

Electrospinning of 100% Carboxymethyl Chitosan Nanofibers

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ABSTRACT

Carboxymethyl chitosan (CMC), one of the most important chitosan derivatives, is synthesized by alkalization of chitosan, followed by carboxymethylation. CMC has higher moisture absorption and moisture retention, higher chelating and sorption abilities as well as better biological properties than chitosan. Polymeric nanofibrous mats produced through electrospinning have high specific surface area and high porosity which are beneficial for various applications. Up to present time, the electrospinning of CMC has only been possible by the addition of polymers such as polyvinyl alcohol or polyethylene oxide. The present study focuses on the electrospinning of 100% CMC. It was found that the solution of CMC (5 – 6%) in trifluoroacetic acid (TFA) was electrospinnable, producing nanofibers containing some beads. However, adding dichloromethane (DCM) to TFA made the electrospinning uniform, and bead-free CMC nanofibers with an average diameter of 260 nm was possible. This study shows that viscosity and surface tension of the electrospinning solution of CMC plays an important role in making CMC solution electrospinnable.

Keywords: Carboxymethyl chitosan; Electrospinning; Nanofibers; Trifluoroacetic acid; Dichloromethane.

INTRODUCTION

Chitosan, a natural polysaccharide, is a copolymer of N-acetylglucosamine and glucosamine found in the cell wall of some fungi, but also produced through the partial deacetylation of chitin [1,2]. Chitosan has many hydroxyl groups, but it also possesses amino side groups, which distinguish chitosan from other polysaccharides [3]. Carboxymethyl chitosan (CMC), an important chitosan derivative, is produced by alkalization of chitosan followed by carboxymethylation. The location of the substitution of carboxymethyl groups determines the kind of CMC i.e. *N*-, *O*-, *N*, *O*-, *N,N*-. The kind of CMC and its degree of substitution (DS) of carboxymethyl

groups depends on the reaction conditions, reagents, and their stoichiometry [4,5]. In comparison to chitosan, CMC has higher moisture absorption and retention and better biological, chelating and sorption properties [6-8]. CMC has attracted attention in research, because of its ease of synthesis and ampholytic character as well as its potential for biomedical and technological applications [9-11] which include controlled drug release, pH responsive drug delivery, DNA delivery as permeation enhancer [12-14], prolonging plasma levels in implantations [15], nerve regeneration [16], ultrafiltration [17,18] and surface modification of films like poly(D,L-lactic acid) (PDLLA) [19]. Nanofibrous mats with high fiber specific surface area and high porosity, are of considerable interest for various applications [20,21]. Considering applications of CMC in the form of films [22] and nanoparticles [23], nanofibrous mats of CMC should have a good potential for biological and technical applications. Up to the present time, only polyblends of CMC with other polymers such as polyvinyl alcohol or polyethylene oxide has been electrospun successfully to nanofibers [5,24]. As the review showed no reports on the electrospinning of 100% CMC, this study focused on this subject.

Electrospinning is well known for producing nanofibers. In electrospinning, polymer solution is ejected from a capillary by a strong electrostatic force and drawn many times before being deposited as a fibrous mat on an electrically grounded collector. The solvent evaporates as the jet travels through the air on its way to the collector [21]. This study deployed a wide range of chemicals as solvents to examine the electrospinnability of the corresponding CMC solutions.

EXPERIMENTAL

Material

Chitosan (MW=2.86×10⁵g/mol, deacetylation degree≈91%) was purchased from Across Company, USA. Monochloroacetic acid, isopropanol, ethanol, methanol, acetone, acetonitrile, dimethyl formamide,

dimethyl acetamide, dimethyl sulfoxide, formic acid (FA), acetic acid (AA), trifluoroacetic acid (TFA) and dichloromethane (DCM) were purchased from Merck Chemical Company, Germany.

Carboxymethylchitosan Preparation

CMC was produced by the method described by Du et al. [5] as follows; Chitosan (2g) was dispersed in 25 mL of aqueous NaOH (50%) and stirred to swell and alkalize for 24 h at room temperature. After filtering, the alkali chitosan was added to 25mL of monochloroacetic acid/isopropanol (1:4 w/v) and allowed to react for 12 h at room temperature. This product was filtered and then dissolved in 100 mL of distilled water.

Precipitation of CMC followed next with the addition of pure ethanol and finally vacuum drying at room temperature. The DS of the produced CMC was measured according to the procedure described by Muzzarelli et al [5, 25] and was recorded as 0.65.

Electrospinning of CMC

The solubility of CMC in some common solvents like methanol, ethanol, acetone, acetonitrile, dimethyl acetamide, dimethyl sulfoxide, dimethyl formamide as well as water and acids, namely FA, AA and TFA was investigated first and then the electrospinnability of the solutions was tested with an electrospinning set up consisting of a dosing pump (TERUMO STC-527, Japan), high voltage source (0-30kV) and a syringe (needle tip = 0.7 mm). An aluminum plate covered with aluminum foil was used as collector. All electrospinnings were carried out at 22 -25 °C and 30-35 % relative humidity.

Characterization

Scanning electron microscope (SEM: 3XL Philips) and x-ray diffractometer (XRD: XPERT-MPD, Philips) analysis were employed for the characterization of the electrospun nanofibrous mats. The conductivity of electrospinning solutions was measured by JENWAY 3540.

RESULTS AND DISCUSSION

In the first stage of this research, the solubility of the fabricated CMC (DS = 0.65) in a series of solvents was tested. *Table I* shows the maximum solubility of CMC in different solvents depending on DS. Then, the electrospinnability of the solutions was examined.

It is worth mentioning that in an earlier research [24], it had been tried in vain to electrospin the solution of 100% CMC (DS = 0.84) in similar solvents. So, lowering the DS in this research was done with the aim of increasing the solubility of CMC in organic solvents.

Although the solubility of CMC with DS = 0.65, in the above mentioned solvents (with the exception of ethanol) was increased by about two to three times in comparison to CMC with DS = 0.84; however, the electrospinning of these CMC solutions proved impossible. It was concluded that, the low solubility of CMC in the solvents leading to too low viscosities was responsible for the unsuccessful electrospinning. Too low concentration of polymer in solution, in other words too low viscosity does not allow the needed chain entanglements for fiber formation during electrospinning.

Following the unsuccessful electrospinning of CMC solution in the above mentioned solvents, the solution of CMC in water was also tried, but failed to electrospin like the two earlier works [5, 24]. This failure can be mainly related to the high surface tension of CMC solution in water as well as the abundance of charges on CMC molecules leading to inefficient chain entanglement of the rigid and extended α -1,4 polyglycosidic chains [5]. It is worth mentioning that high surface tension of polymer solutions makes the elongation of ejected fluid jet during its trajectory very difficult. This increases the chance of fluid jet breakdown.

In the next stage, three organic acids namely, FA, AA and TFA were employed which dissolved CMC completely. The solution of CMC in FA with concentrations of up to 9% (w/v) was not electrospinnable. This can be related to the high surface tension of FA, as during the attempted electrospinning, only droplets were thrown towards the collector. Even adding ethanol to FA with the aim of reducing the surface tension of the CMC solutions in FA was of no help. Moreover, higher concentrations led to too high viscosity for electrospinning.

Nearly, the same results were obtained for CMC solutions in AA with a difference that the higher boiling point of AA led to the deposition of droplets, still containing solvent, when impinging on the collector. This leads to the merging of the droplets.

TABLE I. Maximum Solubility of CMC in different solvents.

Solvent	Solubility×10 ⁻³ (w/v%) *DS=0.84	Solubility×10 ⁻³ (w/v%) DS=0.65
Methanol	5.8	9.5
Ethanol	6	8
Acetone	9	18
Acetonitrile	14	23.2
Dimethyl Acetamide	15	31
Dimethyl Sulfoxide	17.8	47.6

*From reference [24]

The solutions of CMC in TFA with concentrations of less than 5% (w/v) showed no sign of electrospinnability either; however, with concentrations of 5 – 6%(w/v) of CMC in TFA some signs of fiber formation were observed as shown in *Figure 1*; But, as can be seen, the fibers were irregular, fused to each other and big beads were visible with them. Unfortunately, higher concentrations of CMC in TFA i.e. higher viscosities led to the disruption of even the mixture of fibers and beads as in the case of 5 and 6% (w/v).

From these observations, it was deduced that the low flexibility of CMC chains as well as too much charge on its molecules led to too much repulsive force which prevented the chain cohesion required for fiber formation. It is worth mentioning that the elongation of the fluid jet in the electrospinning field is related to the expulsion forces between electrical charges on the jet surface [21].

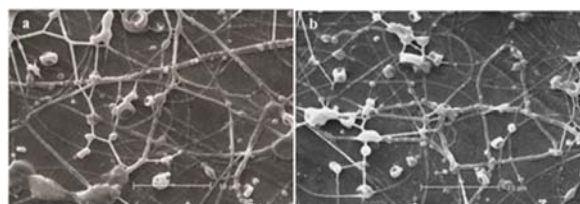


FIGURE 1. SEM images of the electrospun 100% carboxymethylchitosan fibers in trifluoroacetic acid (a CMC concentration= 5%, b CMC concentration = 6%).

To overcome the problem of too much charge, it was decided to add DCM with a low electroconductivity (0.08μs/cm) to TFA. Also, the low boiling point of DCM (39.8°C) helps faster evaporation of the solvents. Therefore, three ratios of TFA/DCM

namely, 90:10, 80:20 and 70:30 (v/v), were chosen and examined. *Figure 2 and 3* show the SEM images of the electrospun 100% CMC (concentrations of CMC in TFA/DCM mixed solvent = 5 and 6% (w/v), respectively).

As *Figure 2* shows, with 5% concentration of CMC in TFA/DCM, increasing the share of DCM from 10 to 30%, leads to less bead formation. When the share of DCM in solvent is 10%, SEM image (*Figure 2a*) showed both beads and fibers.

As the amount of DCM was increased to 20%, the beads became oval and less frequent (*Figure 2b*). Fortunately, with 30% DCM, a network of regular and bead-free nanofibers (*Figure 2c*) with an average diameter of 260±42 nm was obtained. As far as the authors are aware, these 100% CMC nanofibers are prepared for the first time.

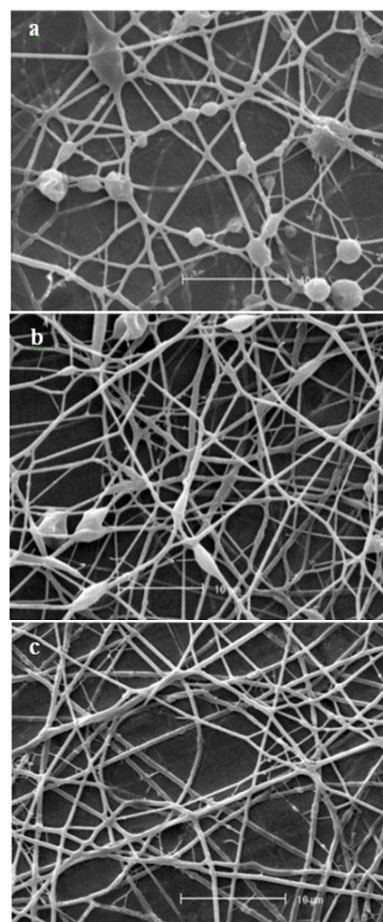


FIGURE 2. SEM images of the electrospun 100% carboxymethylchitosan fibers (5% CMC in dichloromethane / trifluoroacetic acid, a = 10:90, b = 20:80 and C = 30:70).

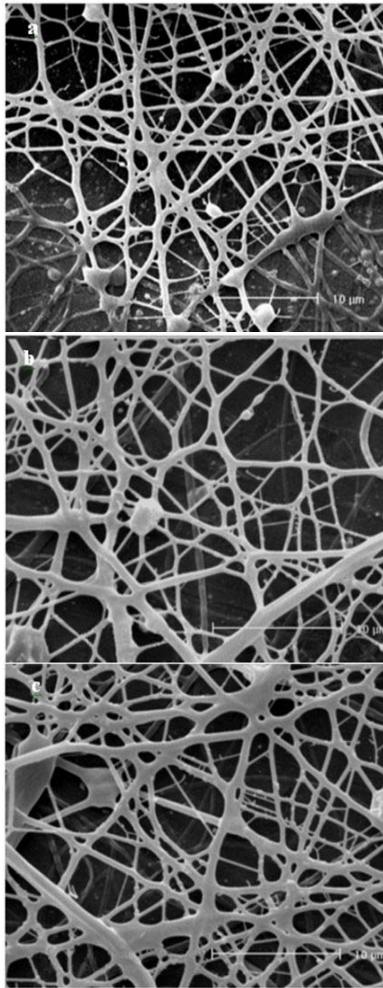


FIGURE 3. SEM images of the electrospun 100% carboxymethylchitosan fibers (6% CMC in dichloromethane / trifluoroacetic acid (a = 10:90, b = 20:80 and C = 30:70).

Figure 3 shows that increasing the concentration of CMC in TFA/DCM (90:10, 80:20 and 70:30) to 6% (w/v), leads to the formation of fused nanofibers in the electrospun web. This is due to the fact that the solvent has not been able to evaporate completely before the nanofibers reach the collector and hence, the nanofibers fuse to each other after reaching the collector. Increasing DCM to more than 30% led to a decrease of CMC solubility in the mixed solvents.

So it is concluded that the optimum concentration for electrospinning CMC in TFA/DCM is 5% (w/v). The optimum condition for electrospinning of CMC with regards to the different apparatus parameters (applied voltage, tip-to-collector distance and feed rate) that were tested in this research is shown in Table II.

Comparing different properties of TFA with other acids deployed in this research, the lower surface tension, lower boiling point, lower acidity constant, and higher viscosity of TFA can be held responsible for making the electrospinning of the rather stubborn 100% CMC possible. The optimum conditions for electrospinning of CMC are, voltage = 14-16 kV, collector-needle distance = 18-20 cm, and feed rate = 0.25 mL/h. Basically, it can be concluded that the electrospinning of 100% CMC requires a specific set of conditions that involves process parameters as well as solution parameters. The XRD pattern of 100% electrospun CMC nanofibrous (Figure 4) exhibits one wide peak at $2\theta = 21^\circ$, indicating that small crystallites constitute a good proportion of 100% CMC nanofibers' microstructure.

TABLE II. Optimum conditions for electrospinning CMC (DS=0.65).

Concentration (w/v)	Solvent	Collector	Voltage (kV)	Electrospinning distance (cm)	Feed rate (ml/h)
5%	TFA/DCM (70:30)	Aluminum plate	14-16	18-20	0.25

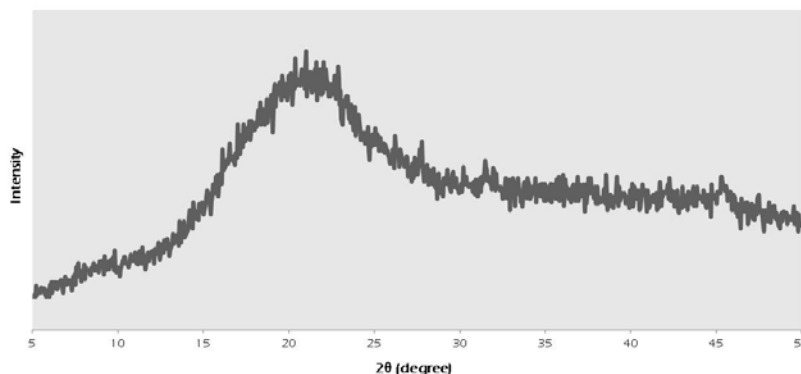


FIGURE 4. XRD pattern of 100% carboxymethyl chitosan nanofiber.

CONCLUSION

Carboxymethyl chitosan (CMC) with a degree of substitution of 0.65 was synthesized by alkalization and then carboxymethylation of chitosan. Among the solutions of CMC in methanol, ethanol, acetone, acetonitrile, dimethyl acetamide, dimethyl sulfoxide, dimethyl formamide, water, formic acid, acetic acid and trifluoroacetic acid, only the solution of CMC in trifluoroacetic acid/dichloromethane was electrospinnable. This research produced the electrospinning of regular and bead-free nanofibers of 100% carboxymethyl chitosan with an average diameter of about 260 nm possible, thanks to the specific properties of trifluoroacetic acid as well as dichloromethane. The optimum concentration of CMC in trifluoroacetic acid /dichloromethane (70 – 30) electrospinning solution was 5%.

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