

Research Article

Electrospun Chitosan Nanofibers: Influence of Temperature and Humidity on the Structure and Chemical Properties

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Article Info	Abstract
Article History	Chitosan is one of the most difficult polymers to use in electrospinning, and the influence of environmental parameters on the electrospinning process has not been thoroughly investigated, making it particularly difficult to obtain uniform fibres with high reproducibility. This study examines the influence of relative humidity (30%–60% RH) and temperature (20–40 °C) on electrospun chitosan fibres, using trifluoroacetic acid as the solvent. Field emission scanning electron microscopy (SEM), attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy, and differential scanning calorimetry (DSC) were employed to evaluate the morphological, chemical, and thermal characteristics of the resulting fibrous structures, respectively. Although ATR-FTIR analysis indicated that the chemical composition remained consistent, FESEM revealed that variations in the temperature and relative humidity during electrospinning led to changes in the fibre diameter, pore size, and porosity. Furthermore, DSC demonstrated that variations in temperature and humidity during chitosan fibre production influenced the melting enthalpy, glass transition temperature, crystallinity temperature, and melting temperature. Ultimately, the optimal temperature (30 °C) and humidity (50% RH) conditions were determined for the given electrospinning parameters.
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1. Introduction

Electrospinning is a versatile and simple process for creating fibres composed of synthetic or natural polymers [1]. Fibrous films are formed by the deposition of these fibres onto a substrate during the electrospinning process, exhibiting structures that mimic the natural extracellular matrix, but with a high surface area-to-volume ratio, owing to the micro/nanoscale fibre diameters, and a high density of microscale pores [2]. Consequently, electrospun fibrous structures have potential in biomedical applications, including tissue engineering [3], wound dressing, and drug delivery [4]; environmental engineering, including filtration, composites, and protective materials [5]; and in protective clothes and sensors [6]. In the

electrospinning process, the polymer is discharged through a highly electrified needle. When the external electrical field force exceeds the electrostatic repulsion and Coulombic forces, the polymer liquid ejects from the tip of the needle. Between the needle and the collector, the polymer jet undergoes a rapid whipping motion, which enables the formation of long, thin fibres [7].

Environmental factors, specifically temperature and humidity, significantly affect the solvent evaporation rate, solution viscosity, fibre morphology, process reproducibility, and jet dynamics [8]. Despite their impact on the quality and characteristics of the resulting fibres and fibrous film structures (i.e., fibre diameter, pore size, and the continuity and reproducibility of the fibres), environmental factors are the least explored and often neglected. Temperature directly influences the rate at which the solvent evaporates from the electrospinning jet, with higher temperatures generally leading to faster evaporation. Humidity can also affect the evaporation rate to varying degrees, depending on the solvent. Higher temperatures typically decrease the viscosity of the polymer solution, influencing the jet's behaviour and fibre morphology. Humidity can significantly alter the pore size and porosity or induce fibre breakage. Moreover, variations in temperature and humidity can make it difficult to reproduce electrospinning results; these parameters must be controlled to improve the consistency and quality of the produced fibres [9]. Therefore, monitoring and reporting these conditions is crucial for achieving consistent and reproducible electrospinning results.

Among the natural and synthetic materials that can be widely used in medical applications through electrospinning, chitosan is a natural polymer that exhibits better biocompatibility than synthetic polymers, as well as biodegradability, low immunogenicity, and hemostatic properties. Furthermore, its amine functional group is strongly reactive with anions, imbuing chitosan with natural antibacterial and antifungal properties [10]. Additionally, the chitosan-free amino group enables the dissolution of chitosan in an acidic aqueous solution to form gels, films, and fibres. However, the electrospinnability of chitosan is limited for several reasons, such as the hydrogen bonding, which disrupts the formation of the polymeric jet by inhibiting the polymeric chains from moving freely. Meanwhile, the repulsive force between the polycationic charges of the chitosan also prohibits fibre continuity, resulting in beading, rather than the formation of smooth cylindrical fibres. The rigid chemical structure and high viscosity also hinder the electrospinnability of chitosan [11]. Chitosan was selected as the subject of the present study because of the challenges associated with fibre continuity and reproducibility, making it even more critical to understand the potential influences of the environmental conditions. Building upon existing research, this study investigates the influence of environmental parameters, specifically relative humidity (RH) and temperature, during electrospinning on the morphological, chemical, and thermal characteristics of fibrous chitosan structures.

2. Literature Review

Several studies have examined the roles of temperature and humidity in electrospinning processes [12,

13]. Desai et al. examined the effect of spinning temperature on chitosan/poly(acrylamide) fibres using aqueous acetic acid as a solvent, showing that heating the solution reduces the viscosity of the electrospinning solution and increases the polymer concentration in the resulting fibres [14]. Furthermore, De Vrieze et al. found that temperature affects both the solvent evaporation rate and the viscosity of the polymer solution, influencing the average diameter of electrospun fibres [8]. They also observed that the effect of humidity on fibre diameter varied depending on the material, with cellulose acetate and poly(vinylpyrrolidone) exhibiting opposite trends. These studies highlight the importance of carefully controlling environmental parameters during electrospinning to achieve the desired fibre properties.

Though electrospun chitosan has previously been investigated, only Van-Pham et al. studied the effects of temperature on the resulting morphological characteristics, namely the fibre diameter [15].

However, changing the temperature and humidity during electrospinning may affect the chemical and thermal characteristics, in addition to morphology. The novelty of the present study is the systematic investigation of the combined effects of temperature (20–40 °C) and humidity (30%–60% RH) on the structural, chemical, and thermal characteristics of electrospun chitosan, addressing this gap in the existing literature.

3. Methodology

3.1. Materials

High-molecular-weight chitosan ($MW = 310,000\text{--}375,000 \text{ g}\cdot\text{mol}^{-1}$) and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification.

3.2. Electrospinning Process

Chitosan (8 wt.%/vol.%) was dissolved in TFA to achieve a homogeneous mixture. The chitosan solution was stirred using a magnetic stirrer and a heating temperature of 50–55 °C. Stirring started at 200 rpm for 10 min and continued at 400 rpm for almost 25 min. The concentration of the chitosan solution and the preparation method were selected following previous work [16].

Immediately after stirring, the prepared chitosan solution was placed in a 10 ml syringe, and a commercial electrospinning apparatus (Inovenso NE-200, Istanbul, Turkey) was used to produce the fibrous structure. The distance between the needle and the collector was 7 cm, and the infusion pump feeding rate was 22 $\mu\text{l}/\text{min}$, following a previous study [16]. Notably, variable voltages (between 15 and 25 kV) were applied to obtain the clearest Taylor cone and optimise the electrospinning process, as determined by the naked eye. Accordingly, the applied voltage was set to 16.5 kV. After setting the process parameters, the fibrous chitosan structure was collected on a flat collector covered with aluminium foil.

The environmental parameters, namely temperature and humidity, were controlled by setting the temperature using the controller on the electrospinning apparatus and using a small humidifier to change

the humidity inside the electrospinning chamber. Fibrous chitosan structures were fabricated under 20 different conditions, using temperatures of 20–40 °C, in 5 °C steps, and humidities of 30%–60% RH, in 10% steps.

3.3. Characterisation Methods

3.3.1. Morphological Characterisation

To characterise the morphology, scanning electron microscopy (SEM) was employed using a field emission microscope (QUANTA, USA) with an accelerating voltage of 20 kV and a resolution of 1.2 nm. The average fibre diameter, pore size, and porosity of the beadless and continuous fibre films were calculated from SEM images using ImageJ software (version 1.52a). At least thirty measurements were conducted for each sample to evaluate the fibre diameter, pore size, and porosity. Three different samples were measured for each combination of environmental conditions.

3.3.2. Chemical Characterisation

Attenuated total reflection Fourier-transform infrared (ATR-FTIR) experiments were performed using a Bruker Vertex 70 instrument (Germany). All spectra were obtained within the wavenumber range of 400–4000 cm⁻¹ by averaging ten scans at 4 cm⁻¹ resolution.

3.3.3. Thermal Characterisation

To investigate the thermal properties, differential scanning calorimetry (DSC) was conducted using a TA Instruments DSC 250. The DSC traces were performed in the range of 20–270 °C under a nitrogen environment, with a heating rate of 10 °C/min.

3.4. Statistical Analysis

All data were reported as the mean plus or minus the standard deviation and analysed using the Student's t-test with OriginPro 2018 (OriginLab Corporation, Northampton, USA).

4. Findings and Discussion

4.1. Morphological Characterisation

From the combinations of five temperatures and four humidities, 20 different fibrous chitosan structures were fabricated, as shown in Figure 1. Most of the SEM images show bead-connected and bead-on-string fibres, but continuous and beadless fibrous structures are observed for four temperatures at 30% RH (i.e., 20, 30, 35, and 40 °C) and two temperatures at 50% RH (i.e., 30 and 35 °C).

Chitosan, a cationic polysaccharide, is one of the natural polymers used for electrospinning, although it can be challenging to obtain high-quality films for multiple reasons [17]. First, the high electric field during the electrospinning process creates a repulsive force with the ionic groups within the chitosan

backbone, leading to weak fibre entanglement, which enhances the formation of beads [18]. Second, the highly crystalline nature of the hydrogen bond formation between chitosan and the solvents, especially organic solvents, complicates the spinning ability [19]. However, TFA interacts with the amino groups of the chitosan and mitigates the interaction between chitosan molecules, which enhances spinnability. Finally, differences in the environmental conditions inside and outside the chamber may still affect the spinnability of the chitosan, even when the conditions are controlled within the chamber.

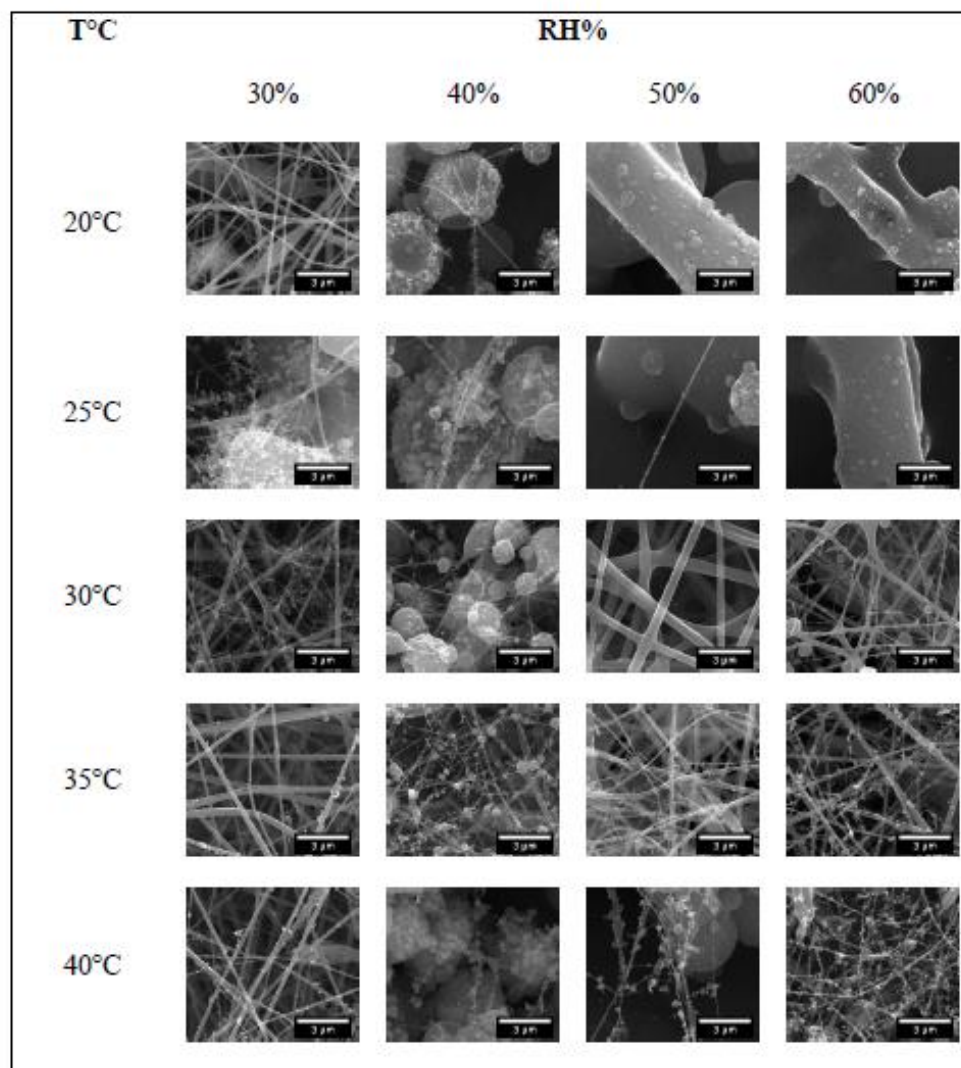


Figure 1. SEM images of the fibrous chitosan structures obtained under various temperature and humidity combinations during electrospinning.

Table 1 shows the average fibre diameter, pore size, and porosity of the fibrous chitosan structures electrospun under 30% RH and different temperatures. Accordingly, the average fibre diameter decreased as the temperature increased. The main reason is that the chamber temperature increases the solvent evaporation rate, thus providing finer and more uniform fibres. Furthermore, at higher chamber temperatures, the viscosity of the solvent-polymer is reduced, allowing the Coulombic forces to increase the spinning performance of the polymer. Similar results were observed by Hardick et al., who electrospun

cellulose acetate with temperatures ranging from 17.5 to 32.5 °C and humidities ranging from 20% to 70% RH and demonstrated a decrease in fibre diameter of 1.9% per 1 °C [20].

Table 1. Average fibre diameter, pore size, and porosity of the fibrous chitosan structures electrospun under 30% RH and temperatures of 20, 30, 35, and 40 °C.

T, RH	Average fibre diameter	Pore size	Porosity
20 °C, 30%	363 ± 2.65 nm	0.039 ± 0.004 µm	49%
30 °C, 30%	347 ± 2.14 nm	0.043 ± 0.006 µm	50%
35 °C, 30%	304 ± 2.48 nm	0.051 ± 0.009 µm	55%
40 °C, 30%	260 ± 2.20 nm	0.063 ± 0.007 µm	60%

Herein, uniform chitosan fibres were obtained at the electrospinning temperature of 35 °C. Accordingly, the electrospinning temperature is a crucial parameter in the electrospinning process, and it should be optimised to obtain a uniform fibrous structure.

Moreover, the pore size and porosity increased as the temperature increased. This is likely due to a decrease in the polymer's viscosity and surface tension [21].

Table 2 shows the average fibre diameter, pore size, and porosity of the fibrous chitosan structures fabricated under 30 or 35 °C and 30% or 50% RH. Note that some samples were excluded from characterisation because of the absence of fibre formation during electrospinning. Furthermore, some of the samples exhibited beads and non-uniform fibre morphology.

According to the results in Table 2, the fibre diameter increases from 347 ± 2.14 to 442 ± 2.72 nm as the humidity of the chamber increases from 30% to 50% RH, at 30 and 35 °C. For lower and higher humidities, we observed the increased presence of beaded fibres (30 °C, 40% RH) and fibres with adsorbed water droplets (35 °C, 50% and 60% RH), as seen in Figure 1. This indicates a complex interplay of factors, including charge dissipation, delayed solidification, and enhanced solvent retention.

The first effect of humidity that acts on the final fibre diameter is the discharge of the electrospinning jet at high humidity due to the increased amount of water molecules in the air, thereby decreasing the number of charges on the electrospun jet. Consequently, the whipping of the jet becomes less vigorous and less elongated, potentially leading to thicker fibres.

Second, humidity affects the solidification rate. Given that chitosan is a hydrosoluble polymer, water absorption in the jet leads to delayed solidification caused by slower evaporation or plasticisation of the polymer. In this case, the jet thins until a capillary instability develops, leading to the formation of beaded fibres. The presence of adsorbed water droplets on the fibres further indicates the occurrence of delayed solidification, attributed to the hygroscopic nature of chitosan and TFA.

While increased humidity generally leads to slower solvent evaporation, the specific characteristics of our chitosan/TFA system, combined with the hydrophilic nature of chitosan, likely promote enhanced TFA retention, reduced jet elongation due to charge dissipation, and delayed solidification. All of these

effects can contribute to the formation of fibres with larger diameters, beaded morphologies, and adsorbed water droplets [9].

Table 2. Average fibre diameter, pore size, and porosity of the fibrous chitosan structures electrospun under 30 or 35 °C with 30% or 50% RH.

T, RH	Average fibre diameter	Pore size	Porosity
30 °C, 30%	347 ± 2.14 nm	0.043 ± 0.006 µm	50%
30 °C, 50%	442 ± 2.72 nm	0.045 ± 0.003 µm	52%
35 °C, 30%	304 ± 2.48 nm	0.051 ± 0.009 µm	55%
35 °C, 50%	311 ± 2.97 nm	0.057 ± 0.008 µm	57%

Notably, needle clogging was observed during electrospinning at high humidity (above 50% RH). Moreover, fusing of the fibres before reaching the collector at the whipping step was observed, although previous reports have shown that increasing the humidity enhances the formation of finer and beadless fibres [20, 22]. Increasing the humidity affects many factors, including polymer hydrophilicity, solvent type, and volatility. Therefore, the effect of the humidity change during the electrospinning process on fibre formation cannot be predicted as well as it can for the temperature change [12]. According to Table 2, increasing the humidity slightly changes the pore size and porosity. The reason for that might be the number of factors affected by humidity variation [22].

4.2. Chemical Characterisation

ATR-FTIR spectroscopy was employed to investigate the chemical characteristics of the fibrous chitosan structures fabricated under two different environmental conditions: 20 °C and 30% RH, and 30 °C and 50% RH. These two samples were selected to analyse alterations in the chemical structure of chitosan fibres resulting from variations in temperature and humidity. Other fabricated samples were excluded because of challenges encountered during electrospinning, non-uniform fibre morphology, or the presence of electrospraying or beads, as depicted in Figure 1.

Figure 2 shows the infrared spectra of the fibrous structures. The chemical composition of chitosan remained consistent across the range of temperatures and humidity levels studied. Furthermore, the intensities of the peaks associated with C-H bending vibrations at 1375 and 1420 cm⁻¹, N-H bending at 1590 cm⁻¹, and C-N stretching at 1320 cm⁻¹ remained relatively constant in both samples. These peaks are indicative of the fundamental building blocks of the chitosan polymer, with C-H bending representing the aliphatic nature of the chitosan backbone, N-H bending corresponding to the amine groups, and C-N stretching reflecting the glycosidic linkages within the polymer chain. The stability of these peaks suggests that the overall amount and chemical environment of these functional groups were not significantly altered by the changes in environmental conditions during electrospinning.

However, closer inspection of the spectra revealed subtle variations in the intensities of the C-O

stretching peaks within the 1000–1150 cm^{-1} region. Specifically, fibres electrospun at a higher temperature and humidity exhibited a lower absorbance in these peaks than those electrospun at a lower temperature and humidity, as designated in the table in Figure 2. This observation warrants further consideration because C-O stretching vibrations are sensitive to factors beyond the simple presence of bonds; several factors may contribute to these observed changes in the FTIR peak intensities. A higher temperature and humidity may influence the conformation of the chitosan chains, enhancing the exposure of C-O, C-O-C, and C=O groups on the fibre surface. Increased humidity may lead to greater water absorption by the chitosan fibres, thus affecting the arrangement or orientation of the polymer chains and, consequently, the intensity of the C-O, C-O-C, and C=O peaks. It is also possible that the electrospinning conditions affect the crystallinity of the chitosan fibres, and a less crystalline structure may expose more of these functional groups.

In contrast, the absence of significant changes in the broad O-H stretching region (3200–3600 cm^{-1}) suggests that the overall hydrogen bonding environment remained relatively stable, implying that the C-O intensity variations are linked to more localised or subtle structural rearrangements.

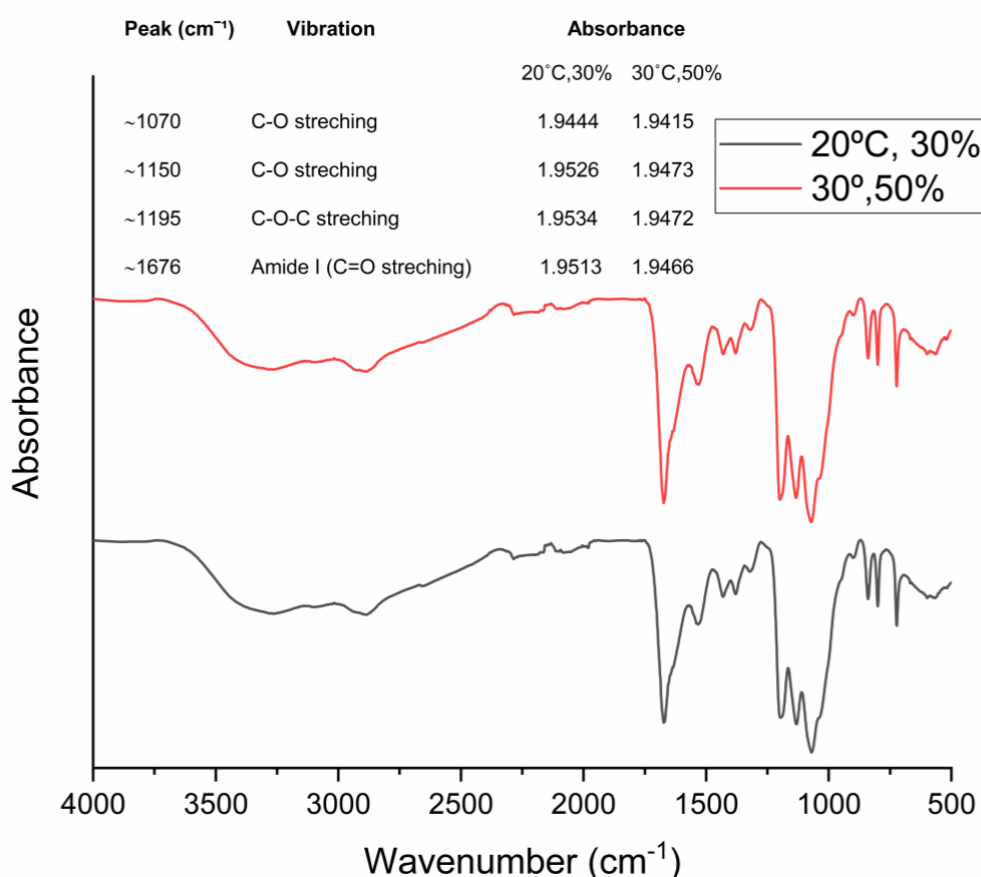


Figure 2. FTIR spectra of fibrous chitosan structures fabricated under two different environmental conditions: 20 °C and 30% RH for spectra (a), and 30 °C and 50% RH for spectra (b).

These findings align with previous reports on chitosan characterised by ATR-FTIR spectroscopy. The presence of all expected functional groups confirms that the fundamental chemical structure of the polymer remained intact despite the variations in the environmental conditions [23]. However, a subtle shift

in the Amide I band from 1650 to 1676 cm^{-1} was consistently observed in both sample sets. This shift may be attributed to the disruption of hydrogen bonding, potential interactions with residual TFA solvent [24] and subtle variations in the degree of acetylation [25]. These factors are known to influence the Amide I band position, suggesting that the observed shift reflects the sensitivity to environmental and processing conditions rather than a fundamental alteration of the polymer's chemical composition.

4.3. Thermal Characterisation

DSC elucidated the thermal properties of fibrous chitosan structures electrospun at various environmental factors, as shown in Figure 3, revealing the energy needed to heat any fabricated sample from 20 to 270°C. Furthermore, the glass transition temperature (T_g), crystallinity temperature (T_c), and melting temperature (T_m) were determined directly from the DSC thermogram, and the melting enthalpies were calculated by integrating the area under the curve. Two samples electrospun under different environmental conditions were selected: 20 °C and 30% RH (sample (a)), and 30 °C and 50% RH (sample (b)).

The T_g , T_c , and T_m were 166.65, 185.37, and 237.28 °C for sample (a) and 184.21, 211.89, and 240.9 °C for sample (b), respectively. Accordingly, the melting temperature, in addition to the crystalline and glass transition temperatures, increased with the rising temperature and humidity in the electrospinning process.

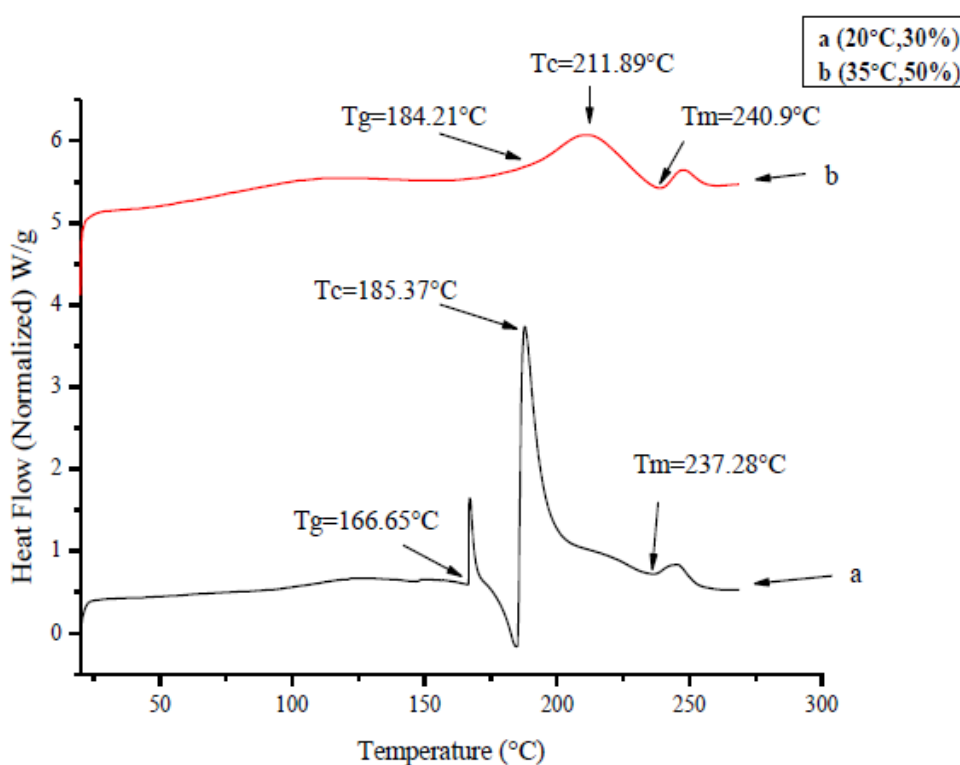


Figure 3. DSC thermograms of the fibrous chitosan structures: a) electrospun at 20 °C and 30% RH, and b) electrospun at 35 °C and 50% RH. T_g : glass transition temperature, T_c : crystallisation temperature, and T_m : melting temperature

The melting enthalpy was found to be 10.634 and 11.842 J/g for samples (a) and (b), respectively. Therefore, the melting enthalpy increased as the temperature and humidity increased in the electrospinning process. Melting enthalpy indicates the crystallinity; hence, higher enthalpy means higher crystallinity [26]. Huang et al. studied the influence of temperature on the structure and crystallinity of poly(vinylidene fluoride), showing that the crystallinity and thermal properties were enhanced by increasing the temperature during electrospinning, consistent with the present findings [27].

5. Conclusion

The effects of temperature and humidity on the morphological, chemical, and thermal characteristics of fibrous chitosan structure obtained during electrospinning were investigated herein. At a constant humidity of 30% RH, the fibre diameter decreased with the increase in temperature from 20 to 40 °C. Conversely, raising the humidity at a constant temperature caused the fibre diameter to increase. Overall, the most uniform fibrous structure with beadless fibres was obtained at 30 °C and 50% RH.

Furthermore, ATR-FTIR showed that the changes in the environmental conditions had no significant effect on the chemical structure of the electrospun chitosan fibres. However, the thermal properties, especially the melting enthalpy, were altered by the changes in temperature and humidity. Therefore, the environmental factors, particularly temperature and humidity, must be considered and controlled during the electrospinning process to obtain uniform and reproducible fibrous chitosan structures.

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