# THERMAL TREATMENT OF GELATIN SOLUTION IMPROVES THE QUALITY OF ELECTROSPUN GELATIN FIBROUS MATS FOR MEDICAL APPLICATIONS

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*Abstract*. Preliminary studies were undertaken to assessment the impact of thermal treatment of commercial gelatin solution on the physicochemical properties of electrospun gelatin fibrous mats. The gelatin (dry granules) was dissolved in aqueous solution and heated up to boiling then was dried at 40 °C. The electrospinning process and cross-linking of electrospun mats using glutaraldehyde (GTA) vapor were achieved. The results revealed that thermal treatment of raw gelatin solution before electrospinning process enhanced the quality of electrospun gelatin fibrous mats, especially in terms of fibers sizes, degradation time, and swelling degree that could provide a basis for improving electrospun gelatin fibers for medical applications.

*Key words*: Gelatin, thermal treatment, electrospun mats, swelling degree, degradation properties.

## INTRODUCTION

Gelatin is a soluble high molecular weight biopolymer extracted by thermal denature of collagen molecules [1, 10]. Gelatin has been widely used for biomedical applications due to its non-toxicity, biodegradability, solubility, and biocompatibility [2, 4, 11]. Recently, electrospun gelatin fibrous mats have become promised as wound dressings or tissue engineering scaffolds [3, 15]. Electrospinning technique has been used to prepare appropriate fibrous gelatin textures having random or aligned fibers with different sizes by tuning processing parameters [9, 14]. In comparison to conventional gelatin mats, electrospun gelatin fibrous mats have controllable fiber sizes and higher surface area, however, the gelatin has many drawbacks during electrospinning process such as disability to electrospun it from aqueous solutions in addition to having large fiber diameters [8, 13, 16]. Moreover, electrospun gelatin membranes have a drawback of their fast degradation making them unsuitable for some medical applications [5, 7]. In order

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to overcome these barriers for electrospinning of gelatin solutions, various approaches have been achieved for obtaining better electrospun gelatin fibrous mats such as using appropriate solvents and chemical additives [6, 12]. However, no studies have been carried out to explore the impact of pre-hydrothermal treatment of gelatin solution on its electrospun fibers properties. Hence, the main objective of the present work is to propose a simple pre-hydrothermal treatment of gelatin solution before electrospinning process as an effective economic method to improve the physicochemical properties of electrospun gelatin fibrous mats. Therefore, the aim of the present study is to investigate the effect of pre-thermal treatment on gelatin solution on the enhancement of the quality of electrospun gelatin fibrous mats, especially in terms of fibers sizes, degradation time, and swelling degree that could provide a basis for improving electrospun gelatin fibers for medical applications.

## MATERIALS AND METHODS

### THERMAL TREATMENT OF GELATIN SOLUTION AND FABRICATION OF CROSS-LINKED ELECTROSPUN GELATIN FIBROUS MATS

Raw Type A gelatin (dry granules) powder (Adwic, El-Nasr Chemical Co., Cairo, Egypt) was used to prepare gelatin solution by dissolving 4 g gelatin in 20 mL of distilled water at 50 °C under stirring. The gelatin solution was heated up to 90 °C then the solution was cooled and dried at 40 °C for 48 h to form solid powders. For preparing gelatin solution for an electrospinning process, 5 mL of glacial acetic acid was added to 1 g of each raw gelatin and thermally treated gelatin powder then all were heated up to 50 °C. The fabrication of electrospun gelatin mats involves an electrospinning setup which consists of a voltage power supply (Glassman High Voltage, Model MK40N1.8) set at 15 kV to provide a strong electrostatic field, a syringe pump (KD Scientific 100 series) to push the gelatin solution through syringe at a flow rate of 0.08 $mL \cdot min^{-1}$ and a drum collector connected to the negative electrode of power supply to collect the fibers. The distance between the drum collector and the stainless steel needle was 15 cm. For cross-linking the electrospun gelatin fibrous mats, the electrospun mats were hung in a sealed glass container containing glutaraldehyde vapors for 3 h. To remove the residual glutaraldehyde, the crosslinked electrospun mats were kept in the oven at 110 °C for 24 h.

### CHARACTERIZATION OF ELECTROSPUN FIBROUS MATS

The surface topography of the fabricated electrospun gelatin mats was investigated using a Scanning Electron Microscope (SEM: JEOL JSM-6510 LV). The functional groups of the electrospun gelatin mats were characterized using

Fourier transformed infrared (FTIR: Bruker Tensor 27). X-ray diffraction analysis (XRD: GNR-APD 20000 pro, H423-vertical diffractometer) was applied to analyze the amorphous and crystalline features of the samples. Differential thermal analysis (DTA: Shimadzu) was carried out to characterize the phase transformations and structural changes of the samples under nitrogen gas. The samples were uniformly heated at a constant heating rate of 1 ºC $min^{-1}$ from room temperature till 300 ºC.

### *IN VITRO* SWELLING-DEGRADATION STUDIES

Both swelling and degradation tests were carried out in parallel where the dried electrospun gelatin mats were cut into square shapes and the weight of the mat ($W_{d1}$) was measured. The mats were immersed in phosphate buffer solution at pH of 7.4 then incubated at 37 °C. For swelling tests, the swollen mats were taken out of the solution at the appropriate time intervals and excess water was removed from their surfaces then weighed ($W_w$). The swelling ratio was calculated according to Eq. (1):

$$swelling\ ratio(\%) = \frac{W_w - W_{d1}}{W_{d1}} \times 100 . \quad (1)$$

For degradation tests, the swollen mats were dried in the oven at 37 °C for 24 h then weighed ($W_{d2}$). The degradation ratio was calculated according to Eq. (2):

$$degradation\ ratio(\%) = \frac{W_{d1} - W_{d2}}{W_{d1}} \times 100 . \quad (2)$$

## RESULTS AND DISCUSSION

### SCANNING ELECTRON MICROSCOPY

This study adjusted and fixed all electrospinning conditions and parameters which can influence fibers sizes of gelatin such as its molecular weight, concentration, and viscosity in addition to other process parameters such as flow rate, distance, and applied voltage. Electrospun gelatin fibrous mats were fabricated by thermal treatment of gelatin solution before the electrospinning process to improve and enhance the quality of electrospun gelatin fibers. The morphological features of electrospun gelatin mats were investigated through SEM analysis. Figure 1 showed SEM images of the surfaces of the electrospun raw gelatin (a) and thermally treated gelatin (b). The fabricated fibers of the two samples have fibrous structures with a regular morphology that are free of bead

defects. On the other hand, there is a dramatic difference between the mean fiber diameters of electrospun thermally treated gelatin (Fig. 1b) and electrospun raw gelatin fibers (Fig. 1a). The mean fiber diameters of electrospun thermally treated gelatin (Fig. 1b) are of about 600 ± 50 nm while those of the electrospun raw gelatin fibers are of 5000 ± 2000 nm (Fig. 1a). The morphological characterization of the electrospun thermally treated gelatin mats gave the best results making it more suitable for the production of electrospun fibers for medical applications. Therefore, for enhancing the quality of the electrospun gelatin fibers, the gelatin solution was heated up to 90 °C. In order to investigate the effect of thermal treatment of raw gelatin solution on the modification of electrospun gelatin structures, XRD, FTIR and DTA analyses were carried out on the raw and thermally treated gelatin fibrous mats.

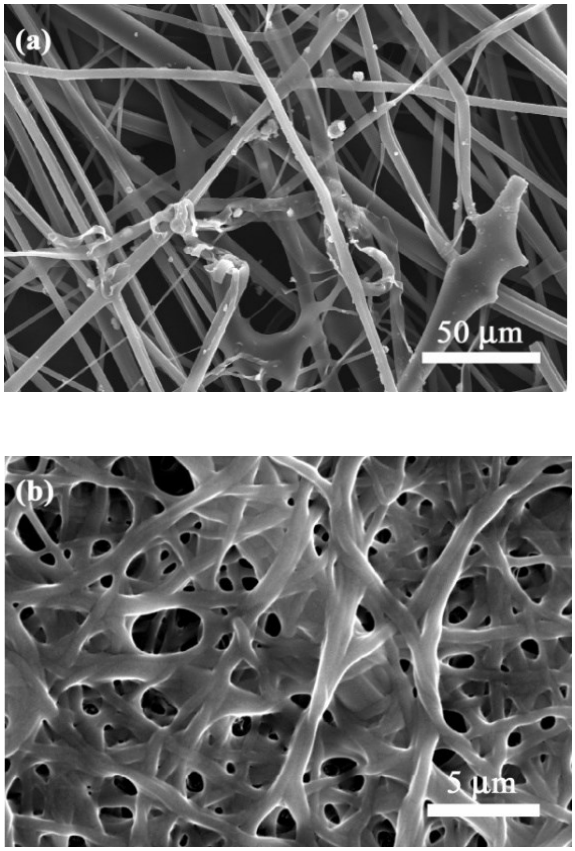


Fig. 1. SEM images of the surfaces of the electrospun mats showing
(a) raw gelatin and
(b) thermally treated gelatin.

## FOURIER TRANSFORM INFRARED SPECTROSCOPY

The FTIR spectra of electrospun raw gelatin and thermally treated gelatin fibrous mats are shown in Fig. 2. Both electrospun raw gelatin (Fig. 2a) and electrospun thermally treated gelatin (Fig. 2b) revealed identical patterns indicating that thermal treatment of gelatin solution did not affect the chemical structure of gelatin. The spectra confirmed the non-occurring of significant changes among the functional groups due to the thermal treatment process. The FTIR spectra revealed the main characteristic vibrational bands belonging to gelatin, in particular, N–H stretching for amide-A (3400 $cm^{-1}$), C–H stretching for amide-B (2945 $cm^{-1}$), C=O stretching for amide-I (1654 $cm^{-1}$), N–H bending for amide-II (1490 $cm^{-1}$), and C–N stretching and N–H bending for amide-III groups (1237 $cm^{-1}$).

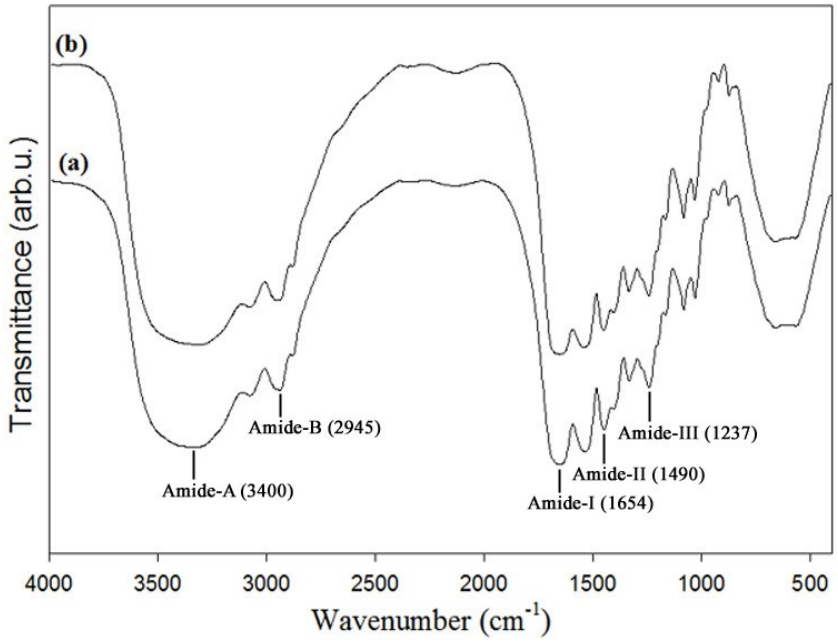


Fig. 2. FTIR spectra of the electrospun mats: (a) raw gelatin and (b) thermally treated gelatin.

## X-RAY DIFFRACTION SPECTROSCOPY

Figure 3 shows the X-ray diffraction analysis of crystalline structures of the electrospun raw gelatin and thermally treated gelatin fibrous mats. Both XRD patterns of the two samples showed an amorphous structure having a large broadening diffraction peak where 2 theta equals 22.5° that can be attributed to the

formation of small crystalline structures owing to the secondary structure of gelatin. On the other hand, the XRD pattern of electrospun thermally treated gelatin (Fig. 3b) has a diffraction peak larger than those of electrospun raw gelatin (Fig. 3a). This change is attributed to physical confirmation of the secondary structure of protein during thermal treatment.

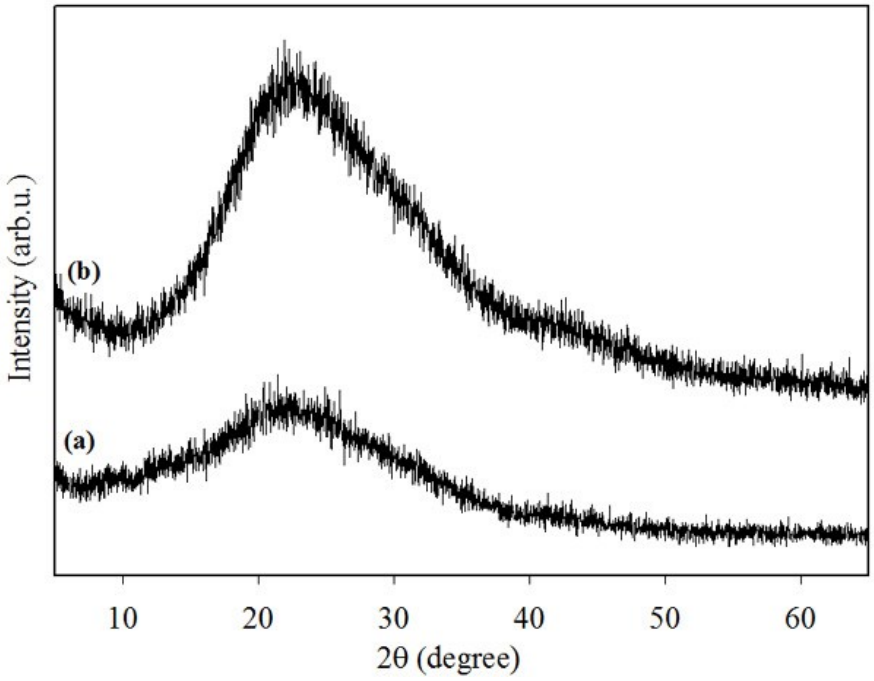


Fig. 3. XRD patterns of the electrospun mats: (a) raw gelatin and (b) thermally treated gelatin.

## THERMAL PROPERTIES OF ELECTROSPUN SAMPLES

Figure 4 showed the DTA of the electrospun samples that investigate the denaturation of electrospun thermally treated gelatin mat comparing with raw gelatin. Both electrospun raw gelatin (Fig. 4a) and electrospun thermally treated gelatin (Fig. 4b) have identical DTA data characterized by the presence of weak endothermic peaks and the absence of the exothermic peaks. The results showed endothermic peaks at 52 °C attributed to the evaporation of adsorbed water and 236 °C which correspond to the denaturation of gelatin. Therefore, the results of XRD, FTIR, and DTA analyses revealed that thermal treatment of gelatin solution has no effect on the chemical structure of gelatin.

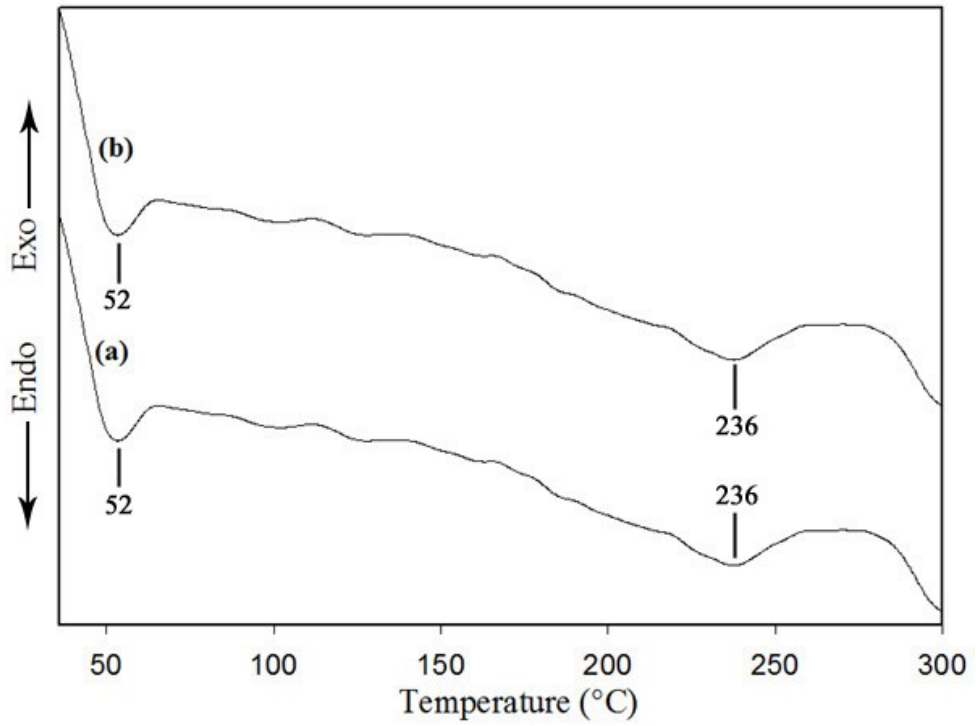


Fig. 4. DTA curves of the surfaces of the electrospun mats:
(a) raw gelatin and (b) thermally treated gelatin.

## SWELLING AND DEGRADATION STUDIES

Table 1 showed the swelling and degradation results for electrospun raw gelatin and thermally treated gelatin fibrous mats which were taken up to 30 h. The results revealed higher swelling ratios and lower degradation ratios for electrospun thermally treated gelatin as compared with those of electrospun raw gelatin. The swelling ratios for thermally treated gelatin samples increased from 920% (in the first 6 h) up to 1450% after 30 h while the corresponding degradation ratios increased from 10 to 52%. On the other hand, the swelling ratios for raw gelatin samples increased from 760% (in the first 6 h) up to 950% after 30 h while the corresponding degradation ratios increased from 47 to 94%. These results indicated that thermal treatment of gelatin solution before the electrospinning process can enhance the quality of electrospun gelatin in terms of the swelling and degradation ratios.

*Table 1*

The swelling and degradation ratios of the electrospun raw gelatin and thermally treated gelatin mats

| Time (h) | Swelling ratio (%) | | Degradation ratio (%) | |
|---|---|---|---|---|
| | Raw gelatin | Thermally treated gelatin | Raw gelatin | Thermally treated gelatin |
| 6 | 760 | 920 | 47 | 10 |
| 24 | 1090 | 1480 | 55 | 31 |
| 30 | 950 | 1450 | 94 | 52 |

## CONCLUSION

The present study aimed to assess the impact of thermal treatment of commercial gelatin solution before electrospinning on improving the quality of electrospun gelatin fibrous mats. The results exhibited a significant decrease in the fiber diameters of electrospun treated gelatin mats to 600 nm while their swelling and degradation ratios underwent obvious improvement. These results confirmed that thermal treatment of raw gelatin solution before electrospinning process enhanced the quality of electrospun gelatin fibrous mats, especially in terms of fibers sizes, degradation time, and swelling degree that could provide a basis for improving electrospun gelatin fibers for medical applications.

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