 2016).

### 3.4 XRD (X-Ray diffraction) analysis

X-ray diffraction spectra of epoxy coated silver nanoparticles shows the peaks which were assigned to diffraction signals of (111), (200) and (220) plane for face centered cubic (FCC) silver Fig. 5. The lattice constant calculated from this pattern was  $4.0869\text{ \AA}$  a value in agreement with literature report ( $4.0855\text{ \AA}$ ) JSPCDS file no 89-3722. This clearly indicates that the epoxy coated silver nanoparticles are crystalline in nature and the peak raised at 200 seen in epoxy coated nanoparticles and epoxy coating but not seen in nano silver. The crystal phase analysis of face centered cubic (FCC) and then high intensity of 111 plane structure supports the enhanced antimicrobial activity of nanoscale silver. It was also confirmed that the epoxy deposited on the surface of Ag particles had no effect on the crystallization behavior of Ag particles.

### 3.5 Dynamic light scattering (DLS) analysis

Dynamic light scattering technique has been used to measure hydrodynamic diameter of the hydrosol (particle suspension).  $\text{AgNO}_3$  was found to be  $84.8\text{ nm}$  Fig. 6(a). Epoxy coated with AgNPs was found to be  $109.8\text{ nm}$  Fig. 6(b).

The recorded value of zeta potential of the silver nanoparticles was  $-57.9\text{ mV}$  Fig. 7(a), Epoxy coated with AgNPs was found to be  $21.5\text{ mV}$  Fig. 7(b) which resulted in the very slight agglomerated state of the formed AgNPs. If the hydrosol has a large negative or positive zeta potential ( $>30\text{ mV}$ ), then the particles tend to repel with each other and show no tendency to agglomerate resulted in polydispersed particles.

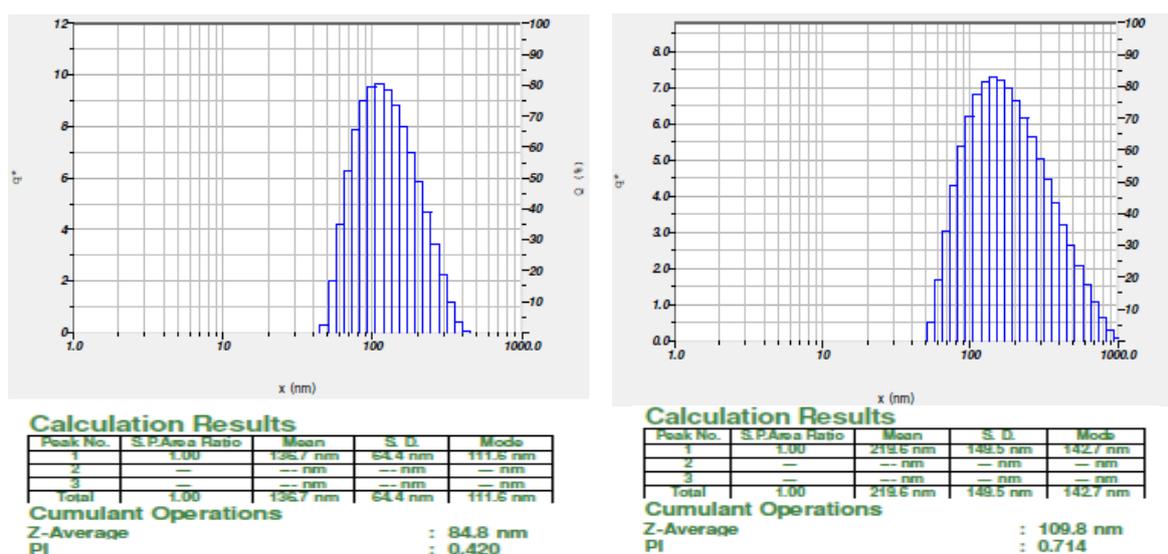


Fig. 6 Dynamic light scattering analysis (a) Particle size of *Alstonia scholaris* mediated synthesis of AgNPs (b) Particle size of epoxy coated *Alstonia scholaris* AgNPs

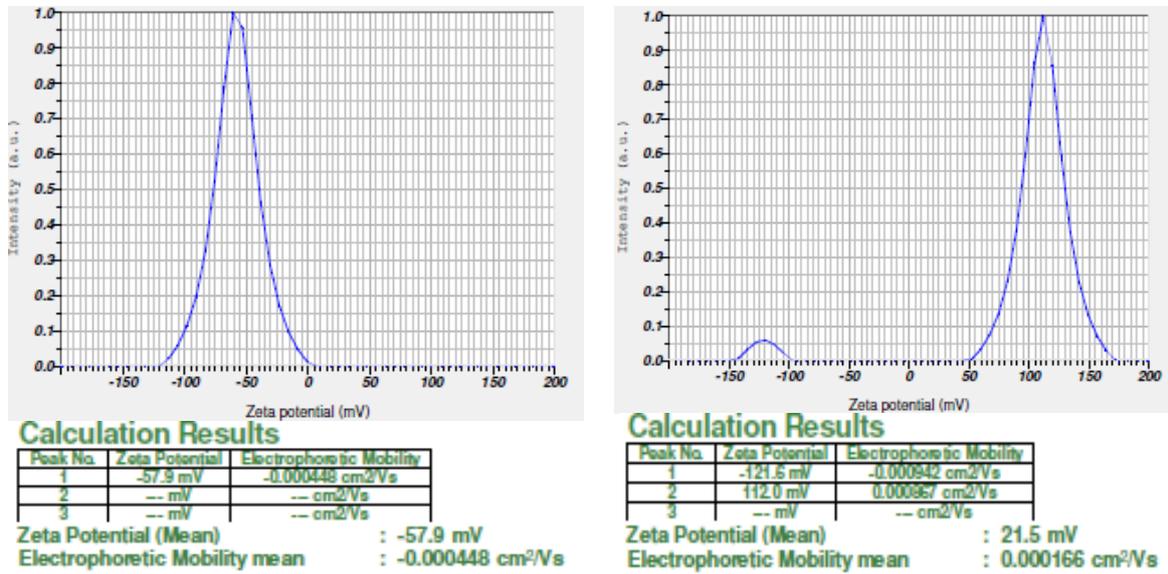


Fig. 7 Dynamic light scattering analysis (a) Zeta potential of *Alstonia scholaris* mediated synthesis of AgNPs (b) Zeta potential of epoxy coated *Alstonia scholaris* AgNPs

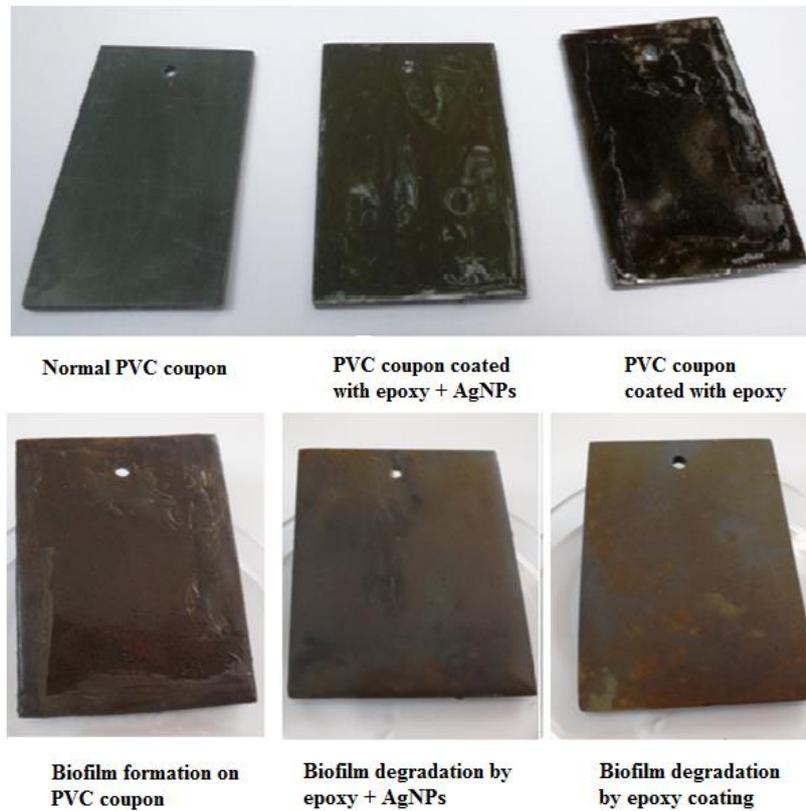


Fig. 8 PVC coupons with epoxy coating and Silver nanoparticles mediated epoxy coating on PVC materials and its anti-scaling (biofilm) activity

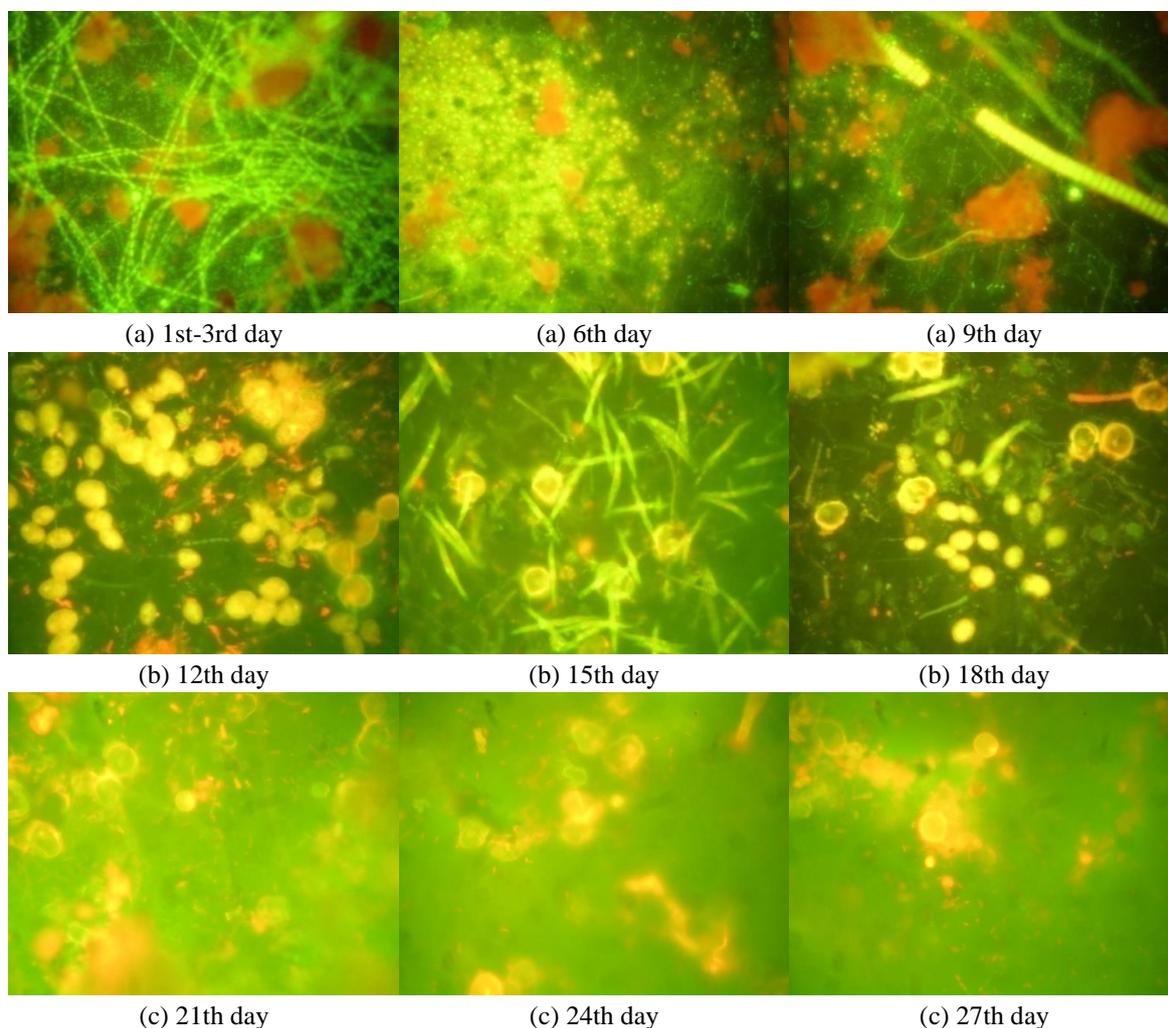


Fig. 9 Epi-fluorescence microscopic pictures showing the anti-biofilm activity from 1<sup>st</sup> to 27<sup>th</sup> days clearly

### 3.6 Anti Biofilm (Degradation studies) activity

Epoxy coatings PVC coupons were removed from drinking water samples after the completion of incubation period 27days which the pure epoxy coating was found to be formation of biofilm is lower when compared to normal PVC coupon showed visual deterioration or dissolution during this period. It was found that sample of the epoxy coated with Ag nanoparticles on PVC coupons shown maximum inhibition of biofilm formation. Incorporating 1 wt% of nano-Ag particles with a mean diameter of 50 nm into the epoxy coating reduced the biofilm rate of the epoxy coated PVC coupons. It merits further investigation to see whether a higher dosage of Ag nanoparticles can provide better long term anti-biofilm performance for the epoxy coating. The epoxy coating modified with Ag nanoparticles showed a significantly enhanced anti-biofilm property this was shown in Fig. 8 and Epi-fluorescence microscopic observation shown the maximum anti-biofilm

Table 1 Total viable count showing anti-biofilm activity towards biofilm degradation

Samples	Total viable counts (CFU/cm <sup>2</sup> )					
	1 <sup>st</sup> day	5 <sup>th</sup> day	10 <sup>th</sup> day	15 <sup>th</sup> day	20 <sup>th</sup> day	25 <sup>th</sup> day
PVC coupon	$0.4 \times 10^3 \pm 0.1$	$2.6 \times 10^4 \pm 0.2$	$3.6 \times 10^6 \pm 0.5$	$4.2 \times 10^7 \pm 0.7$	$5.7 \times 10^8 \pm 0.9$	$6.9 \times 10^8 \pm 1.1$
Epoxy coated with PVC coupon	$7.2 \times 10^2 \pm 0.7$	$5.8 \times 10^3 \pm 0.6$	$4.1 \times 10^4 \pm 0.5$	$3.1 \times 10^6 \pm 0.4$	$2.6 \times 10^7 \pm 0.3$	$1.3 \times 10^8 \pm 0.2$
AgNPs with epoxy coated on PVC coupon	$6.4 \times 10^2 \pm 0.6$	$5.2 \times 10^2 \pm 0.5$	$4.1 \times 10^3 \pm 0.4$	$3.2 \times 10^4 \pm 0.3$	$1.8 \times 10^5 \pm 0.2$	$0.7 \times 10^6 \pm 0.1$

Each value is the  $\pm$ SE of five replications

activity with nano silver epoxy coating Fig. 9.

Nonetheless, the modification with nanoparticles does not always enhance the stiffness of the epoxy coatings. A 70% increase was observed for the nano-Ag modified epoxy coating, the total cell count of anti-biofilm property was given in Table 1. The anti-biofilm activity of these composite surfaces was quantified based on the inhibition of biofilm formation using Epi-fluorescence microscopic study, These epoxy coated silver nanoparticles are capable of killing microorganisms upon contact by inhibiting the biofilm formation in the aqueous environments. Both epoxy and epoxy/Ag nanocomposites although the optimal antimicrobial conditions remain to be fully established, the results highlight a better anti-biofilm activity of epoxy/Ag compared to epoxy (Ansari, Khan *et al.* 2014). The intact epoxy coatings with Ag nanoparticles provided a much higher degree of biofilm protection than epoxy coating. Interconnected regions of Ag nanoparticles in the mixed epoxy coating appeared to provide an anti-biofilm pathway to the underlying PVC coupons so that biofilm i.e., anti-scaling occurred on the surface of coupons (Martinez-Gutierrez, Boegli *et al.* 2013).

#### 4. Conclusions

In this study, phyto-genic silver nanoparticles were produced using the *Alstonia Scholaris* bark extract as reducing and stabilizing agent of silver ions.

- The incorporation of nanoparticles into epoxy resins offers environmentally benign solutions to enhancing the integrity and durability of coatings, since the fine particles dispersed in coatings can fill cavities.
- The anti-biofilm of pure, mixed epoxy coatings and epoxy coated with phyto-genic Ag nanoparticles applied on reinforcing PVC coupons were evaluated by means of total viable count and Epi-fluorescence microscopic studies.
- This composite has a strong potential use in environmentally friendly antimicrobial coatings. The appearance and usefulness of nanoparticles brings many advantages and opportunities to paint and coating industry.
- In addition, epoxy coatings containing nanoparticles offer significant barrier properties for corrosion protection and reduce the trend for the Coating to blister or delaminate.

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