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Probing Thermal and Antibacterial Behaviors of Plant Oil-Based Alkyd Resins Loaded by Bio Reduced Ag Nanoparticles

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Abstract

In this research, extracts of *Elaeagnus angustifolia* leaf is used to reduce the lifetime of silver nanoparticles for the first time. The photosynthesized nanoparticles are homogeneously dispersed into Alkyd resin with 0.5 and 1.0 weight percentage. The two-stage Alcoholysis-poly esterification method was used to synthesis Alkyd resin in 210°C. First, mono glyceride is formed from triglyceride and glycerol, and then Alkyd resin is produced from mono glyceride and phthalic anhydride. The prepared samples including pure Alkyd resin and Alkyd resin with 0.5 and 1.0 weight percentage of silver are investigated by FT-IR, UV-vis, SEM, TGA and XRD analysis. In addition, to study on the antibacterial effects of the loaded nanoparticles, growth and reproduction of two well-known bacteria, i.e., *Staphylococus aureus* and *Klebsiella pneumoniae*, in a nutrient agar containing resultant disk-like nanocomposites are evaluated. The results showed that nano composites containing antibacterial silver has non-growth bacteria halo. While pure Alkyd resin don't have this capability. The more silver amount in polymer matrix, the less bacteria resistance against it and the non-growth halo diameter increases. Finally, the presence of silver nanoparticles in polymer matrix will lead to anti-bacterial property which can be used in the containers keeping food stuff, medical and dental equipment.

Keywords: Photosynthesis; Alkyd resin; Silver nano composites; Antibacterial behavior; Thermal analysis

Introduction

Alkid resins are products which are arisen by compaction or triglyceride oil. Alkid resins are derived by polyols and a dicarboxylic acid or anhydride acid carboxylic. The word "alkid" is the primary modified name of alcid and reflects this fact that they are derived by alcohol and organic acids. Fatty acid in these compounds forms a flexible cover [1].

There are two kinds of dried alkid resins (including semi-dried) and un-dried ones. Both of them are produced by acids or dicarboxylic anhydrides like phthalic anhydride or maleic anhydride and polyols such as trimethylolpropane, glycerin or pentaerythritol. Alkids are used in materials such as resins and colors too.

Alkid resins usually are classified by three methods including: long-oil, average oil and short-oil. These terms represent the percent of the ratio of existing dried oil compound in resins which are called short-oil if the used oil is less than 45% and average-oil in the range of 45%-55% and long-oil if greater than 55% [2].

Alkid resins are synthesized by a two-stage method alcoholosis and polyesterization. In this two-stage process, Oil reacts with glycerol and turns to mono glyceride cursor. Then it reacts with phthalic anhydride in order to synthesize alkid resins.

Due to the new properties and characteristics reflected by nanometer-dimension materials in industries, nowadays, there is much tendency towards to their process and application. Basically, characteristics relative to ratio of the surface and volume of the material in nano metric scale show significant changes. In other words, surface and volume characteristics of the material in nano metric scale are related and have relationship, surface molecules can cause so much hardness in metals and also can produce electronic machineries and pharmaceuticals with better performance and yield.

Using materials and nanostructures whose range is 1-100 nanometer are considered as a new scope of nanotechnology. Also these materials can bring about solutions for technological and environmental problems. Nanoparticles provide completely new or enhanced characteristics according to size, distribution and structure. Different physical and chemical methods are used to synthesize the silver and other metal nanoparticles.

The effect of Nanoparticles on nano-compounding properties can be summarized as follow. Of course, you must be noted that all these cases are not observed in a nano-compounding concurrently.

a) Hardness promotion, durability and dimension stability,

b) Toughness promotion and impact durability,

c) Thermal softness temperature promotion,

d) Mechanical loss promotion,

e) Electrical properties promotion,

f) Weight and value promotion.

Although polymers with anti-bacterial properties are known and produced, but anti-bacterial properties of alkid resin silver nano compounds are not studied. The main aim of the project is production of silver nano-particles in producing alkidresin-silver nano-compounds with studying their thermal and anti-bacterial behavior.

First of all, silver nano-particles are produced using the Extracts of *Elaeagnus angustifolia* leaf and $AgNO_3$ solution. Then produced nano-

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particle is added to alkid resin with different weights percent's, and then cooking agent i.e., Methyl-Ethyl Keton peroxide (MEKP(and Cobalt-Actoate is added to them and cooking reaction is done during a thermal stage. Finally, nano-compounding with different densities of silver nano particles will be put on the seeding Agar bacteria environment than the minimum preventing density for bacteria growth will be discussed.

Materials and Methods

Chemicals

The used materials in this research are big oil, glycerol, phtalic anhydride, lead oxide catalyzer (II), silver nitrate. Their specifications are shown in Table 1 completely. All above-mentioned materials are provided by Merck and Aldrich companies and are used as provided without any purification.

Deionized water and the Extract of *Elaeagnus angustifolia* leaf, also Whatman paper number 1 were used for filtering.

The necessary materials and organisms to make microbial tests

a) *Staphylococcus aureus* bacteria

b) *Klebsiella pneumoniae*

c) Disc Blanc 6 mm

d) Moller Hinton Agar planting environment

e) Ethanol 70% for disinfecting

The needed equipment for microbial test includes plate, bacteria insemination standard loop, sterile blade, sterile plastic dish and swap.

Devices and techniques

For distinguishing crystalline structure as well as calculating of nano-particles size, X-ray diffraction device model Brunker- D_8 ADVANCE with Nickel filter equipped with radiation source of Cu/K_α with wavelength of =1/54056λ in the angle range of 2θ=5-90° stationed in solid state research laboratory of Damghan university was used. To study morphology of polymer samples surface, the pictures of samples in powder form was provided after fixing on Carbon glue base by gold mark layer by Scanning Electron Microscope (SEM) model ZEISS-DSM-960A and Hitachi S4160 located in campus of science school and engineering-technical school of Tehran university. Thermogravimetric Analysis (TGA) on samples was made by TGA device model Bahar Termo Analyse Gmbh STA 503 under Argon atmosphere with thermal rate of 15°C/min in Damghan university. UV-Vis Spectroscopy of samples was done by model varian 50 spectrometer. Infrared spectrum of raw materials and target samples of this research was registered using FT-IR model Turmo 220 spectrometer.

Preparing the extracts of *Elaeagnus angustifolia* leaf

At First, the *Elaeagnus angustifolia* leaves were collected and washed with distilled water and dried for 5 days in the shadow and then were changed into powder by mill. 20 grams of plant powder was poured in 200 ml deionized water and boiled for 30 minutes in oil bath with 103°C. After cooling, the resulting extracts (Figures 2 and 3) filtered with Wahtmann Filter Paper No 1 and be kept in 4°C for the next tests.

Silver nanoparticles synthesis

First, a blend of 30 ml plant extract and 500 mL $AgNO_3$ 1 mM shall be ready and then was exposed to sunlight for 24 hours. The solution color got dark. The settlement was separated from the solution by centrifuge device, washed by deionized water and then was dried in a normal oven under 85°C temperature for two hours and hereby Nitrate nano-particles was prepared.

Alkid resin synthesis

Alcoholysis-Polyesterization reaction was done in a circle-bottom dish equipped with a mechanical mixer, a thermometer and an entrance door for Hydrogen gas. A mix of 33.6 gram (0.04 mol) big oil, 7.36 gram (0.08 mol) Glycerol and 0.05 wt% (regarding the oil) of PbO were exposed to heat in 230°C temperature and mixed with constant rate of 500 rpm under nitrogen atmosphere for 60 minutes to constitute mono glyceride. Monoglycerid constitution was confirmed by methanol solubility test.

One part of the resin was mixed with three part of Methanol in a sample glass and in the room temperature. Solving the resin completely in Methanol arises clear liquid indicative of mono glyceride constitution. Then the reaction mix will be cooled to 12°C and 0.12 mol phtalic anhydride will be added along with 1.98 gram extra glycerol (0.27%). Now, reaction temperature will increase to 210°C and the heating will continue until the acidity stay between 20-30 mg KOH/g.

Alkid resin nano-compounds synthesis containing 0.5 and 1 weight percentage of silver

Alcoholysis-Polyesterization reaction was done in a circle-bottom dish equipped with a mechanical mixer, a thermometer and a hydrogen gas input. A mix of 33.6 gram (0.04 mol) big oil, 7.36 gram (0.08 mol) glycerol and %wt 0.05 (with regard to oil) of PbO were exposed to heat in 230°C temperature and mixed with constant rate of 500 rpm under Nitrogen atmosphere for 60 minutes to constitute monoglycerid. Monoglycerid constitution was confirmed by Methanol solubility test.

One part of the resin was mixed with three part of Methanol in a sample glass and in the room temperature. Solving the resin completely in Methanol makes a clear liquid, indicative of monoglycerid constitution. Then the reaction mix will be cooled to 120°C and 0.12 mol phtalic anhydride will be added along with 1.98 gram extra glycerol (0.27%).

Item	Chemical Formula	Reagent Aspect	Mol Mass g/mol	Density g/cm³	Fusion Temperature °C	Boiling Temperature °C	Purity Percent
Silver nitrate	$AgNo_3$	White crystalline	169.87	4.35	212	444	≤ 99
Glycerol	$C_3H_8O_3$	Odorless & colorless liquid	92.09	1.261	17.8	290	≤ 99.5
Phthalic anhydride	$C_8H_40_3$	White flakes	148.1	1.53	131	295	≤ 98
Big Oil	-	Yellow and clear liquid	-	-	24	-	-
Lead (II)	PbO	Yellow or red	223.20	9.53	888	1477	-

Table 1: The specifications of raw materials.

Now, reaction temperature will increase to 210°C and the heating will continue until the acidity is between 20-30 mg KOH/g. This method was done for nano-compound containing 1% weight of silver.

Studying and measuring anti-bacteria properties of silver alkid-resin nano-compounds

Linear planting: In the linear planting method which is a qualitative separation method for getting separated colonies, plate is divided into four divisions. In this method, the aim is to dilute the number of bacteria. Hence, insemination loop will be heated after planting of each part. Heating the loop destroys the residual bacteria and reduces the number of them. Each part indicates decreasing in the number of thousands of bacteria cell existing in primitive insemination sample. The colonies are constituted after 24 hours incubation in 37°C.

Provision of microbial suspension

Klebsiella pneumonia bacteria (negative gram) and *Staphylococcus aureus* (positive gram) was provided by Pastor institute in lipophilized ampules. After bacteria survival, the mentioned bacteria were planted linearly on Moller Hinton Agar environment. For provision of microbial suspension from 24-hour linear planting of bacteria, 4 or 5 colonies were removed with laboratory loop and were completely mixed in a sterile tube containing 5 ml sterile physiology serum and the resulting mixture got vertex in order to get a uniform suspension from bacteria. Then the tube was put for 30 minutes in 37°C and the tube darkness was compared with standard darkness of McFarland Standard No. 0.5 (15×10^8 cfu/mL) and in case of difference, suspension darkness was reached to standard darkness of 0.5 McFarland adding sterile physiology serum or bacteria colony. For more certainty, the optic absorption of suspension and the standard solution in wavelength of 600 nm was read by Spectrophotometer. The Blanc of device is distilled water.

Determining the anti-bacteria properties of alkidresin-silver nano-compounds using the method of disc emission

In sterile conditions under Laminar Fluor with sampler 100 microliter from prepared microbial suspension was poured on plates containing planting environment of Moller Hinton Agar with 5 ml thickness and was distributed with L-shape and sterile on plate surface as the surface of plate was covered with a microbial layer completely. Then, the discs with 6 ml diameter (from Paadtan Company) were put on the plate surface with sterile pans with suitable distances and the discs were fixed in their situation using the tip of the pans. 50 micro liters from each one of the provided nano-compounds (with different densities) was poured in sterile distilled water on the discs.

After 24 hours incubation in 37°C, the diameter of non-growth halo was measured using ruler on the basis of ml. The test was done with three repetitions and the average of results was reported.

Results and Discussion

FT-IR analysis of samples of extracts of *Elaeagnus angustifolia* leaf and the solution of the extract and silver nitrate

The extracts of *Elaeagnus angustifolia* leaf analyzed by FT-IR. Figure 1a shows the FT-IR spectrum of the extract provided by *Elaeagnus angustifolia* leaf in the range of 500-4000 cm^{-1}. Photochemistry analysis of the extract showed that it consists of chemical compounds including: Flavonoids and Phenols. The Target spectrum shows the active groups presence in the extract. The peak of 3267 cm^{-1} show the absorption frequency of phenol OH which there is abundant in the extract and the peak of 1636 cm^{-1} show the absorption frequency of bending OH and the peak of 2107 cm^{-1} is related to C-H asymmetric pull vibration.

Figure 1b shows the FT-IR spectrum of nano-particle of silver suspended in the bed of the extracts of *Elaeagnus angustifolia* leaf in the range of 500-4000 cm^{-1}. Bio-reduction of silver ions is done by chemical compounds existing in plant extract. More amounts of Flavonoids play a significant role in silver ions reduction reaction. Also, Carbonyl group is oxidized to metal silver for silver ion reduction. Those peaks observed in the spectrum of extract FT-IR will be seen here. This spectrum shows that silver ions reduction might be due to the presence of polyols.

Analysis of alkidresin FT-IR spectrum and alkidresin-silver nano-composites

The resulting FT-IR spectrum in this research is studied in the range of 500-4000 cm^{-1} wavelength. The specified peaks were found in 3471 cm^{-1} which were due to pull vibration of O-H as well as in 2853-2924 cm^{-1} which were due to aliphatic pull vibration of C-H. The existing peaks are appeared in 1724 cm^{-1} for pull vibration of C=O of triglyceride, in 1599 cm^{-1} for C=C and in 1452 cm^{-1} for C-H bond. Also, the existing peaks are due to pull vibration of C-O-C in 1069 cm^{-1} and to kinetic vibration of Methylene in 741 cm^{-1} (Figure 2).

XRD spectrum analysis of silver nano-particles, alkidresin, 0.5 and 1 weight percentage of alkidresin-silver nano-composite

XRD spectrum observed in Figure 3 is indicative of presence of synthesized silver nano-particles crystalized from the extracts

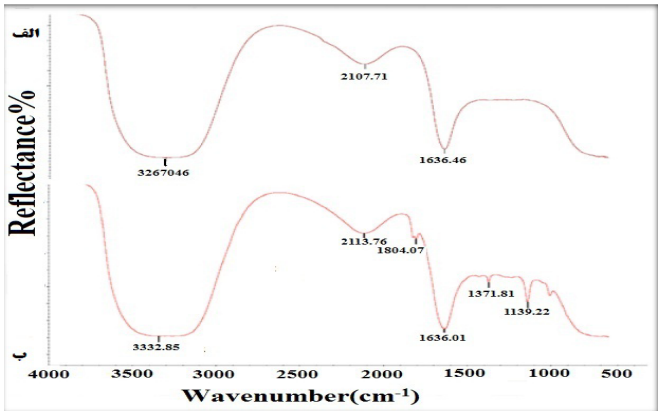

Figure 1: FT-IR Spectrum A) extract b) extract with silver.

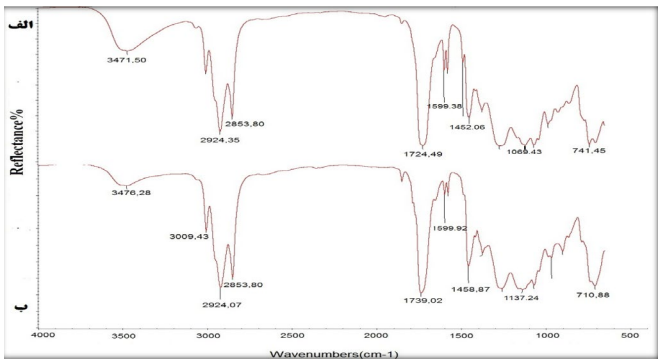

Figure 2: FT-IR spectrum A) alkidresin and b) alkidresin-silver.

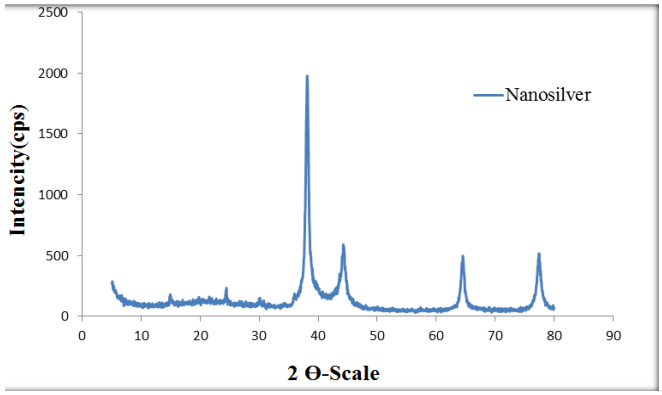

Figure 3: XRD spectrum of silver nanoparticles.

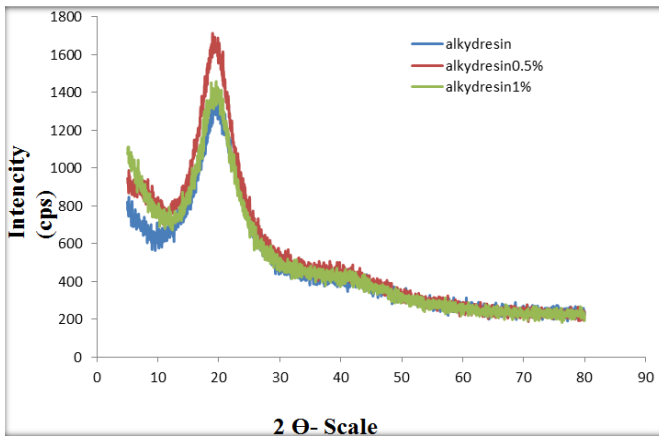

Figure 4: XRD spectrum, alkidresin, 0.5% silver alkidresin, 1% silver alkidresin.

of *Elaeagnus angustifolia* leaf. Generally, the Increase of the height of the peak and existence of sharp regions in spectrum is indicative of more crystallinity in the target composition. Figure 3 shows the XRD spectrum of silver nano-particles. As is shown in the figure, in this spectrum, a sharp peak is appeared in about 2θ=38° which can be considered as a clear signal for nano-particle. As is clear from the figure, the existence of four index peaks in angles 38, 44, 64, 77 is a confirmation for the constitution of silver nano-particle.

Figure 4 shows the XRD alkidresin, alkidresin 0.5% and 1% weight percentage of silver. As is observed, a sharp peak is appeared about 2θ=20° which can be considered as a clear signal for alkidresins and also the weak fluctuations in 2θ=38° indicate the presence of silver nano-particle in polymer matrix.

TGA analysis of alkidresin and nano-composite alkidresin-silver 5%

This graph is considered as an important indicator of candescent for polymers. The resulting sample shows a three-stage weight loss from the room temperature to 600°C. Pure alkidresin meets weight loss in 78.9°C while exposing silver nano-particles in polymer matrix causes the increase of thermal resistance and the temperature which causes nano-composite weight loss is 205°C. Increasing the percentage of silver in matrix polymer associate with increased thermal stability of nano-composite and has more remaining coke than pure alkidresin (Figure 5).

According to Table 2, IDT is the temperature which only the 5% polymer chains of the samples is destroyed and changed into coke. With temperature increase in coke yield column (CY), the remained percentage of samples (95%), has turned into coke and in fact, the residue polymer for each one of samples in 600°C is mentioned in Table 2.

UV-Visible spectrum

According to spectrum reported, in the range of 405 nm is indicative of the presence of silver nanoparticles constituted in plant extract whereas it doesn't show any λ_{max} in this interval. This strong strip is related to surface Plasmon resonance of silver nano-particle. Silver has the most conductivity and in addition, its absorption peak is more strong and clear than other nobel metals. In visible spectrum of noble metals, when the particles size reaches to nanometer scale, a strong absorption is observed which its origin is surface Plasmon resonance (Figure 6).

SEM pictures

Figure 7 shows the pictures of Scanning Electronical Microscope (SEM) of silver nano-compounds in different magnifications. As the pictures show clearly, the resulting nano-particles are seen in the form of compact mass particles which have uniformity and lumpy surface and are sticked together (Figure 8).

Figures 9 and 10 shows morphology of alkidresin nano-composites containing 0.5 and 1% silver weight percentage. The picture (a) shows the morphology of alkidresin nano-composites containing

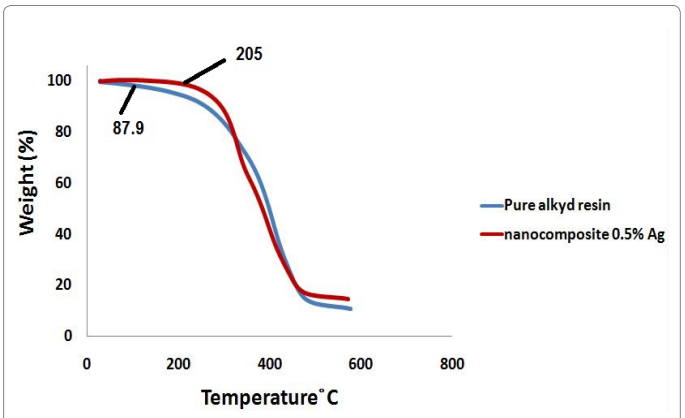

Figure 5: TGA analysis, alkidresin, alkidresin-silver nano composite.

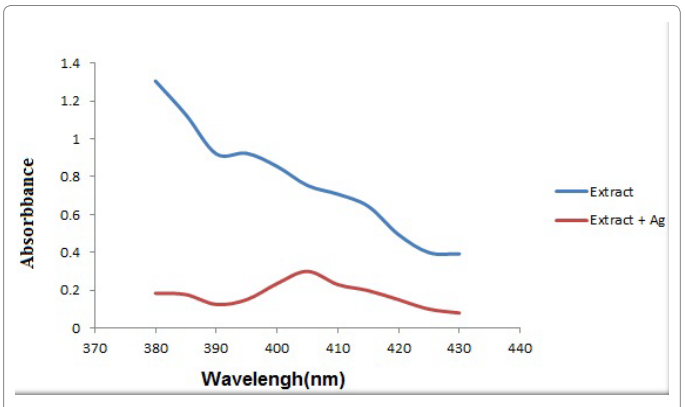

Figure 6: UV-visible spectrum, the extracts of *Elaegnus angustifolia* leaf and the extract with silver.

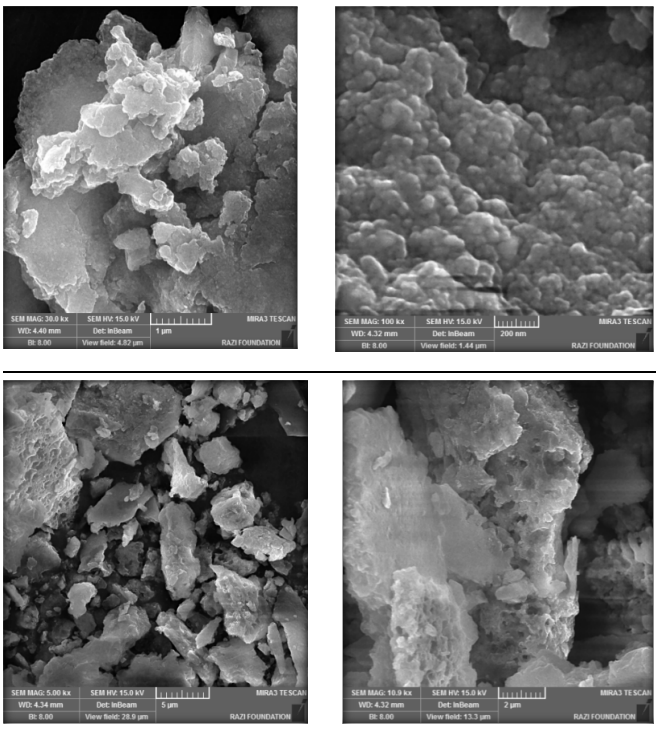

Figure 7: SEM micrograph of silver nanoparticle provided by the extracts of *Elaegnus angustifolia* leaf and $AgNO_3$ solution in different magnifications.

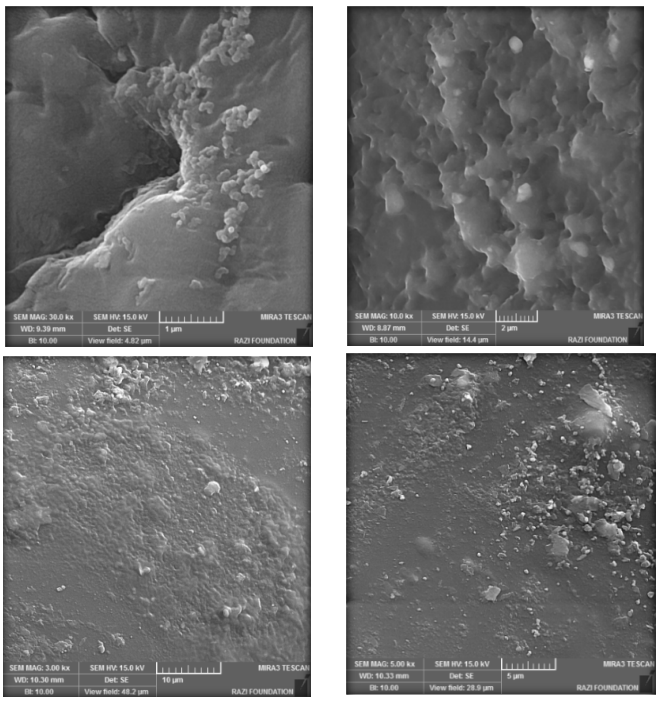

Figure 8: Alkid resin SEM micrographs.

0.5% silver weight percentage and the picture (b) shows Alkidresin nano-composites containing 1% silver weight percentage in different magnifications. In both pictures Nanoparticles are seen as spherical compact bullets in polymer bed and are dispersed pretty uniformly [3-26].

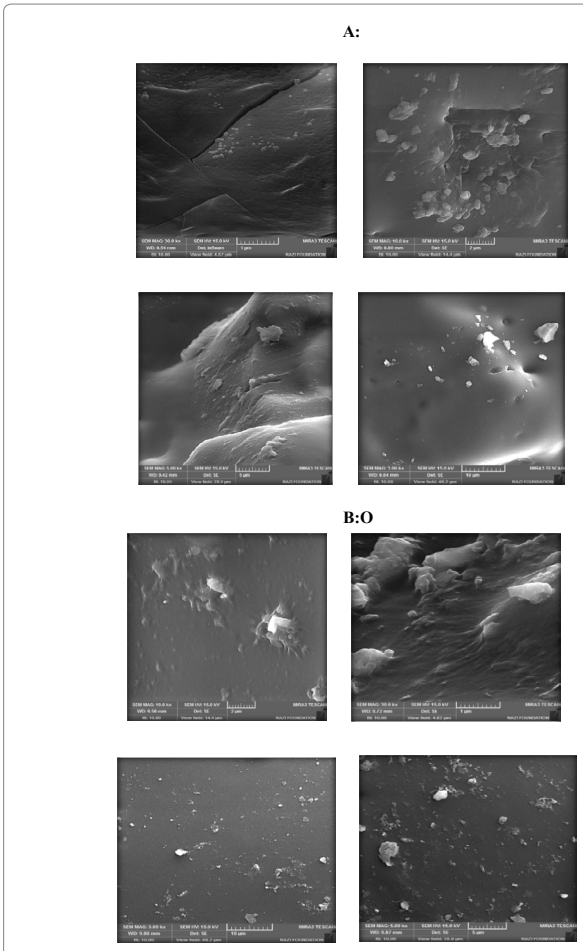

Figure 9: A) Alkidresin nano-composite of 0.5% silver. B) Alkidresin nano-composite of 1% silver.

Code	IDT°C	CY (%)
Alkid-resin	87.9	11
Silver alkid resin 0.5%	205	15

Table 2: An explanation of TGA analysis.

Biological studies

As it is seen in the picture, after 24 hours, we studied the amount of inhibition of bacteria growth. With constitution of non-growth halo and its measurement, we found that 1% nano composite has fewer diameters than 2.5% nano composite because. The reduction of resistance of bacteria versus silver (Table 3).

Conclusions

Silver nanoparticle was prepared by the *Elaeagnus angustifolia* leaf in size of 16-24 nm with the method of green chemistry and this method is important due to adaptability with environment. Also a two-stage method of alcoholysis-polyesterization is used for preparing nano-composites. This method evenly spreads the nanoparticles in polymer matrix and prevents the accumulation. Studying FT-IR Spectrums, the presence of OH group in the extract makes silver ion decrease into metal silver and because there is no definition of limitations in FT-IR for Ag ، its presence in polymer matrix hasn't induced change in IR peaks. Presence of silver in polymer matrix leads to polymer resistivity increase which was observed in TGA Diffract gram analysis clearly. The

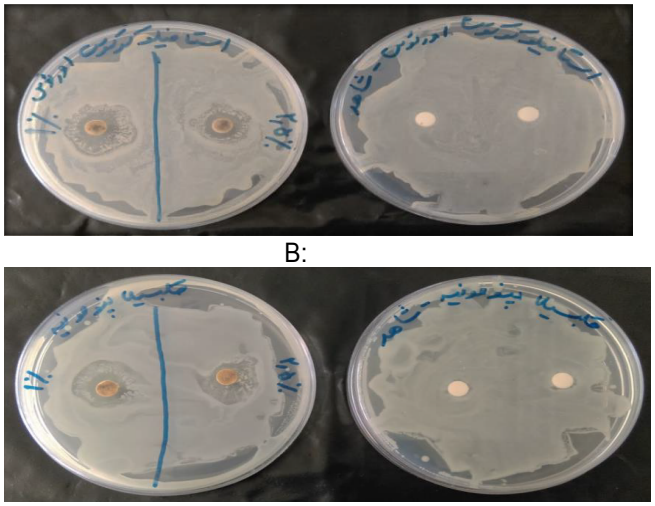

Figure 10: Anti-bacteria picture, a) *Staphylococcus aureus* and b) *Klebsiella pneumonia*.

Sample	Inhabitation Area (mm)	
	Klebsiella pneumoniae	***Staphylococcus aureus***
Pure polymer	-	-
Nano-compounding 1%	12	11
Nano-compounding 2.5%	13	12

Table 3: The size of non-growth halos.

remained coke in nano-composite containing 0.5% sliver was less than alkidresin.

Silver metal reflects absorption when it is in the path of UV beam due to surface vibrations and this process is the same with surface Plasmon resonance. As observed, UV-Visible spectrum in the range of 405 nm confirmed the presence of silver in the extract. When particle sizes reach to nanometer scale in visible spectrum of noble metals, a strong absorption with the origin of surface Plasmon resonance is observed.

Beam X diffraction was used to determine crystallinity amount of silver nanoparticles confirming silver presence in polymer matrix. Generally, the increase of peak and existence of sharp areas in diffract gram is indicative of more crystallinity in the target compound. 4 index peaks were appeared for silver nanoparticles, the most important of them was in the interval of =θ238°. Alkidresin showed its respective peak in 2=θ20°. On the other hand, due to low concentration of nanoparticles in nano-composite samples, we can't expect the peaks related to nanoparticles appear in high resonance. Here, due to very low concentration, the peaks related to silver in polymer matrix have experience very weak vibrations. Using the Shearer equation, the size of nanoparticles was 16.04 nm.

$t=0.9\lambda/B\cos\theta_B$

SEM analysis of baked resins indicates a good surface property and as it is clear from the pictures, silver nanoparticles are dispersed in polymer surface evenly.

Silver showed the anti-bacteria properties, alkidresin didn't have any anti-bacteria property in the normal state, but with constitution of polymer matrix with silver and production of 1 and 2.5 weight percentages, nano-composites anti-bacteria property was appeared with constitution of non-growth halo. The more silver amount in polymer matrix, the less bacteria resistance against it and the non-growth halo diameter increases.

Finally, the presence of silver nanoparticles in polymer matrix will lead to anti-bacterial property which can be used in the containers keeping food stuff, medical and dental equipments. Green chemistry process is adaptable to environment, non-toxic and valuable.

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