Chapter 4

Characterization of the sheets processed through the machinery

Collagen, the most abundant protein, known as a good wound dressing material, is used in the treatment of burns and wounds. The collagen sheets prepared using automated machinery were characterized for their physiochemical properties and results are discussed in this chapter.

4.1 X-RAY DIFFRACTOMETRY (XRD)

The Collagen samples were analyzed on a Bruker 500 X-ray powder diffractometer using a source of Cu Kα with the wavelength of 1.5406 Å. The patterns were recorded in the 2θ range of 10°–90° in steps of 0.001° with a counting time of 1 s in each step.

4.2 INFRARED SPECTROSCOPY (FTIR).

FTIR spectra of the collagen sheets was measured at a resolution of 4 cm⁻¹ in the frequency range of 4000–500 cm⁻¹ using Nicolet 360 FTIR Spectrometer.

4.3 SCANNING ELECTRON MICROSCOPY

The sample was coated with gold ions using an ion coater (Fisons sputter coater) under the following conditions: 0.1 Torr pressure, 20 mA current and 70 s coating time. Surface structure was visualized by scanning electron microscope (JSM 5300 Scanning microscope) using 15kV accelerating voltage.

4.4 ATOMIC FORCE MICROSCOPY

The shape and surface morphology of collagen sheets were investigated using atomic force microscopy (AFM, Agilent Pico LE Scanning Probe Microscope) under normal atmospheric condition.
4.5 MECHANICAL PROPERTIES

Three dumbbell shaped specimens of 4mm in width and 10mm in length are punched out from the prepared films. Mechanical properties such as tensile strength (MPa) and elongation at break (%) were measured using a Universal testing machine (INSTRON model 1405) at an extension rate of 10cm/min. The force and elongation at break were calculated and given by the formulae.

\[
\text{Tensile strength (N/mm²)} = \left( \frac{\text{breaking force (N)}}{\text{crosssectional area of sample (mm²)}} \right) \times 100
\]

\[
\text{Elongation at break (%) = } \left( \frac{\text{Increase in length at breaking point (mm)}}{\text{Initial length (mm)}} \right) \times 100
\]

4.6 WATER ABSORPTION CAPACITY

Estimation of water absorption capacity was done by the method of (Rao et al 1996). The water absorption capacity was determined by swelling small pieces of each sample of known weight in distilled water at room temperature. The swollen weight of the sample was determined by first blotting the samples with filter paper followed by accurately weighing the sample. The weights of the swollen pieces were recorded every 1h, 2h, 3h and after 24h. Percentage swelling of the samples at a given time was calculated from the formula.

\[
\text{Es} = \left( \frac{W_s - W_o}{W_o} \right) \times 100
\]

Where, \(W_s\) is the weight of the sample (moist) at a given time, \(W_o\) is the initial weight of the sample, \(Es\) is the percent of swelling at a given time.
4.7 RESULTS AND DISCUSSIONS

The XRD pattern of collagen sheet developed from the automated machinery is shown in Fig 4.1. The characteristic collagen diffraction peak observed matches with pure collagen. It should be mentioned here that the processed sheet from the machinery does not show the presence of chromium in comparison to the conventionally heated collagen sheets that show trace amount of collagen (Wang et al 2014).

![Collagen film from our inbuilt machine](image)

**Fig.4.1. XRD patterns of collagen sheet from the continuous collagen sheet forming machinery**

The FTIR spectrum of collagen sheet developed from the machinery is shown in the Fig 4.2. The major peaks at 1644 cm\(^{-1}\), 1557 cm\(^{-1}\) and 1240 cm\(^{-1}\) are assigned to amide I, amide II and amide III bonds respectively. The prominent peak at 2957 cm\(^{-1}\) represents the CH\(_2\) - CH\(_3\) stretching vibration which is a characteristic of collagen (Sionkowska et al 2004).
Fig. 4.2. FTIR spectrum of collagen sheet from the continuous collagen sheet forming machinery

Fig 4.3 and 4.4 illustrates the SEM images of collagen sheet from the developed machinery. The images reveal that the surface of the developed sheets are smooth. The porous nature of the collagen sheet was observed and uniformity in thickness is seen throughout the sheet unlike those prepared in batch process.

Fig. 4.3. SEM images of collagen sheet prepared using the continuous collagen sheet forming machinery.
Fig.4.4. SEM images of collagen sheet prepared using the continuous collagen sheet forming machinery.

The topological information about a sample with nanometer-order resolution is depicted with an AFM shown in the Fig 4.5(c) and Fig 4.5(d). AFM pictures of transverse sections of collagen film developed from the machinery is shown in the Fig 4.5(a) and Fig 4.5(b). The sample shows collagen structure with uniaxial and well defined image. Bigger structures on the collagen sheet surface possibly represent agglomeration of collagen fibrils, whereas small dots represent individual fibers (Nocedal et al 2006).

Fig.4.5. (Continued)
Fig. 4.5. The topological information with nanometer-order resolution

The collagen sheet developed using the machinery exhibited tensile strength of 6.06 ± 0.8 MPa and elongation at break was observed to be 31.25 ± 2%. The results confirm that the sheets processed are suitable for wound dressing applications. (Ramnath et al 2012)

The water absorption capacity of a biomaterial is an important feature when it is applied onto an open wound surface. A biomaterial with better water absorbing properties absorbs wound exudates and keeps the wound dry, thereby prevents air borne infection. In the present study, sheets processed from the machinery show increased water absorption capacity with increase in time and exhibit a maximum of 190± 10% which is comparable with that of the conventionally prepared sheets.
Fig. 4.6. Water Absorption Studies of the Collagen Sheets Developed from the continuous collagen sheet forming machinery.

The characterization study reveals the sheets developed using the machinery are comparable to the sheets developed using the conventional batch process.