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# Preparation and Characterization of Silver Nanoparticle/Aloe Vera Incorporated PCL/PEO matrix for wound dressing application

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Polymeric wound dressing materials have remarkable mechanical, structural, and biocompatible behavior. In this work, a polymer matrix of Polycaprolactone (PCL)/Polyethylene Oxide (PEO) incorporated with Aloe Vera (AV) extract and silver nanoparticles were prepared for wound dressing application. Initially, the phytochemicals from AV were extracted by Soxhlet apparatus, and then the aloe extract was used as a reducing agent to synthesize silver nanoparticles (Ag NP). Ag NP's formation was confirmed by the presence of a characteristic UV absorbance peak at 420 nm. Ag NP's average diameter and shape were found to be between 10-50 nm and spherical, respectively. AV extract and Ag NP were incorporated into PCL/PEO polymer solution to prepare the polymer matrix by solution casting method. Box-Behnken design (BBD) was used to study the effect of Ag NP concentration, AV extract percentage, and PEO weight percentage concerning PCL on wound dressing application. Water Vapor Transmission Rate (WVTR) and swelling properties of all the sample were tested and found that the PEO and AV extract plays a major role in both swelling and WVTR irrespective of Ag NP concentration. The antimicrobial property of synthesized Ag NP was studied against gram-negative bacteria Escherichia coli with control samples (PCL and PCL/PEO), Ag NP with 150 mg concentration showed a higher zone of inhibition than the other concentrations. Thus, the prepared PCL/PEO polymer matrix incorporated with AV extract and Ag NP can be used as an effective wound dressing material.

**Keywords**: Ag nanoparticles, Box-Behnken design, Polycaprolactone, Polyethylene oxide, Water vapour transmission rate

Skin is the vital organ and the largest of the human body. It gives us a sense of touch, heat, and cold. It also acts as a barrier and protects our body from the external environment. Another major function of the skin is to regulate the body temperature and serve as a production house for vitamin D. It also gives us protection against radiation. Also, it helps to regulate blood pressure in our body. If any injury or wound occurred deep towards the skin, the basal layers are damaged, and cells' re-epithelialization becomes questionable with specified underlying diseases[1-3]. An injury to anybody's tissues caused by any physical means or any interruption to tissues is defined as 'wound,' and the restoration process of these damaged tissues is termed as wound healing. Chronic wound refers to a hard wound and wound concerning skin ulcers with other underlying diseases such as diabetes[4]. Earlier wound dressings were primarily meant for protecting the wound from secondary infection and to absorb wound exudate. Later wound dressings were prepared to protect and promote wound healing, relieve pain, and maintain moisture balance.

Traditional medicine has a long history of exceptional benefits in accelerated wound healing. It can tender an enhanced micro-environment for wound healing, prevent inflammation, and promote immunity. Moreover, they are highly biocompatible with body tissues and body fluids and will not have any adverse cytotoxic effects. Plant extracts are being used for wound repair, anti-inflammatory, anti-tumor, and phytochemical activity due to active flavonoids, terpenoids, and other bioactive components[5]. Among a wide range of wound healing medicinal plants, AV – the plant of healing which is very well related to herbal medicine therapy is used in the current research work as a biomolecule to be incorporated in wound dressing as well as acts as a reducing agent in

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the green synthesis of Ag NP. The wound dressing scaffold in the present work was prepared using the solution casting method.

Biodegradable polymers are being widely accepted as wound dressing agents with a combination of bioactive agents. All polymers are not suitable and cannot be used for wound dressing or medical applications. Biodegradable synthetic polymers such as PEO [poly (ethylene oxide)], PLLA [poly(L-Lactide)], PLGA [poly(glycolic acid)], and PCL [poly(caprolactone)] are being used for wound dressing applications in combination with naturally derived biopolymers such as collagen, gelatin, chitosan, silk, and fibrinogen. Treating chronic wounds with traditional medicines has been attempted by the researchers to heal the wound faster and prevent infection. The solution casting method to prepare scaffolds was one of the simple and easiest techniques to incorporate plant extracts with incorporated nanoparticles in dressing materials[6]. These polymeric scaffolds have been recommended as scaffolds for tissue engineering of bones, skin, cartilage, blood vessels, ligament, kidney, and liver. Studies on plant extracts, drugs, and their pharmacological activity have increased because of their safety and lesser side effects. Blending polymeric substrates with plant-derived extracts with incorporated nanoparticles provide required mechanical stability, improved cell regeneration, proliferation, and hydrophilicity of the matrices, which is a primary requisite for wound healing and moisture balance at the wound site.

PCL is a highly hydrophobic semi-crystalline polyester. It is approved by FDA (Food and Drug Administration) as a biodegradable synthetic polymer and widely accepted for its biocompatibility and mechanical properties. Its limitation lies in its hydrophobic property and slower degradation kinetics, and it is mostly preferred for long-term drug delivery applications. PCL has a degradation profile lasting several months with the low glass transition and melting temperature. It has better structural and mechanical stability. They show excellent adhesion, proliferation, and regeneration property[7,8]. PEO is a water-soluble polymer, and it acts as a binding, thickening, water retention, lubricity, and film & pore formation agent. Medicinal plants are considered potent healers as they naturally facilitate repair mechanisms. AV - the plant of healing one of the oldest medicinal plants extremely used to treat skin conditions such as burns, cut, and eczema. Biochemical constituents of AV contain many nutrients and active compounds, including sugars, cellulose, polysaccharides, hemicelluloses, anthraquinones, saponins, vitamins, enzymes, minerals, lignin, sterols, salicylic acid, and amino acids. The presence of these active ingredients makes AV a potential candidate for wound healing[9].

Nanoparticles are particles with a size range of 1-100 nm. Based on size, distribution and morphology, the nanoparticle exhibits improved properties. Properties like higher surface to volume ratio increase with decreasing size of the particle, larger surface area which is relevant for higher catalytic activity. As the nanoparticle's surface increases, their biological effectiveness also increases due to the increase in surface energy of the nanoparticle[10,11]. Among the various metal nanoparticles, Ag NP has been known to have improved inhibitory and antibacterial effects[12,13]. The current study focuses on Ag NP green synthesis using AV extract and the feasibility of preparing a wound dressing material using Ag-AV extract and PCL/PEO polymer matrix.

## Materials and Methods

### Experimental

The materials used for the scaffold's preparation include AV powder procured from a local herbal medicine shop. Chloroform (MW 119.38 g/mol), Methanol (MW 32.04 g/mol), Silver nitrate (MW 169.88 g/mol), Diluted ammonia (MW 17.031 g/mol), Ferric chloride (MW 162.2 g/mol), Glacial acetic acid (MW 60.05 g/mol), and Sulphuric acid (MW 98.079 g/mol) were purchased from SRL chemicals Pvt. Ltd. PCL (MW 70,000~90,000 g/mol) and PEO (MW 1000 g/mol) are purchased from Sigma - Aldrich India. Phosphate buffer solution and Nutrient Agar are purchased from Himedia, India.

### Extraction of AV extract

AV powder procured from the local herbal medicine shop was weighed and refluxed in chloroform: methanol mixture in a volumetric ratio of 6:4 for 6 h at 60°C. Later, using the Whatman filter, the insoluble components were filtered, and the procedure was repeated twice[14].

### Characterization of AV extract

#### *Phytochemical Analysis of AV Extract*

*Test for flavonoids*

To 3 mL of AV extract, 10% of $NH_4OH$ (diluted ammonia) was added, and the colour change was observed and recorded[15].

*Test for Phenolic compound*

2 mL of AV extract was diluted by 2 mL of distilled water (DW). To this, a ferric chloride

solution of few drops was added and mixed well. The colour change was observed and recorded[15].

*Test for glycosides*

With 2 mL of AV extract, 2 mL of glacial acetic acid was added. The mixture of few drops of ferric chloride solution was added, then concentrated sulphuric acid was added along the tube's sides. The difference in colour change was noted and recorded[15].

*Test for Tannins*

To 0.5 mL of AV extract, 1 mL of DW, and 2-3 drops of ferric chloride solution was added. The colour change was observed and recorded for the presence of tannins[15].

*Test for Anthraquinones*

To 2 mL of AV extract, 1 mL of diluted $NH_3$ solution was added, and a change in colour difference was observed and recorded[15].

*Green synthesis of Ag NP using AV extract*

The Ag NP was synthesized using AV extract from silver nitrate. For synthesis, 5 mL of 2 M silver nitrate solution was added to 30% ammonia solution of 2 mL, followed by 5 mL addition of AV extract. Finally, the solution volume was adjusted to 50 mL by the DW and stirred under a magnetic stirrer at 250 rpm for 24 h. A colour change of faint yellow colour was observed after 24 h of reaction, indicating the formation of Ag NP[16].

**Characterization of AV and Ag NP**

The prepared AV extract was analysed using UV-visible spectrophotometer, whereas the Ag NP were characterized by UV-visible spectrophotometer, SEM (Scanning Electron Microscope), and TEM (Transmission Electron Microscope).

**Preparation of wound dressing by PCL/PEO polymer matrix with incorporation AV/Ag NP**

Wound dressing material was prepared by incorporating AV/Ag NPs into the PCL/PEO matrix. For this Box-Behnken design was used with three independent variables namely Ag wt% (100 mg, 125 mg, 150 mg), AV wt.% (20%, 30%, 40%) and PEO wt.% w.r.t. PCL (1%, 3%, 5%) for the preparation of wound dressing (Table 1).

Table 1 — Independent variables considered for the design

| Independent Variables | −1 | 0 | +1 |
|---|---|---|---|
| PEO (%) | 1 | 3 | 5 |
| Ag (mg) | 100 | 125 | 150 |
| AV (%) | 20 | 30 | 40 |

**Box-Behnken Design (BBD)**

BBD[17,18] belongs to the experimental designs of surface response methodology. BBD is a three-level incomplete factorial design that offers a class of rotatable or nearly rotatable second-order design. The independent variables are coded as −1, 0, and +1 and are placed at equally spaced values. Combinations such as all the factors at the highest or lowest level are usually avoided in BBD. Thus, the design was useful in avoiding unsatisfactory results that might occur in experiments under extreme conditions. The number of experiments (S) required for the preparation of wound dressing by BBD is given by:

$$S = 2(k - 1) + Co$$

where k is the number of factors and $C_0$ is the number of central points. Table 2 gives the number of samples prepared as per BBD. Thus, based on the Box-Behnken design, wound dressing scaffolds with different concentrations were prepared using a solution casting method. PCL and PCL+PEO scaffolds prepared similarly by solution casting method were used as control samples.

**Functional study of wound dressing material**

***Water Vapour Transmission Rate (WVTR)***

The WVTR of the developed wound dressing was determined by following this procedure. The prepared dressing material cut into a pre-determined size of diameter 1 cm was used for the study. Later the same was mounted on the centrifuge tube's mouth, filled with water to 3/4$^{th}$ of its volume. Further, the

Table 2 — Box-Behnken design for the experiment

| Sample ID | PEO | Ag | AV | PEO wt.% | Ag wt.% | AV% |
|---|---|---|---|---|---|---|
| S1 | −1 | −1 | 0 | 1 | 100 | 30 |
| S2 | 1 | −1 | 0 | 5 | 100 | 30 |
| S3 | −1 | 1 | 0 | 1 | 150 | 30 |
| S4 | 1 | 1 | 0 | 5 | 150 | 30 |
| S5 | −1 | 0 | −1 | 1 | 125 | 20 |
| S6 | 1 | 0 | −1 | 5 | 125 | 20 |
| S7 | −1 | 0 | 1 | 1 | 125 | 40 |
| S8 | 1 | 0 | 1 | 5 | 125 | 40 |
| S9 | 0 | −1 | −1 | 3 | 100 | 20 |
| S10 | 0 | 1 | −1 | 3 | 150 | 20 |
| S11 | 0 | −1 | 1 | 3 | 100 | 40 |
| S12 | 0 | 1 | 1 | 3 | 150 | 40 |
| S13 | 0 | 0 | 0 | 3 | 125 | 30 |
| S14 | 0 | 0 | 0 | 3 | 125 | 30 |
| S15 | 0 | 0 | 0 | 3 | 125 | 30 |

centrifuge tube was kept in an environmental chamber at 37°C with 35% RH (relative humidity). The centrifuge tubes were weighted at a regular time interval to determine the weight loss across the dressing material[19]. From the data, a plot was drawn between weight loss *vs* time, from which the slope was calculated using the formula:

$$WVTR = \frac{slope\ X\ 24}{A} g/m^2/day$$

where A is the area of scaffolds in $m^2$.

***Degree of swelling***

The degree of swelling or the water-absorbing capacity% (WAC) of the prepared wound dressing with and without AV extract was measured in phosphate buffer solution (PBS) of pH 7 at 25°C for 24 h[14]. The difference in weights was calculated using the formulae.

$$Swelling\% = \left[\frac{(w_s - w_d)}{w_d}\right] * 100$$

$W_s$ was the weight of each sample after submersion in the buffered saline, and $W_d$ was the sample's weight before submersion in the buffered saline, respectively.

***Study of antibacterial property***

The antibacterial activity of the developed wound dressing was determined by the disc diffusion method. First, nutrient agar of 2.4 g was taken in the conical flask and dissolved by 100 mL of DW. The conical flask was plugged with cotton and sterilized. A few nutrient agar quantities were poured into the Petri plates from the sterilized conical flask and allowed to solidify. Later, a swab stick was dipped into the nutrient broth containing the bacterial cells and was swabbed in above the nutrient agar in the Petri plates. The prepared dressing was placed in a Petri plate, and the antibacterial activity was analysed from the inhibition zone length after 24 h[20].

## Results and Discussion

**UV-Visible spectrometer analysis of AV extract**

The UV-Visible spectroscopic analysis of AV extract was shown in (Fig. 1). The spectral peak was observed between 280 and 300 nm, which indicates the successful extraction of AV[14,21].

***Phytochemical analysis of AV extract***

*Test for flavonoids*

An intense yellow colour was formed, which indicates the presence of flavonoids in the extract. An important flavonoid present in aloe vera is called 'rectin,' which is known to act during wound healing by promoting healing and preventing infection[15] (Fig. 2A).

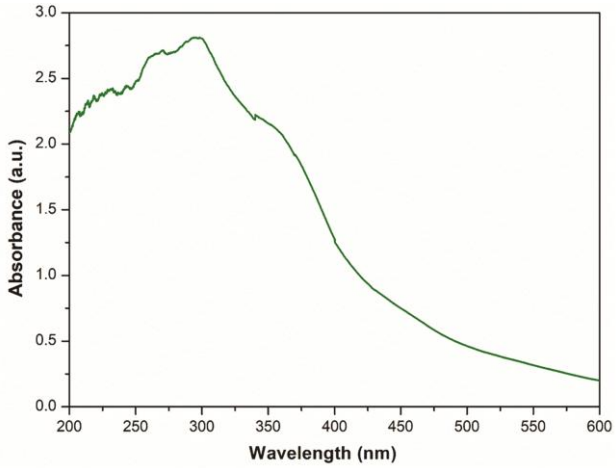


Fig. 1 — The UV-Visible spectrum of AV extract

*Test for Phenolic compounds*

A dark green colour was formed, which indicates the presence of phenolic compounds. Phenolic compounds have antioxidant and anti-carcinogenic property[15]. The phenolic compounds present in AV include anthraquinones, its derivatives, aloesin, 2'-O-feruloyl aloesin, aloeresin A, barbaloin, isobarbaloin, aloenin, *etc* (Fig. 2B).

*Test for glycosides*

A reddish-brown colouration was observed at the junction of two layers, with a bluish-green colour in the upper layer was observed, which confirms the presence of anthrone - glycosides in the AV extract. Glycosides have anti-inflammatory effects. AV contains anthrone glycosides of aloe –emodin, Stereoisomers of C-10-glycosides of anthrone, Isobarbaloin, aloinozides A and B, 8-O-methyl-7-hydroxy-aloin A and B, and chrysophanol; these are active compounds biologically involved in the wound healing process[15] (Fig. 2C).

*Test for Tannins*

Green black colour was observed, which indicates the presence of catecholic tannins. Tannin is a compound that tends to shrink or constrict body tissue, rendering them resistant to proteolytic enzymes (Fig. 2D).

*Test for Anthraquinones*

A pink-red colour was observed, which indicates and confirms the presence of anthraquinones. They have antiallergenic action. Major components of

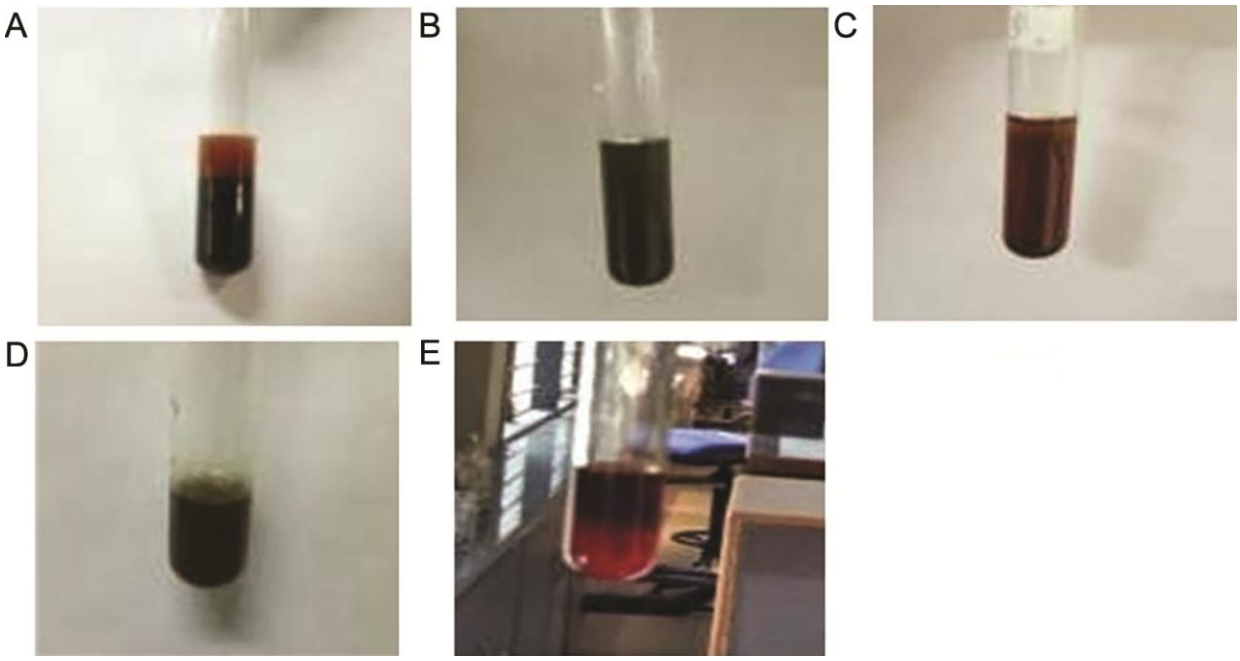


Fig. 2 — Phytochemical analysis of AV extract

aloe anthraquinones include hydroxyanthraquinone derivatives mixture of aloin A and B, 7-hydroxy aloin isomers, and diastereoisomeric 10-C glycosides of Aloe-emodin anthrone[15] (Fig. 2E).

**Characterization of Ag NP**

***The UV-visible spectrum of Ag NP***

The Ag NP's UV- visible spectroscopic measurements were carried out on a JASCO model V-570 dual-beam spectrophotometer, and the spectra are shown in (Fig. 3). For Ag NP, the reference will in the range of 400 to 430 nm[21,22], and the sample that we have prepared shows a peak at 420 nm, which confirms the formation of Ag NPs using AV as a reducing agent.

***SEM and TEM Studies of Ag NP***

Figure 4 shows the SEM and TEM images for the Ag NP. An accelerating voltage of 5 kV was used to capture the SEM image. SEM results show that the synthesized Ag NP is spherical in shape and is uniformly distributed in size. TEM results also confirm that the synthesized Ag NP were spherical in shape, and from the image, it was found that the average diameter of the Ag NP is between 10-50 nm[21,23,24].

***Functional study of wound dressing material***

*Water Vapour Transmission Rate (WVTR)*

Moisture balance remains to be optimum for optimal wound healing. WVTR for the prepared

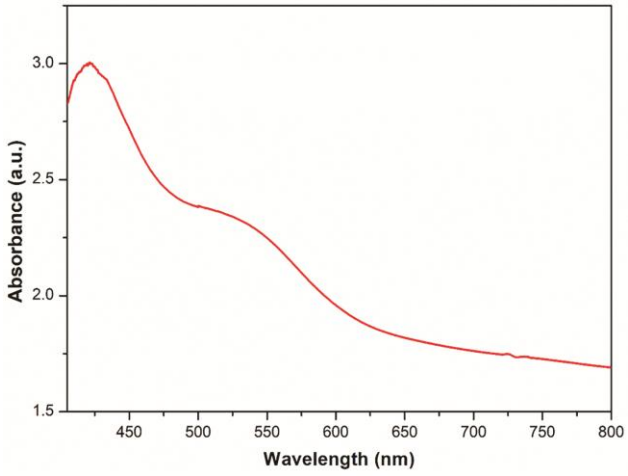


Fig. 3 — UV-Visible spectrum of Ag NP

wound dressings were calculated as weight loss vs. time for every 2 h for up to 6 h. An ideal wound dressing should have a water loss between 2500-5000 g/m$^2$/day for optimal wound healing[20,25]. From Table 3, the result shows the WVTR of the samples S3, S6, S11, S13, S14 was observed to provide an optimal moisture environment locally to promote wound healing. Figure 5 shows the influence of process parameters on WVTR. The graph shows that by increasing AV and PEO concentration, the WVTR also increases linearly with time. From the Figure 5, it was predicted that the PEO and AV range of 1-2% and 20-30% will provide the optimal WVTR.

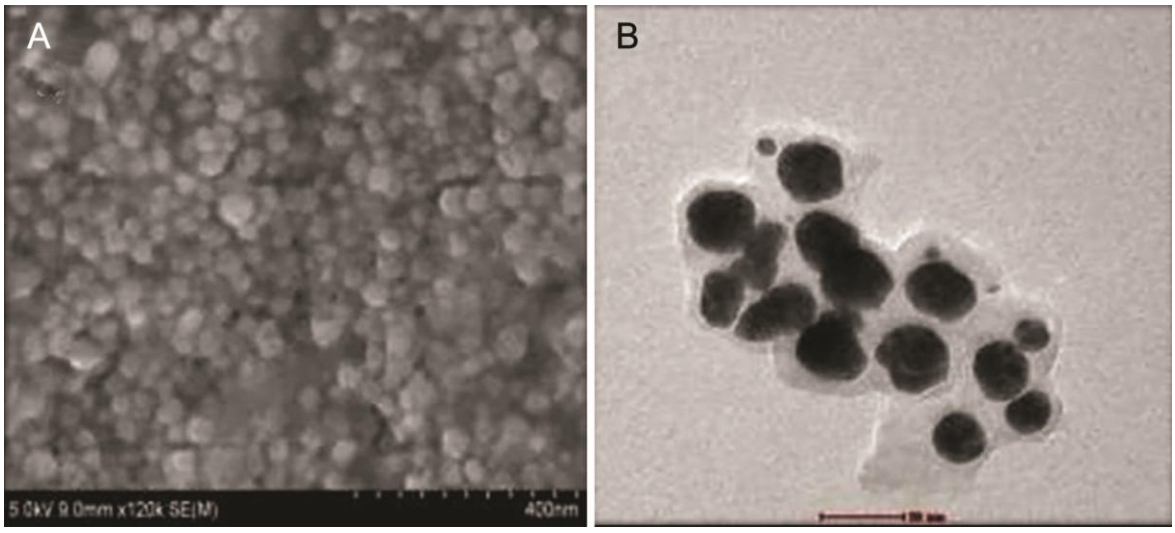


Fig. 4 — (A) SEM; and (B) TEM image of Ag NP

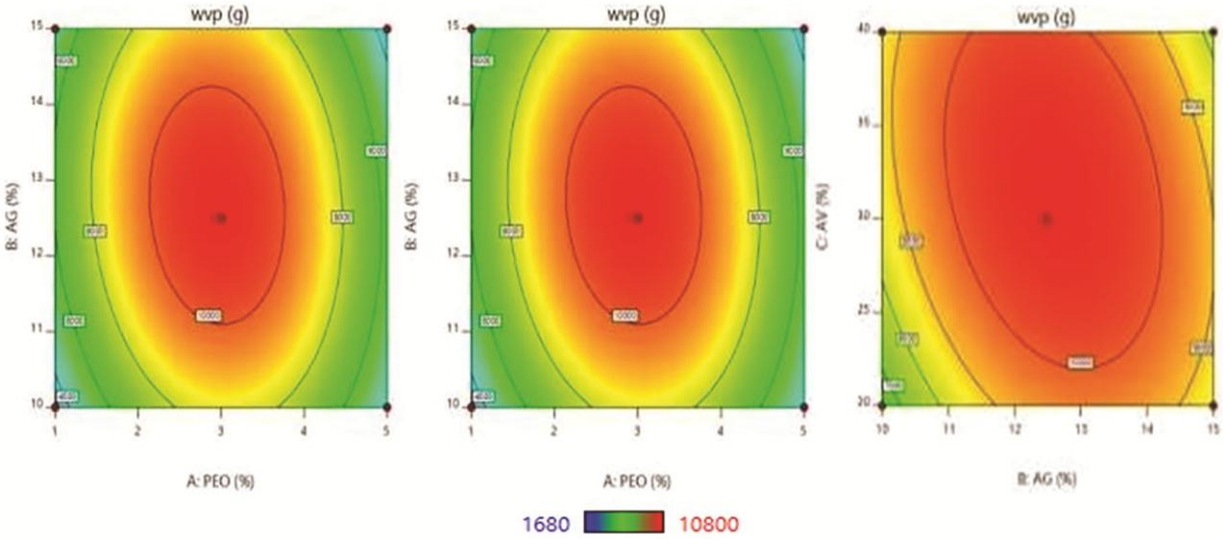


Fig. 5 — Influence of process parameters on WVTR

Table 3 — WVTR of the prepared scaffolds

| Sample ID | WVTR ($g/m^2/day$) |
|---|---|
| S1 | 6240 |
| S2 | 1680 |
| S3 | 4560 |
| S4 | 9600 |
| S5 | 6240 |
| S6 | 4080 |
| S7 | 10800 |
| S8 | 10800 |
| S9 | 10800 |
| S10 | 6240 |
| S11 | 3210 |
| S12 | 7440 |
| S13 | 4080 |
| S14 | 4800 |
| S15 | 7920 |

It was also observed that by varying the concentration of Ag wt.% does not affect the WVTR. Table 4 gives the regression equation predicted for WVTR.

*Degree of swelling*

A wound dressing material should possess good water uptake and water vapour. Permeability for maintaining proper moisture balance at the wounded site. Water uptake and permeation particularly depend on the polymer type and materials used to prepare the dressing. The wound exudates and other fluids coming out of the wound environment need to be absorbed to accelerate the healing process. The rate at which it happens was a significant factor for faster healing of the wound. Moreover, quick absorption will promote

autolytic debridement of the wound. Figure 6 shows that the weight of the sample decreases initially for a period of 1 h due to the solubility of PEO in water as well as the leaching of Ag and AV into the media. Further, the weight of the sample increases for the period of 5 h and remains constant. It was found that the amount of water uptake increases with the increase of AV composition in the dressing and thereby implying that the scaffold has become hydrophilic with the incorporation of the AV extract[14,20].

Figure 7 shows the influence of process parameters on swelling. From the contour plot, it was predicted that PEO and AV wt.% play a major role in swelling characteristics irrespective of Ag wt.%. Thus, PEO and AV range from 1- 3%, and 30-40% will provide an optimal wound dressing material. Table 5 gives the regression equation predicted for WVTR.

Table 4 —The regression equation for WVTR

| | Regression Equation | F-value | *P*-value | $R^2$-Value |
|---|---|---|---|---|
| WVTR | −78502.5000+9030.00*PEO +9576.0*Ag+990.0*AV -108.00*PEO*Ag-12.0*PEO*AV −28.80*Ag*AV-1237.50*$PEO^2$ −331.20*$Ag^2$-9.30*$AV^2$ | 7.74 | 0.0066 | 0.9087 |

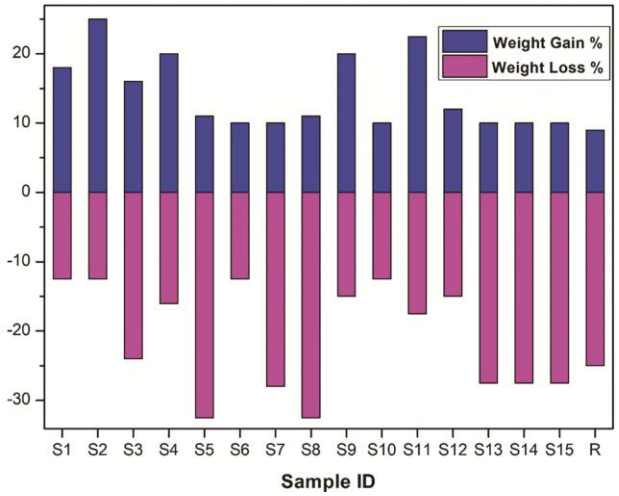


Fig. 6 — Weight loss and weight gain of the prepared sample

*Study of Antibacterial property*

The antibacterial activity of prepared wound dressing material was evaluated by observing their inhibitory zone length against Gram-negative (*E. coli*) bacteria[26]. It is evident from (Fig. 8) that the sample containing Ag NP had a good antibacterial effect against *E. coli*. In contrast, the reference samples (PCL, PCL+PEO) have shown no activity against the test bacteria. The inhibitory zone diameter of the samples with different Ag concentrations was 100 mg (2.1 cm), 125 mg (2.3 cm), 150 mg (2.5 cm)[27]. Based on the results obtained, the AV/Ag NP incorporated polymer scaffold can successfully inhibit bacterial growth at the wound site.

Table 5 — The regression equation for swelling

| | Regression Equation | F-value | *P*-value | $R^2$-Value |
|---|---|---|---|---|
| Swelling | 193.98-1.34*PEO - 3.083*Ag+1.1825*AV - 0.018*PEO*Ag +0.025*PEO*AV-0.001*Ag*AV +0.58*$PEO^2$ +0.012* $Ag^2$ -0.0182*$AV^2$ | 7.40 | 0.0076 | 0.9049 |

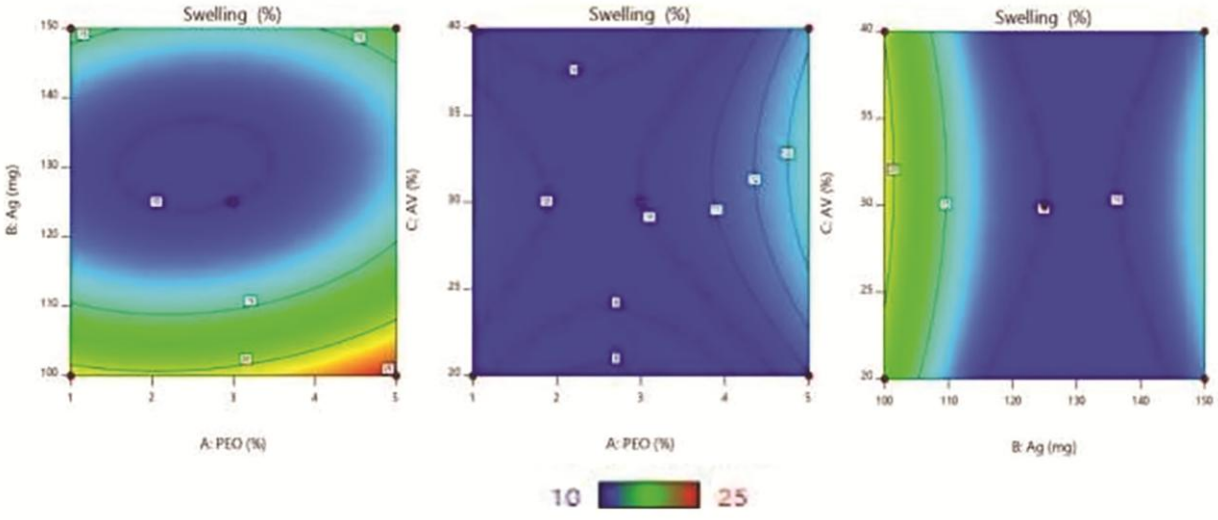


Fig. 7 — Influence of process parameters on swelling

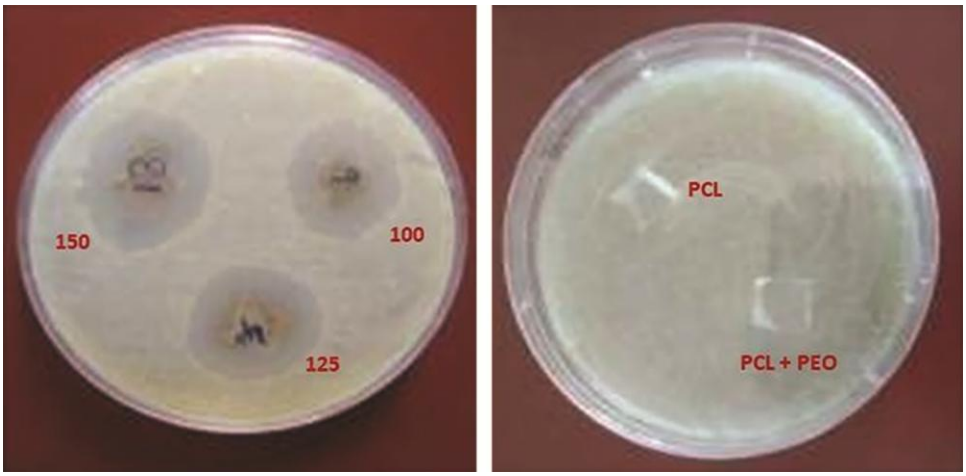


Fig. 8 — Antibacterial activity against *E. Coli*

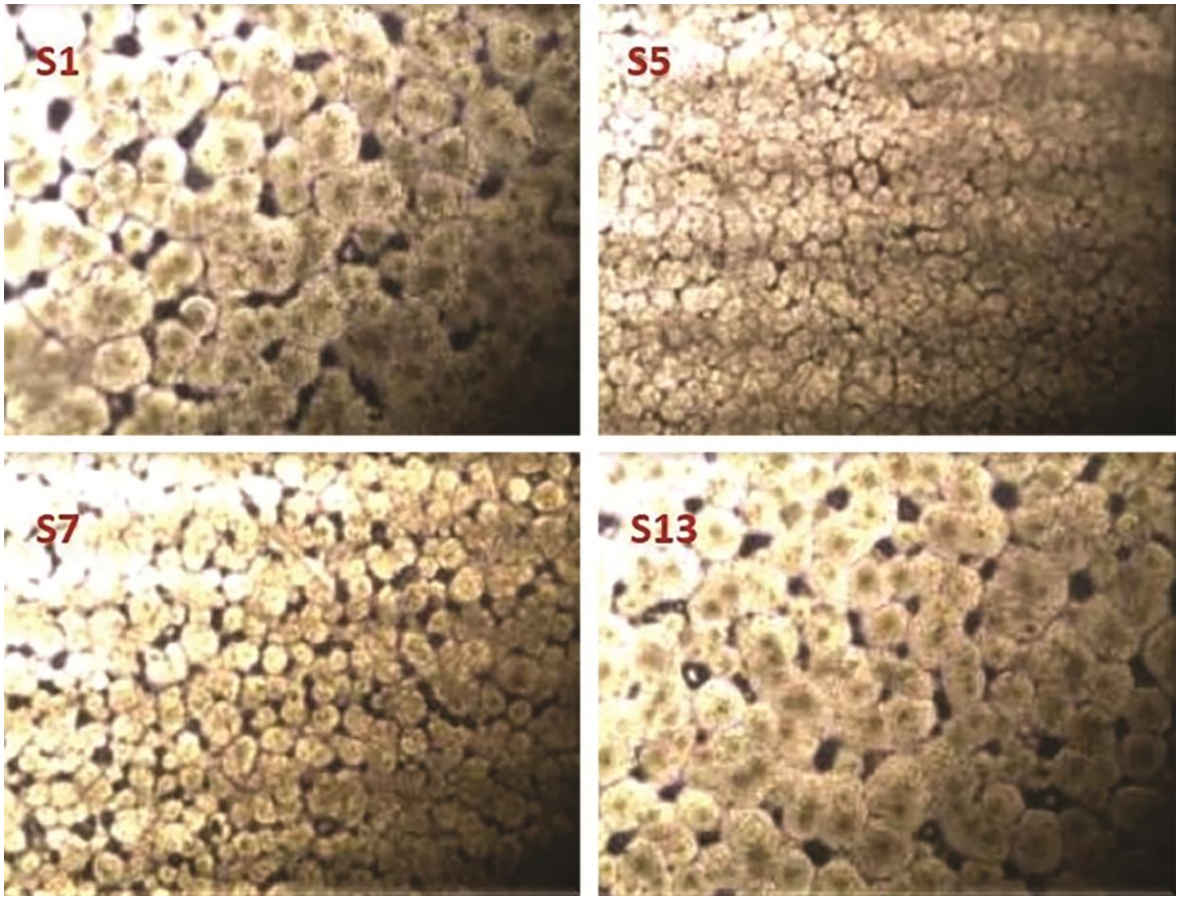


Fig. 9 — Optical microscope images for the sample

**Porosity Analysis**

The dressing porosity was an important parameter for wound healing because it offers a diffusion of oxygen and nutrients at a higher rate. Porosity, pore size, and pore interconnectivity can be controlled by varying the material composition of scaffold[28-31]. Figure 9 shows the optical microscope images of the prepared wound dressing material. It was clearly seen from the image that the porosity totally depends on the PEO wt.%. Porosity increases with increasing wt.% of PEO in the dressing; the reason is due to leaching of PEO as it is a water-soluble polymer. The

porosity of the material also plays a major role in releasing AV extract to the wounded site.

## Conclusion

The AV extract was successfully extracted from the AV powder using chloroform: methanol. By using the AV extract, Ag NP was prepared by the green synthesis method. Later the prepared AV/Ag NP was incorporated in PCL/PEO polymer matrix by solution casting method. The prepared Ag NP was confirmed through UV-visible spectroscopy, SEM, and TEM. SEM and TEM studies showed that the prepared Ag NP is spherical in shape, ranging from 10-50 nm. The effect of process parameters, namely AV, Ag, and PEO, was studied on WVTR and swelling using the Box-Behnken method. The WVTR and swelling study showed that AV of 20-30%, Ag of 100-125 mg, and PEO of 1-3% provides optimal results for preparing wound dressing material. Therefore, the samples with these combinations S3, S4, S11, S13 provided better results. The pore size analysis using an optical microscope showed that the pore size increases with increasing PEO concentration. The prepared dressing material also showed better antibacterial activity against *E. coli* bacteria, and the antibacterial activity increases with increasing wt.% of Ag NP. Overall, the prepared AV/Ag NP incorporated PCL/PEO polymer matrix can be used as an effective wound dressing material.

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## Conflict of interest

All authors declare no conflict of interest.

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