Additional Information

Effect of neutralization and cross-linking on chitosan electrospun membranes thermal degradation

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Abstract

hermal degradation of as-electrospun chitosan membranes and samples subsequently treated with ethanoland cross-linked with glutaraldehyde has been studied by thermogravimetry (TG) coupled with an infrared spectrometer. The influence of the electrospinning process and cross-linking in the electrospun chitosan thermal stability was evaluated. Up to three degradation steps were observed in the TG data, corresponding to water dehydration reaction at temperatures below 100°C, loss of side groups formed between the amine groups of chitosan and trifluoroacetic acid between 150 and 270°C and chitosan thermal degradation that starts around 250°C and goes up to400°C. The Kissinger model was employed to evaluate the activation energies of the electrospun membranes during isothermal experiments and revealed that thermal degradation activation energy increases for the samples processed by electrospinning and subsequent neutralization and cross-linking treatments with respect to the neat chitosan powder.

Keywords: Chitosan, electrospun membranes, thermal degradation kinetics, activation energy, cross-linking

1. Introduction

Chitosan, a natural polysaccharide composed of (1, 4) - linked β-$_D$– glucosamine is the N-deacetylated derivative of chitin, the second most abundant natural polymer in the world afterward cellulose [1-4]. Chitin is widely found in crustaceans and insects and can be converted to N-deacetylated product by homogeneous or heterogeneous alkaline N-deacetylation [2, 4, 5]. The degree of deacetylation (DD), defined as the ratio of N-deacetylated (amino groups) to N-acetyl groups, that defines chitin and chitosan is not fully well-defined [6]. However, deacetylated chitin with a DD higher than 50-95 % have been named as chitosan [5].

Structural modifications to impart desired properties and functions, lead to a biomaterial with potential technological and scientific applications. Chitosan have multidimensional properties, highly sophisticated functions and a wide range of applications in biomedical engineering, due to its biological properties, such as biodegradability, biocompatibility, cellular binding capability, acceleration of wound healing, hemostatic and anti-bacterial properties and low toxicity [4].

Chitosan can be used in a variety of physical shapes, including beads, films, sponges, tubes, powders and fibers since it is quite soluble under acidic aqueous solutions [1]. Electrospinning is a versatile production method to produce porous fibers membranes with diameters ranging from few micrometers to several nanometers [7, 8]. This process uses a high voltage source to inject charge of a certain polarity into a polymer solution or melt, which is then accelerated, in the form of a thin jet, and when the applied electric field is strong enough to overcome the surface tension of solution the jet is drawn into a fiber that is collected on a surface of a grounded target [8-11].

Chitosan was successfully electrospun into fibers from dissolution of the polymer in trifluoracetic acid (TFA) [12, 13] or a co-solvent system of TFA with dichloromethane (DCM) [14], and it was reported that the last solvent improve fiber spinability. Further, Ohkawa et al. [12] refers that the success of the chitosan dissolution in TFA was probably a result of the formation of salts between TFA and amino groups along the chitosan chain leading to a smaller interaction between chitosan molecules, which results in a more stable solution to perform electrospinning. However, the resulting chitosan membranes lose their fibrous structure when in contact with a neutral or weak basic aqueous media,

due to the dissolution of the trifluoracetate salts that are formed when chitosan is dissolved in TFA [15]. In order to remove those salts a neutralization process was applied in which the polymer mats were immersed in a sodium hydroxide (NaOH) or sodium carbonate (Na_2CO_3) aqueous solution [15], while other authors use absolute ethanol as neutralizing agent of chitosan [14, 16, 17].

Greiner and coworkers [13] reported that nanofibers of water-soluble polymers obtained by electrospinning decompose more rapidly in contact with water. In order to improve polymer stability to aqueous environments, a cross-linking process is required. This can be achieved by chemical methods that exploit chemical agents to modify chitosan groups. Chitosan cross-linking can be achieved by glutaraldehyde (GA), genipin or diisocyanates that leads to intermolecular bridges forming crosslinks between the polymer structure [6], reducing crystalline degree present in the electrospun chitosan fibers with a small decrease in the average fiber diameter [18, 19].

Thermogravimetric analysis (TGA) has been used to investigate the kinetics of the chitosan thermal degradation. It has been reported that the degradation of chitosan occurs in two separate steps, where the first step corresponds to dehydration and the second one to chitosan weight loss [20]. The first step is described as a result of the strong affinity of polysaccharides with water, as they can be easily hydrated [21]. Taboada et al. [22] refers that the water loss occurs in the temperature range between 25-140 ℃, where the chitosan presents a mass loss between 4 and 7 %, and a strong polymer weight loss occurs between 190-410 ℃ via the cleavage of glycosidic linkages. Hong et al. [23], found that the activation energy (E_a) of chitosan thermal degradation process change with the degree of conversion during the thermal degradation process and three weight loss steps were proposed: the first corresponds to the volatilization of low molecular materials with E_a between 0-140 kJ mol^{-1}, the second with a E_a between 140- 160 kJ mol^{-1} caused by thermal degradation of chitosan main chains, and in the last step wan increase of E_a with increasing degree of decomposition during thermal degradation of residual carbon was observed.

In a previous work, neutralized and cross-linked electrospun chitosan membranes were prepared. It was found that the degree of deacetylation does not suffer changes during the processing and a small decrease of the mean fiber diameter was observed [16, 19]. This work reports the influence of the processing technique, neutralization and cross-linking with GA on chitosan thermal degradation properties. Thermal degradation kinetics was

analyzed and discussed using the Kissinger's mathematical model in order to characterize the thermal behavior and degradation kinetics of chitosan electrospun membranes.

2. Materials and methods

Chitosan medical grade polymer was purchased from Novamatrix (Protasan UP B 80/20) with 80-89 % degree of deacetylation and apparent viscosity of 20-199 mPa.s [24]. Dichloromethane (DCM, 99 %) and trifluoracetic acid (TFA, 99 %, ReagentPlus) were purchased from Sigma-Aldrich. All materials were used as received from the provider. Electrospun membranes were processed according to the method explained elsewhere [16, 19]. Briefly, Protasan powder was dissolved in a TFA/DCM solution (70:30 % v/v) volume ratio for a 7 % (weight / total solvent volume) of polymer. The solution was prepared under a constant and vigorous magnetic stirring (JPSelecta, Agimatic-E) at room temperature until complete dissolution of chitosan. The polymer solution was placed in a commercial plastic syringe fitted with a steel needle with an inner diameter of 0.5 mm, a distance between the metallic tip and the grounded collector of 150 mm and an applied voltage of 25 kV with a PS/FC30P04 power source from Glassman was applied. A syringe pump fed the polymer solution into the tip at a rate of 1 ml.h^{-1}. As-spun chitosan fiber mats were neutralized in a vapor chamber (JPSelecta Vacuo-Temp), at low pressure with ethanol (99 %, Merck) for 72 h at 40 ºC. After this process the samples were desiccated at 80 ºC, for another 72 hours to remove the ethanol excess. Cross-linking of as-spun and neutralized membranes was performed in a vapor chamber (JPSelecta Vacuo-Temp), at low pressure. 10 mL of glutaraldehyde (GA, 50 % water, Panreac) was placed at the bottom of the chamber and vaporized at 25 ºC, for 24 h.

Fluor quantification present in the chitosan sample and the compositional changes that occur during neutralization and cross-linking were characterized by microanalysis in an Energy Dispersive X-ray (Oxford Instrument X-Max detector) attached to scanning electron microscope JSM - 6300 from JEOL. Samples were previously coated with carbon in vacuum and spectra were taken at 5 kV of acceleration voltage and silicon was employed as standard.

Simultaneous thermogravimetry coupled with Fourier transform infrared spectrometer (TGA-FTIR) was performed on a thermal analyzer (TGA, SDTQ600 TA instruments) and a FTIR (Spectrum one). For TGA analyses, the samples were heated to 40-800 ºC, at

different heating rates (between 5 and 40 ºC.min^{-1}) under a nitrogen flow rate of 25 mL.min^{-1}. The transfer line used to connect TGA and FTIR had an internal diameter of 1.5 mm, and the temperature was maintained at 523 K. The FTIR spectra were collected at 8 cm^{-1} resolution.

3. Results and discussion

3.1. Electrospun membrane morphology

Chitosan was electrospun into a flat grounded collector, and the obtained fibers shown a smooth, bead free, continuous and random oriented (figure 1) [16]. It was observed that samples treated with ethanol and cross-linked with GA retain their integrity with cylindrical fibers, similar to the as-spun fibers (figure 1b). Finally, it was observed that neutralization and cross-linking does not had significant influence on average fiber diameter and distribution (figure 1), but a fiber shrinkage around 10 % was observed after neutralization with ethanol [16].
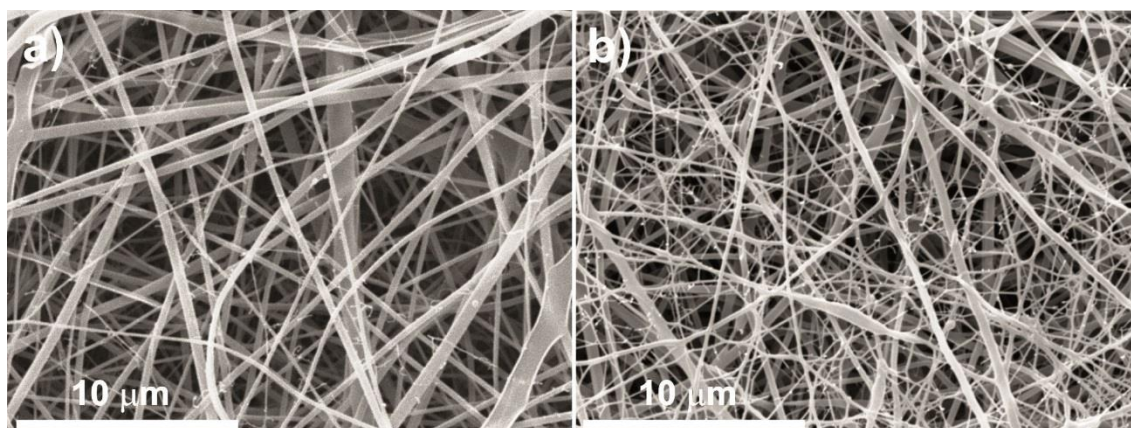

Figure 1 – Electrospun chitosan membrane prepared from a 7 % (w/v) polymer solution, travelling distance of 15 cm, needle inner diameter of 0.5 mm, flow rate of 1 mL.h-1 and a applied voltage of 25 kV: a) as-pun fibers and b) neutralized with ethanol followed by GA cross-linking.

### 3.2. Effect of the electrospinning process and chemical treatments on thermal degradation

In the present work, the influence of the processing technique and the subsequent neutralization and cross-linking on thermal degradation of chitosan was studied by thermogravimetric analysis (TGA) under dynamic conditions.

For pristine chitosan powder two main steps were observed (figure 2): the first step, that takes place below 100 ºC, corresponds to the dehydration process due to water evaporation that was absorbed in the sample, with a weight loss of approximately 8 %. The second thermal step occurred between 250-375 ºC with a weight loss around 45 % and is related to polymer decomposition and release of volatile products (figure 2) [25].
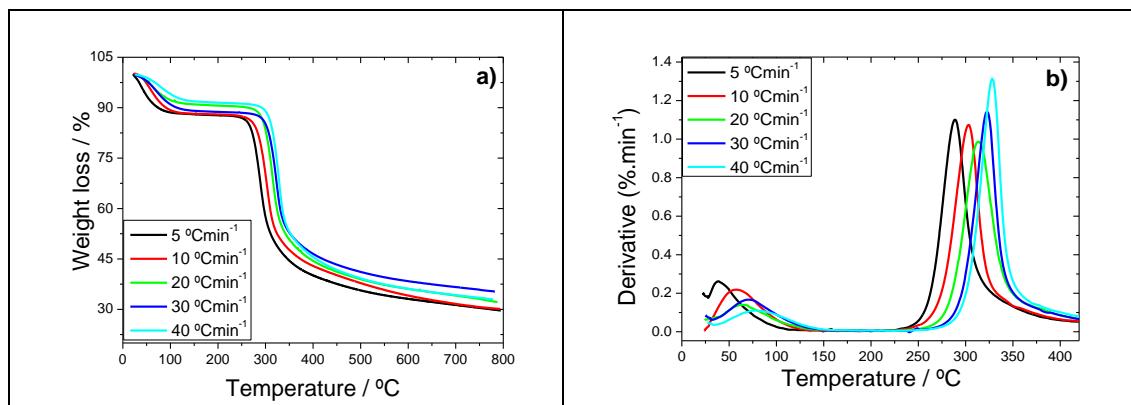
Figure 2 - TGA (a) and DTG (b) thermograms of pristine chitosan for different heating rates (5, 10, 20, 30 and 40 ^{0}C min^{-1}).

Figure 3 shows the effect of the electrospinning process, neutralization and cross-linking treatments on chitosan thermal stability. The electrospun membranes showed three major degradation steps. The first, at low temperatures, below 100 ºC, is associated to dehydration and is quite similar to the one observed for the as-received material (figure 2 and 3) between 150-270 ºC and 270-400 ºC, respectively (figure 3a). In the derivative (DTG) curves three peaks can be observed, corresponding to the thermal decomposition of chitosan fibers (figure 3b).
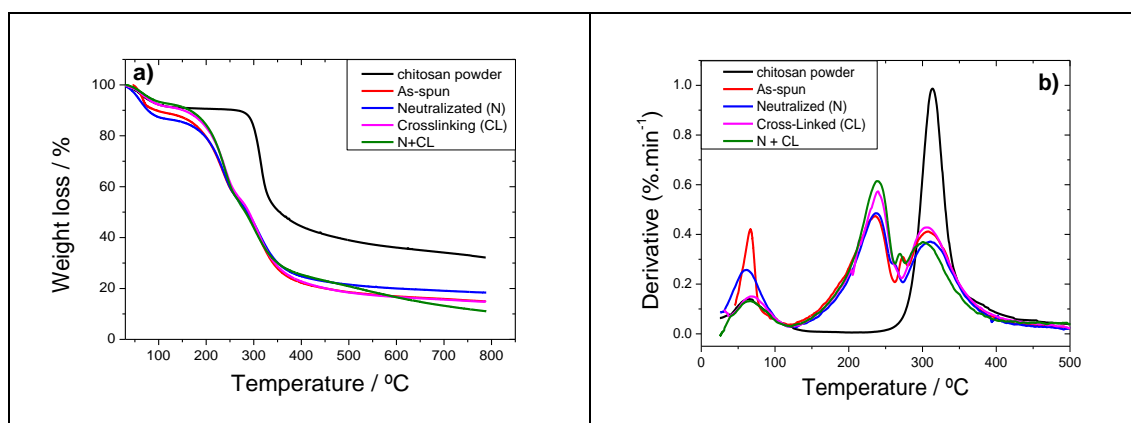

Figure 3 - TGA (a) and DTG (b) thermograms of the pristine chitosan and as-spun membranes.

The second degradation process shown by the derivative peak presents a maximum around 240 ºC which is absent in the pristine chitosan powder (figure 2), and can be ascribed to the loss during heating scan of side groups formed by the association of TFA and the amine groups of chitosan. Films of chitosan formed by solvent casting from a solution of pristine chitosan in TFA were prepared and submitted to TGA analysis showing similar thermograms (data not shown) to those of electrospun mats (figure 3), what indicates that the new degradation peak is not the result of a special orientation or organization of chitosan chains due to electrospinning process.

To prove the origin of this relaxation peak, films of chitosan were analyzed by TGA at different heating rates in the range between 200 and 300 ºC and then to elemental analysis to detect the presence of fluoride in the sample. The results shown in Figure 4 reveals that fluoride is still present in the original film (a similar pattern was shown by electrospun mats, results not shown), but when the sample is taken to temperatures in the range of the second stage degradation peak, the fluoride peak decreases (figure 4a and b). The salts produced by the interaction of TFA and amine groups of chitosan during the dissolution and electrospinning process persists in the as-spun mats, and favor polymer dissolution under aqueous environments. Sample cross-linking with GA renders the electrospun mats insoluble in water but TFA-amine salts persists in the fiber membranes. Further, chitosan films neutralized with ethanol showed the presence of fluoride in the sample, and for the samples treated with NaOH is absent of fluoride as it can be observed in figure 4d.

TGA is a sensible technique to detect the presence of these side groups in the samples. In order to ensure that the second degradation stage was due to the presence of trifluoracetate salts, chitosan samples were neutralized with NaOH. TGA of these samples shown a similar trend to the one obtained for the pristine chitosan powder, i.e., without any TFA residues (Figure 5), which is in accordance to the elemental analysis results obtained for the samples neutralized with NaOH (figure 4d).
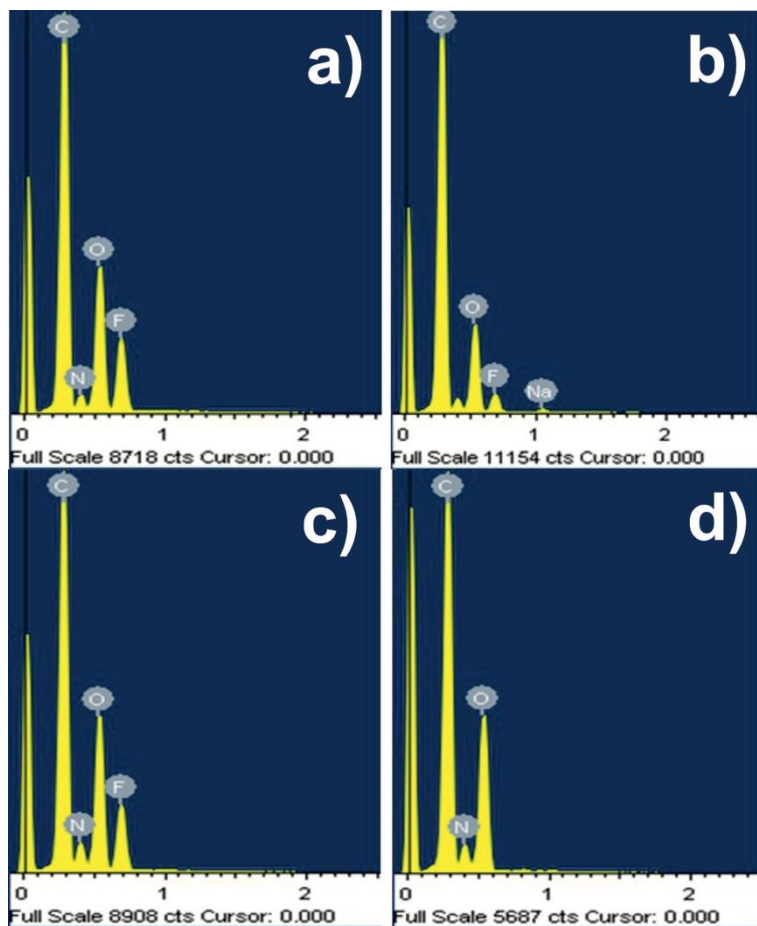
Figure 4 – Elemental analysis of: a) pristine chitosan dissolved in TFA/DCM before thermal annealing, and b) after annealing at 300 ° C; c) pristine chitosan dissolved in TFA/DCM and subsequent neutralization with ethanol and d) neutralized with NaOH.
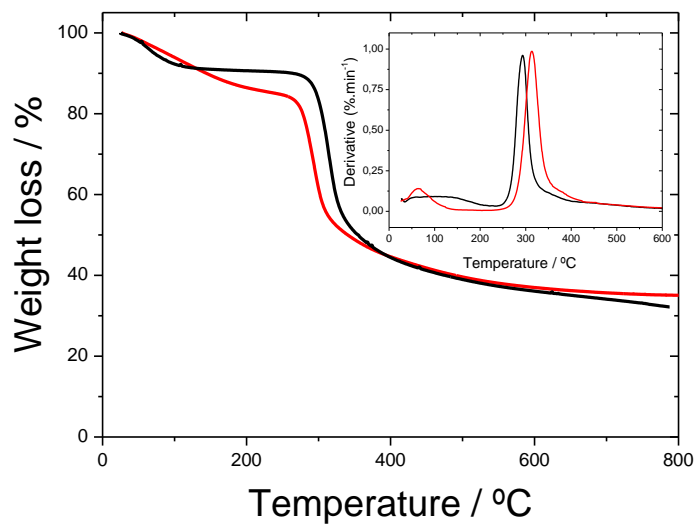
Figure 5 – Thermogravimetric data obtained for pristine chitosan and for the electrospun membrane neutralized with NaOH.

Comparing the onset temperatures of as-received chitosan and electrospun membranes, it is possible to observe that the electrospinning process decrease their thermal stability and this is related to the release of TFA compounds produced during the polymer dissolution and posterior electrospinning process. Further, the amount of absorbed water found for all the prepared samples is quite similar between them, revealing that processing techniques does not had influence in sample hydration (table 1). Moreover, sample neutralized with ethanol showed a higher amount of water absorbed which may be due to the strong capacity of ethanol to establish linkages by hydrogen bonds with water (table 1).

Table 1 - Parameters obtained from de TGA curves of pristine chitosan and chitosan membranes.

MEMBRANES	WATER ABSORPTION (%)	RESIDUE AT 400 ºC (%)	RESIDUE AT 600 ºC (%)
Pristine chitosan	8.6	44.5	36.0
As-spun	8.8	22.3	17.0
Neutralizated (N)	12.9	24.8	20.0
Cross-linking (CL)	8.1	22.8	17.0
Neutralization + Cross-linking (N+CL)	7.2	25.5	17.0

3.3. FTIR analysis of gas products

During chitosan thermal degradation, the released gases were analyzed by FTIR spectroscopy using the experimental conditions described above. It was observed that electrospun membranes release more gaseous products than as-received chitosan, leading to conclude that the chitosan dissolution in a TFA/DCM solution provide less thermal stability to the electrospun membranes, which is in accordance to the observed in figure 2 and 3. From the obtained results, three major absorption bands were perceived: at 3735 cm^{-1} represents the water dehydration that occurs in the first step (figure 2 and 3) [26]; the absorption bands at 1660 cm^{-1} and 1560 cm^{-1} are assigned to the C=O in the amide group (amide I band) and NH bending vibration in the amide group, respectively [27]. At 300 ºC (figure 6), the peaks at 1660 cm^{-1} and 1560 cm^{-1} became broader, which indicate changes that occurs to chitosan during thermal degradation due to the formation of unsaturated structures. The absorption band at 1380 cm^{-1} was assigned to the CH$_3$ of chitosan amide group [27]. Furthermore, Zeng et al., [25] propose a chitosan pyrolysis mechanism and formation of volatile compounds such as pyrazines, pyridines, pyrroles and furans that are generated from the polymer thermal degradation. Compounds like pyrazine are the most important products among the volatiles that were identified. A strong absorption band was observed at 1150 cm^{-1} for the electrospun samples heated until 300 ºC (figure 6), corresponding to the C – F stretching band due to the – NH$_3^+$CF$_3$COO$^-$ salts previously formed during polymer dissolution in a TFA/DCM mixture, and proves the existence of such residues, probably located in the side groups of the electrospun polymeric chains [28, 29]. The absorption band at 1150 cm^{-1} was also observed for the chitosan samples submitted to ethanol neutralization and cross-linking with GA (data not shown).
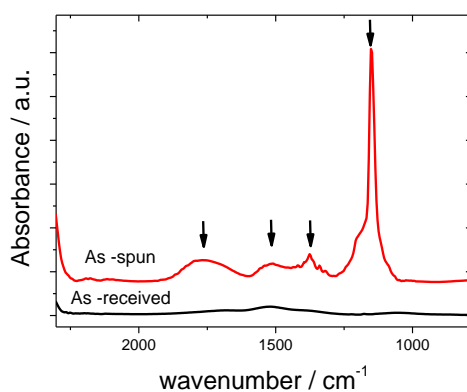
Figure 6 - FTIR spectra of as-received and as-spun chitosan membranes along degradation from TG analysis, obtained at 300 ºC.

3.4. Thermal degradation kinetics

Polymer thermal stability can be characterized by the kinetic parameters of the thermal degradation [30]. Kissinger developed a mathematical model which is used to determine the activation energy from plots of the logarithm of the heating rate *vs* reciprocal temperature at the maximum reaction rate, in constant heating rate experiments [31, 32]. In the Kissinger method the activation energy (E_a) can be obtained without a precise knowledge of the reaction mechanism, according to the following equation [31]:

$$\ln\left(\frac{\beta}{T_p^2}\right) = \frac{\ln(AE_a)}{T} + ln[n(1 - \alpha_p)^n] - \frac{E_a}{RT_p} \tag{1}$$

where T_p and α_p are the absolute temperature and the conversion at the maximum weight loss rate, respectively.

In the present work, the degradation kinetics was evaluated by the method proposed by Kissinger (equation 1), in which the E_a for the water evaporation reaction (first step), degradation of TFA salts present in the membrane (second step) and chitosan thermal degradation (third step) can be calculated by plotting $\ln \beta/T_p^2$ *vs* $1000/T_p$. Figure 7 shows that the fitting straight lines were nearly parallel, which implies that this method was suitable to apply to the chitosan polymer membranes. According to the Kissinger's mathematical model for the degradation kinetics, the slope of straight lines is proportional to the E_a, and the obtained results for the different thermal degradation steps are reported in table 2. All linear fittings were obtained with R > 0.97.
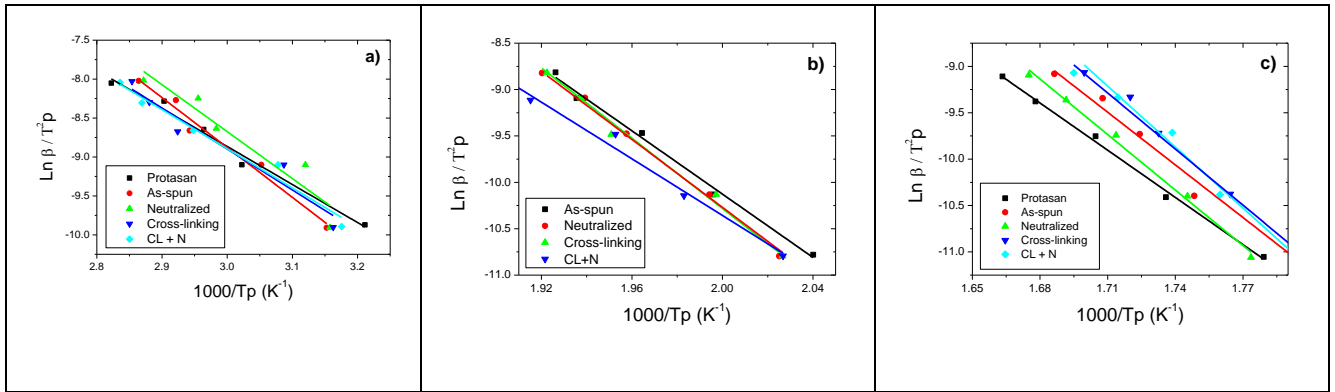
Figure 7 - Kissinger plot of $\ln \beta / T_p^2$ versus $1000/T_p$ for the as-received and chitosan membranes: a) water dehydration, b) second step degradation process and c) third step degradation process.

The values obtained for water evaporation E_a (first step) are higher after the electrospinning process and decrease after cross-linking with GA in vapor phase, which probably is related to the water affinity of TFA salts formed during polymer dissolution in a TFA/DCM solvent mixture and to the strong capacity of ethanol establish linkages by hydrogen bonds with water. Moreover, the difference between the activation energy for pristine chitosan and electrospun fibers with and without neutralization is around 10-13 kJ.mol^{-1}, which is comparable to the energy of the hydrogen bond [33], which confirms the interaction between the TFA salts and water and ethanol. Further, for the samples submitted to neutralization and cross-linking the activation energy for the dehydration process decreases to a value similar to the one found for pristine chitosan. For the second degradation process, related to the TFA salts produce by the interaction between the TFA/DCM solvents and the polymer, the E_a is higher for the sample submitted to ethanol neutralization process when compare to the as-spun mats and decreases for the cross-linked samples. Moreover, activation energy for the chitosan thermal degradation (third step) is around 141.9 kJmol^{-1} and increases with the subsequent neutralization and cross-linking (table 2).

It has reported that chitosan E_a follows in the range between 140- 160 kJ .mol^{-1} caused by thermal degradation of chitosan main chains [22, 23]. Thermal degradation process of chitosan may be a random degradation of polymer chains and it was reported that the degradation starts from the scission of the C-O-C bond at a weak point. Electrospinning and cross-linking have a strong influence on the amount of the amorphous phase present in the chitosan fiber mats. The reaction of the chitosan with GA vapor induces a reorganization of the polymeric chains, and intra- and inter-molecular hydrogen bonds of

chitosan network would break apart. Cross-linking induces discontinuities along the polymer chains which hinder crystal formation leading to a more amorphous polymer mats. Also, leads to formation of a new C=N bonds of imine group, decreasing the primary amine bond when the chitosan fibers which as higher bond strength when compared to C–O, C–H, C–C, and O–H present in polymer chain, which implies that a greater energy is needed to supply in order to start its thermal degradation process. This can explain the increase of the E_a observed for the cross-linked samples (Table2).

Table 2 - Values of E_a obtained by Kissinger mathematical model.

SAMPLES	E_a (kJ.mol^{-1})		
	First step	**Second step**	**Third step**
Pristine chitosan	41	-	142
As-spun	53	142	157
Neutralizated (N)	50	157	165
Cross-linking (CL)	43	152	177
Neutralization + Cross-linking (N+CL)	42	141	167

4. Conclusions

The influence of the electrospinning processing and the subsequent neutralization and cross-linking on the thermal degradation of chitosan was studied by TG. It was observed that chitosan shows up to three degradation steps: the first step was attributed to a dehydration process associated to evaporation of water absorbed below 100°C, and the second to chitosan degradation and release of volatile products that occurs between 250 and 375°C. A third step, which is absent in the original chitosan powder appears between 150 and 270°C in electrospun chitosan fibers and chitosan films dissolved in TFA/DCM. FTIR spectra revealed the presence of NH3$^+$CF3COO$^-$ salts produced by the interaction of TFA and amine groups of chitosan during the dissolution and persist after the electrospinning process, favoring polymer dissolution in aqueous environments. After

neutralization with ethanol in a vapor chamber, NH3$^+$CF3COO$^-$ salts still can be found in the polymer fibers, which decrease material thermal stability. Thermal degradation activation energy of chitosan fiber mats (third step) was slightly higher for the samples treated with ethanol and with GA, when compared to the values found forthe chitosan powder.

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