Synthesis, Characterization and Antibacterial Activity of Gelatin-Herb Nanocomposite

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ABSTRACT:
Bio nanocomposites open an opportunity for the use of novel, high performance, light weight nanocomposite materials making them to replace conventional non-biodegradable polymer matrix materials. The present study focused on the synthesis of gelatin-herb bio nanocomposite by multiple emulsion and solvent evaporation method. The synthesized bio nanocomposite was characterized by UV, FTIR and SEM analysis techniques. The average size of the composite particle was found to be in the range of 56 – 96 nm. The composite showed antibacterial activity against bacterial strains.

Key words: bio nanocomposite, UV, FTIR, SEM, Antibacterial activity.

INTRODUCTION:
A nanocomposite is a multi-phase solid material where one of the phases has one, two or three dimensions of less than 100 nm or structures having nano-scale repeat distances between the different phases that make up the material [1]. Bio nanocomposites are a novel class of nano sized materials. They show a remarkable advantage of exhibiting biocompatibility, biodegradibility and in some cases functional properties provided by either the biological or other added materials [2][3] The present work focused on the synthesis of gelatin-herb bio nanocomposite by multiple emulsion solvent evaporation method [4]. Gelatin is a biopolymer, nontoxic, biocompatible and its chemical composition, in many respects similar to that of parent collagen. It finds its application in variety of fields like pharmaceutical, biomedical, cosmetics, food science [5] etc. The herb chosen for this work was Andrographis paniculata belongs to Acanthaceae family has a number of important medicinal uses, in the treatment of upper respiratory infection, ulcerative colitis and rheumatic symptoms [6][7].

METHODS AND MATERIALS
Chemicals: All the chemicals used in the synthesis were AR grade. Chemicals used in this synthesis such as Gelatin, Tween-80, Span-80 and Tri poly phosphate (TPP) were purchased from HIMEDIA and used as such and distilled water used for the synthesis. The leaves of the herb were collected from in and around Chennai. The leaves were shade dried, powdered and sieved.

Synthesis: Gelatin-Andrographis paniculata nanocomposite was prepared by following steps.

Preparation of Gelatin solution: About 1 gram of gelatin was dissolved in 100 ml of water.

Preparation of Herbal extract: 20 grams of herbal powder was suspended in 100 ml of methanol and incubated overnight. The supernatant liquid was filtered twice using Whatman no. 1 filter paper.

Preparation of Herb – Gelatin Composite: About 10 ml of the herbal extract was added drop wise to the prepared gelatin solution under constant stirring.

Preparation of Herb – Gelatin Nanocomposite: 3 % gelatin solution was added with 5 % (W / V) Tween – 80 and placed on a magnetic stirrer for 1hr. The herbal extract was added to the emulsion and kept in the magnetic stirrer for 5 min. simultaneously 5 % Span – 80 was prepared with Palm oil and stirred for 10 min. Both the solutions were mixed in 9: 1 ratio stirred well for 5 min. To this 0.01g of TPP was added and kept in the water bath at 50°C and then cooled. Nanocomposite was segregated from the Palm oil by repeated washing with Petroleum ether.

Characterization: The gelatin – herb nanocomposite and the gelatin solution were analyzed by UV-Visible spectrophotometer of the model SHIMADZU 1650 PC and FTIR spectroscopy using model of SHIMADZU IR 1650 PC. SEM images were obtained by Field Emission Microscope (FESM) Dst- Nano emission model. Antibacterial activity of the synthesized nanocomposite was carried out by Agar Well Diffusion method.

RESULTS AND DISCUSSION
Ultraviolet Spectroscopy:
The gelatin - herb nanocomposites, and gelatin were analysed using UV-Visible Spectrophotometer. The UV-Visible

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The spectrum of gelatin - herb composite is shown in figure- 1.

**Figure–1:** UV Spectrum of Gelatin and Gelatin- Herb Nanocomposite

The UV spectrum of gelatin shows that it is transparent in the UV and visible region and its optical properties are hard to characterize by spectroscopic method. whereas the UV spectrum of gelatin- herb nanocomposite showed 3 peaks at 412 nm, 317 nm and 331 nm which indicates the weak electronic transition between gelatin and the herb.

**FT-IR Spectroscopy:**

FT-IR spectroscopy was scanned using IR model of SHIMADZU 1650PC. The samples were mixed uniformly with potassium bromide at 1:5 ratio respectively. The KBr disc was prepared by compressing the sample and KBr at a pressure of 5 tons for 5 minutes in a hydraulic press. The disc was scanned in the range of 400-4000 cm\(^{-1}\) to obtain FT-IR spectra. FT-IR spectra of gelatin and gelatin -herb nanocomposite are shown in figure -2.

**Figure -2:** FT-IR spectrum of Gelatin and Gelatin- Herb Nanocomposite

The Fourier transform infrared spectrum of gelatin shows various vibration bands at 3441 cm\(^{-1}\) for N-H stretch coupled with hydrogen bonding (HB), 2932 cm\(^{-1}\) for alkenyl C-H stretching, 2874 cm\(^{-1}\) for CH\(_2\) asymmetrical stretching, 1641 cm\(^{-1}\) for C=O stretching, one hydrogen bonding coupled with COO\(^{-}\) band at 1535 cm\(^{-1}\) for N-H band coupled with CN stretch, 1448 cm\(^{-1}\) for CH\(_2\) band, 1255 cm\(^{-1}\) for NH band and 1096 cm\(^{-1}\) for C-O stretch.

FT-IR spectrum of Gelatin-Herb nanocomposite showed a mixture of characteristic absorptions similar to that of the pure gelatin. The intensity of the absorption peaks for the nanocomposite found to be lower than that of gelatin, because of the result of formation of intermolecular hydrogen bonding between gelatin and herb.

**SEM Analysis:**

SEM was used to investigate the distribution of herbal nano particles in the gelatin matrix. The SEM micrograph of the gelatin- herb nanocomposite is shown in the Figure– 3. The average diameter of the nanocomposite particle was found to be in the range of 56 – 95 nm. SEM images of surface and cross sectional area of the film showed the nanoparticles were present in small aggregates near the surface as well as dispersed through the gelatin matrix.

**Antibacterial Activity:**

The extremely small size of the nanoparticles mean they exhibit enhanced or different properties when compared with the bulk material. The extremely small size of nanoparticles results in the particles large surface area relative to their volume, which allows them to easily interact with the other particles and increases their antibacterial efficiency. Antibacterial activity towards *Staphylococcus aureus* and *Escherichia coli* was evaluated using agar plate method and it is shown in figure 4 and 5. Antibacterial activity were tabulated in Table 1.

**Figure -3:** SEM image of Gelatin- Herb nanocomposite

**Figure 4 and 5:** Inhibitory effect of (1) Gelatin, (2) Herb, (3) Gelatin Herb composite, (4) Gelatin - Herb nanocomposite,(5) Streptomycin against *S. aureus* (Fig-4) and *E. coli* (Fig-5).
Table – 1: Antibacterial activity of Gelatin, herb, Gelatin herb composite, Gelatin -herb nanocomposite and streptomycin

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zone of Inhibition in mm</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>Gelatin</td>
<td>-</td>
</tr>
<tr>
<td>Herb</td>
<td>10</td>
</tr>
<tr>
<td>Gelatin Herb Composite</td>
<td>9</td>
</tr>
<tr>
<td>Gelatin Herb Nanocomposite</td>
<td>12</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>16</td>
</tr>
</tbody>
</table>

As shown in figure - 4 and 5 the zone of inhibition appeared around the gelatin, Herb, gelatin-herb composite and gelatin-herb nanocomposite. For nanocomposite the inhibition zone was larger than that of composite, gelatin and herb which shows nanocomposite has enhanced antibacterial property.

CONCLUSION
Bio nanocomposite was synthesized by multiple emulsion solvent evaporation method and were characterized by UV, FTIR and SEM techniques. UV & FTIR confirmed the formation of composites. The morphology of nanocomposite was examined by FESEM. The SEM results showed that the nanocomposites were in the size range of 56-96 nm. The antibacterial effects of nanocomposites against S. aureus & E. coli were examined by Agar well diffusion method. The results revealed a nanoparticle formation within the gelatin matrix. It was proved that the synthesized bio-nano composite had an excellent antibacterial activity.

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