Modification of Waste Leather Trimming with \textit{in situ} Generated Silver Nanoparticles by One Step Method

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Abstract
Waste leather trimming (WLT) was modified by \textit{in situ} generation of silver nanoparticles (AgNPs) and silver oxide nanoparticles (Ag$_2$ONPs) by one step thermal assisted method. The modified WLT was analyzed by FTIR, SEM, EDS, XRD and TGA techniques and antibacterial analysis. The generated silver based nanoparticles had a mean size of 93 nm. The FTIR spectra revealed no major changes between WLT and modified WLT. The XRD studies indicated additional peaks in the diffractogram of modified WLT which belonged to AgNPs and Ag$_2$ONPs. The unmodified WLT had four inflection temperatures at 89, 303, 454, and 785°C while these values for the modified WLT were 84, 211, 305, and 328°C indicating a slight lowering of thermal stability due to the catalytic activity of the generated silver based nanoparticles. Further, the modified WLT formed the inhibition zones against \textit{E.coli}, \textit{P.aeruinoso}, \textit{S.aeureus} and \textit{B.lichinomonas} bacteria with diameters of 33, 36, 29, and 30 mm respectively. Hence, the modified WLT can be considered as filler for making low cost antibacterial biocomposites.

Keywords: Industrial waste, Leather trimming, \textit{In situ} method, Silver and silver oxide nanoparticles, Antibacterial activity

1 Introduction
The science and technological development is benefiting the society to the maximum extent and also meeting the needs of the people. In recent times, these activities have become part of our daily life [1]. Unfortunately, the scientific achievements are not only meeting the needs of the people but also creating lot of pollutants...
in the environment. Hence, the researchers are concentrating on environmentally benign materials and innovative industrial methods for maintaining environmental protection and society wellbeing [2]–[7]. One of the major global problems is disposal of solid wastes and one possible solution is to convert them into useful and harmless products, utilizing environmental friendly methodologies.

The solid waste materials such as tamarind fruit shells, hen egg shells, coconut shells, etc., are occupying huge land areas polluting the surrounding environment and ground water [8]. Leather industry is one of the major growing industries of the world resulting huge amounts of waste materials in the environment. The leather processing is also responsible for unfavorable impact on the surrounding areas [9]. The leather making process also generates large quantities of solid waste materials such as shaving, trimming, buffing dust, etc which form more than half of the raw hide being rejected as waste [10].

A few researchers have reported the ecofriendly methods of utilizing solid wastes. For instance, some agricultural wastes and used coffee bean powder were utilized in polymer matrices for preparing bio composites [11], [12]. The biodegradable composite films were prepared using the waste tamarind nut powder in polypropylene carbonate [13] and cellulose [14] for packing applications. The kitchen waste of egg shells is creating lot of pollution posing health problems and spreading diseases [15]. The egg shell powder (ESP) was used as a filler in poly lactic acid [16] and polypropylene carbonate [17] for making biodegradable films. Recently, a hydrothermal method was adopted to modify ESP by in situ generating copper nanoparticles [18]. The leather waste (shavings, buffing dust, trimming etc.) is employed as an important protein source, agricultural fertilizer, thermal insulator, building concrete material, chrome recovery for bio energy and as filler to make polymer composites [10], [19]–[26]. Waste leather buff was utilized as filler in epoxy resin matrix and made useful composites [27]. Waste leather buff was also used as a reinforcing filler in poly lactic acid to make useful biocomposites in which the modulus increased by 25% with mere 10wt% filler content [28]. In one study, leather waste was used in preparing light weight construction materials to add value to the waste generated in leather industries [29]. In another study, AgNPs were generated by in situ method to modify tamarind fruit shells powder (TFSP) to impart antibacterial activity [30]. Some researchers generated bimetallic nanoparticles in cellulose cotton fabrics by in situ method, utilizing plant parts like leaves, stem etc. [31], [32].

In the present study, waste leather trimming (WLT) was selected as it is generated in huge quantities in leather industries. Moreover, WLT is available in pure form compared to leather shaving, buffing dust etc. Hence, the authors made an attempt to modify WLT with in situ generated silver based nanoparticles by single step thermal assisted method. The modified WLT was analyzed by X-ray diffraction (XRD), Thermogravimetric analysis (TGA), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). The modified WLT was also tested for its antibacterial properties for potential use as filler for making low cost antibacterial biocomposites.

2 Experimental

2.1 Materials

The vegetable tanned waste leather trimming (WLT) was collected from local leather industries in India and ground down to around 2 to 3 mm size in a high speed grinder. The obtained WLT was light brown in color. Silver nitrate (Sigma-Aldrich Chemicals Ltd., Mumbai, India) and deionized water were also utilized in this research work.

2.2 Modification of WLT

WLT was first rinsed in mild soap solution. It was subsequently cleaned with conductivity water a number of times until all the impurities were removed and then dried. Single step thermal assisted method was employed to in situ generate silver based nanoparticles on the surface of WLT following the procedure given elsewhere [30]. In brief, in a glass beaker, 50 mL of aqueous 5 mM AgNO₃ solution (source solution) was prepared. This beaker was then kept on a magnetic stirrer with hot plate maintained at 80°C and to this hot source solution, 4 g of dried WLT was added and stirred at a speed of 300 rpm for 24 h. The light brown colored WLT changed to chocolate brown, preliminarily indicating the in situ generation of silver based nanoparticles in the modified WLT. In some similar studies, we already reported that the color of the
nanocomposites was different from that of the matrix and the change in color was a preliminary indication of the \textit{in situ} generation of metal nanoparticles \cite{17, 18, 30–32}. The presence of the reducing chemical groups in the matrix such as hydroxyl, amino, polyphenols etc played an important role in reducing the metal salts into their corresponding nanoparticles \cite{17, 18, 30}. Finally, the modified WLT was separated, cleaned rigorously with conductivity water for number of times and dried.

3 Characterization of the WLT and Modified WLT

To visualize the generated AgNPs and study the particle size distribution, the scanning electron microscope (Zeiss EVO 18) operated at 10KV was used to record the micrographs and EDS of gold sputter coated modified WLT. To study the possible interactions between the AgNPs and WLT, the FTIR spectra of WLT and modified WLT were recorded using Smart iTR ATR Nicoet iS10 FTIR spectrophotometer in the spectral range of 4000–500 cm$^{-1}$. To confirm the generation of silver based nanoparticles in the modified WLT, the X-ray diffractograms of WLT and modified WLT were recorded using Brucker D8 Advance X-ray diffractometer operated at 40 KV and 25 mA in the 2θ range of 10° to 85° at a scanning rate of 4° per min. To study the effect of AgNPs on the thermal stability of the modified WLT, the derivative thermograms of WLT and modified WLT were recorded employing a thermogravimetric analyzer (TGA NETZSCH STA 2500) at a heating rate of 10°C/min in an Argon atmosphere.

The disc method was adopted to assess the antibacterial activity of the materials under study \cite{30}. The bacterial cultures of both Gram-positive (\textit{S. aureus} (MTCC 76) and \textit{B. licheniformis} (MTCC 73637)) and Gram-negative (\textit{E. coli} (MTCC 1652) and \textit{P. aeruginosa} (MTCC 2453)) bacteria were used in the test. Zones of clearance indicating the antibacterial activity in each case were photographed. Using the clearance zone images, the size of the inhibition zones was measured using ImageJ software. More details are provided in the antibacterial analysis section.

4 Results and Discussion

4.1 Physical observation of change in color

The digital images of WLT and modified WLT with generated silver based nanoparticles are depicted in Figure 1(a) and (b) respectively to visualize the change of color.

![Figure 1](image1.png)

Figure 1: Digital images of (a) waste leather trimming (WLT) and (b) modified WLT.

![Figure 2](image2.png)

Figure 2: SEM images of modified WLT at (a) $\times$25K and (b) $\times$50K (some generated AgNPs are indicated by arrows). (c) EDX spectrum and (d) Histogram (particle size distribution).

From Figure 1, it can be visualized that the WLT was light brown in color while the modified WLT appeared chocolate brown. The change in color preliminarily indicates the formation of silver based nanoparticles in the modified WLT \cite{17, 30}. However, in order to confirm the generation of silver based nanoparticles in the modified WLT the SEM coupled EDS analysis was carried out.

4.2 SEM with EDS

The recorded SEM images of the modified WLT with generated silver based nanoparticles are presented in Figure 2(a) and (b) at two magnifications.

The corresponding EDS image is presented in...
Figure 2(c) and the particle size distribution histogram in Figure 2(d). The generated silver based nanoparticles on modified WLT were found to be spherical in shape as shown in Figure 2(a) and (b). The observed two peaks from EDS [Figure 2(c)] were related to silver element. These results confirm the in situ generation of silver based nanoparticles in modified WLT. Figure 2(d) indicates that the size of the generated silver based nanoparticles was between 54 nm and 120 nm and most of them were in 91–100 nm range with an average size of 93 nm.

4.3 FTIR studies

The interaction between WLT and the generated silver based nanoparticles in the modified WLT was investigated by FTIR studies. The spectra of the WLT and the modified WLT using 5 mm aq.AgNO₃ source solution are shown in Figure 3.

It is revealed from Figure 3 that the spectra of WLT and modified WLT exhibited similar peaks with variation of intensity between them. Further, it can also be seen that the intensity of spectrum of modified WLT was increased. The common characteristic peaks with wavenumber 3300 (N-H), 1660 (C=O), 1530 (NH coupled C-N), 1445 (C-N, C-C) and 1230 cm⁻¹ (C-H) indicate the presence of protein amide functionalities in WLT and modified WLT [20]–[24], [33]. Hence, it can be inferred that the generated silver based nanoparticles in the modified WLT did not bring major structural changes in the modified WLT.

4.4 XRD studies

XRD analysis was used to investigate the nature of the generated silver based nanoparticles in the modified WLT. The recorded diffractograms of the WLT and modified WLT are shown in Figure 4(a).

It was already established that WLT was amorphous without any characteristic peaks [34]. From Figure 4(a), it can be observed that the diffractograms of both WLT and modified WLT were similar except the presence of some additional sharp peaks in the modified WLT. In order to clearly observe the additional peaks in the modified WLT, its diffractogram was expanded in the 2θ = 26° to 80° range [Figure 4(b)]. From Figure 4(b), it can be observed that the diffractogram of modified WLT has the peaks at 2θ = 38.2° (111), 46° (200), 64.5° (220) and 76.7° (311) which belonged to the generated AgNPs [35]. The other sharp peaks at 2θ = 27.6° (110), 32.1° (111), 54.5° (220) and 67.2° (222) were related to Ag₂ONPs [17]. Hence, the X-ray analysis indicated the generation of both AgNPs and Ag₂ONPs in modified WLT. The generation of Ag₂ONPs besides the AgNPs was possible due to the oxidation of some of the generated AgNPs. Similar observation was also made by earlier researchers in the case of modified Thespesia lampas natural fibers having silver based nanoparticles [17].

4.5 TG analysis

Thermal degradation of the materials under study was examined by TG studies. The derivative thermograms of WLT and modified WLT are shown in Figure 5.

In both cases, the thermal degradation was observed in four stages as shown in Figure 5. The four inflection
temperature regions arose due to the evaporation of moisture and other volatiles, degradation of collagen, tannins and formation of char [26], [36] respectively. The catalytic activity of the silver based nanoparticles in the synthetic reactions was reported in the literature. In one study, PVC/ silver nanoparticles composites were prepared and their structural, thermal, dielectric and catalytic properties were evaluated [37]. In this study, the composite films especially with 10% silver loading showed improved catalytic performance during the reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) in the presence of aqueous sodium borohydride. In another study, the catalytic activity of silver nanoparticles supported on silica spheres [38], [39] with synthesized silver nanoparticles and loaded them onto polymer-inorganic composite materials and their regulated catalytic activity was studied. In the NaBH₄ reduction of 4-nitrophenol, they demonstrated that nanocomposite polymer chains not only favored the mass transfer of the reactant but also modulated the catalytic activities of the AgNPs under controlled temperature. Recently, AgNPs were synthesized by one step using Isoimperatorin and evaluated their photo catalytic and electrochemical activities. It was reported that the as-prepared Iso-AgNPs exhibited excellent electrocatalytic activity in the reduction of hydrogen peroxide [40]–[42] synthesized silver nanoparticles in the presence of poly acrylate using sodium borohydride as a reducing agent. They observed that these solutions having the AgNPs even after one thousand fold dilution, could catalyze the reduction of 4-nitrophenol to 4-aminophenol. In the present study, the four inflection temperatures in the case of WLT were found to be 89, 303, 454, and 785°C respectively while for the modified WLT the corresponding values were 84, 211, 305, and 323°C. These observations clearly indicate that the modified WLT exhibited lower thermal stability than WLT due to the catalytic effect of silver based nanoparticles on the thermal degradation. Such reduction in the thermal stability of the composites by the generated silver nanoparticles was reported for some other systems also [43]–[45].

4.6 Mechanism

Most of the leather waste contains collagen protein [7], [46] as a major constituent. The collagen protein (in WLT, in the present study) contains mainly three amino acids. They are glycine, proline and hydroxy proline. Glycine is the major amino acid constituent present in collagen protein [47]. The hydroxyl and amide functional groups present in glycine undergo reduction of silver ions into silver based nanoparticles. The mechanism of generation of silver based nanoparticles in WLT involving the glycine is presented in Figure 6.

![Figure 6: Mechanism of formation of AgNPs and Ag₂ONPs on WLT.](image)

The amide group in glycine reduces two silver ions to form glycine-silver complex. The formation of glycine-silver complex due to the affinity of glycine with metal ions was reported by earlier researchers [48]. This complex on rearrangement yields silver nanoparticles. The reduction of two Ag⁺ ions into Ag⁰, using two hydroxyl groups was reported in the literature [49], [50]. The presence of amide group involved in reduction was confirmed by FTIR spectra with a peak at 3300 cm⁻¹ [51], [52]. In case of formation of Ag₂ONPs, one silver ion was reduced by amide group, owing to
high catalytic activity and affinity with oxygen and the other silver ion attached to OH functional group of COOH of glycine forming Glycine-Ag complex which further on rearrangement forms Ag$_2$ONPs and an aldehyde [53], [54]. The formation of AgNPs and Ag$_2$ONPs was in accordance with XRD studies. Hence, the modified WLT with in situ generated silver based nanoparticles can be used as antibacterial filler in the preparation of hybrid nanocomposites [55].

4.7 Antibacterial activity

For medical applications, the modified WLT should possess antibacterial property. It is well known that the isolated AgNPs inhibit the growth of the bacteria [17], [30]. In order to assess the antimicrobial activity of the modified WLT against the standard lethal *P.aeruginosa* and *E.coli* (G$^-ve$) and *B.licheniformis* and *S.aureus* (G$^+ve$) pathogens, the antibacaterial test was conducted using nutrient agar as a culture medium. The medium consisted of peptone (5 g/L), yeast extract (1.5 g/L), beef extract (1.5 g/L), sodium chloride (5 g/L) and agar (15 g/L). The nutrient agar was a general purpose medium supporting growth of a wide range of non-fastidious organisms. The peptone component provided organic nitrogen while the beef extract and yeast extract contributed vitamins, carbohydrates, nitrogen, and salts. Over this media, the selected bacterial strains with a loading of 106 cells/mL were introduced separately and incubated for 12 h. After this, the unmodified and modified WLT were introduced over the inoculated media and left in incubation chamber maintained at 37°C for 24 h. The clear zones formed which indicate the inhibition of bacteria by the modified WLT were photographed. In each case the experiment was repeated three times [56]. The images of the formed zones of clearance in each case (which indicate the ability to inhibit the growth of bacteria) are presented in Figure 7(a)–(d).

Using Figure 7, the diameters of the clear zones exhibited by all the bacteria were measured and the corresponding values are presented in Table 1.

It can be clearly observed from Figure 7 and Table 1 that WLT did not show any clear zones and unable to kill the pathogenic bacteria tested. On the other hand, the modified WLT with generated silver based nanoparticles exhibited antibacterial activity with clear zone diameters in the range of 29–33 mm against all the bacteria under test. From the above observations, it can be inferred that the modified WLT possessed good antibacterial property and hence, can be considered as antibacterial filler to prepare polymer green composites.

**Table 1**: Clear zones formed by WLT and the modified WLT against pathogenic bacteria

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Code</th>
<th>Diameter of Inhibition Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WLT</td>
<td>1</td>
<td>No zone formation</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>No zone formation</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>No zone formation</td>
</tr>
<tr>
<td>Modified WLT</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>30</td>
</tr>
</tbody>
</table>

4.8 Comparative study

Earlier, we prepared some biodegradable nanocomposite films with *in situ* generated AgNPs by bioreduction method using different medicinal plant leaf extracts as reducing agents [43], [45], [56]. We also modified Thespesia lampas natural fibers [17] and waste tamarind fruit shell powder [30] with *in situ* generated AgNPs by hydrothermal method. For comparison of the two methods, the number of steps in making the nanocomposites, particle size range and range of the
The modified vegetable tanned waste leather trimming (WLT) with in situ generated silver based nanoparticles was made by one step thermal assisted method. The modified WLT was analyzed by FTIR spectroscopy, SEM coupled EDS, XRD and TGA and also tested for its antibacterial activity. The generation of silver based nanoparticles in the modified WLT was revealed by the SEM associated EDS analysis and found the nanoparticles to be with a mean size of 93 nm. No specific spectral changes were found between WLT and the generated silver based nanoparticles in the modified WLT. The formation of AgNPs and Ag$_2$ONPs in the modified WLT was established by XRD results. The thermal studies revealed that the modified WLT exhibited lower thermal stability than WLT due to catalytic effect of generated AgNPs and Ag$_2$ONPs. The modified WLT showed excellent antimicrobial activity and hence, can be considered as low cost antibacterial filler in the preparation of antibacterial polymer hybrid green composites.

<table>
<thead>
<tr>
<th>Nanocomposite</th>
<th>Leaf Extract/</th>
<th>Number of Steps</th>
<th>Particle Size Range (nm)</th>
<th>Inhibition Diameter Range (mm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose/AgNP films</td>
<td>Ocimum sanctum</td>
<td>2</td>
<td>81–140</td>
<td>16–22</td>
<td>[57]</td>
</tr>
<tr>
<td>Cellulose/AgNP films</td>
<td>Cassia alata</td>
<td>2</td>
<td>70–79</td>
<td>8–17</td>
<td>[45]</td>
</tr>
<tr>
<td>Cellulose/AgNP films</td>
<td>Terminalia catappa</td>
<td>2</td>
<td>11–110</td>
<td>5–13</td>
<td>[43]</td>
</tr>
<tr>
<td>Thespesia lampas/AgNP fibers</td>
<td>Hydrothermal</td>
<td>1</td>
<td>61–120</td>
<td>19–37</td>
<td>[17]</td>
</tr>
<tr>
<td>Tamarind fruit shell powder/AgNP</td>
<td>Hydrothermal</td>
<td>1</td>
<td>51–120</td>
<td>26–39</td>
<td>[30]</td>
</tr>
<tr>
<td>Waste leather trimmings/AgNP</td>
<td>Hydrothermal</td>
<td>1</td>
<td>54–120</td>
<td>29–33</td>
<td>Present work</td>
</tr>
</tbody>
</table>

Table 2: Comparison of the number of steps, particle size range and diameters of inhibition zones of some nanocomposite cellulose films with in situ generated AgNPs by bioreduction method and the modification of some waste materials with in situ generated AgNPs by hydrothermal method.


*B. Ashok et al., “Modification of Waste Leather Trimming with in Situ Generated Silver Nanoparticles by One Step Method.”*