

Electrospinning alginate/polyethylene oxide and curcumin composite nanofibers

Javier Gutierrez-Gonzalez ^a, Esther Garcia-Cela ^{b,c}, Naresh Magan ^b, S. Sameer Rahatekar ^a

^a Enhanced Composites and Structures Centre, Cranfield University, Bedfordshire MK43 0AL, UK

^b Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, Bedfordshire MK43 0AL, UK

^c School of Life and Medical Sciences, University of Hertfordshire, Hatfield, UK

Abstract

Manufacturing a sodium alginate (SA) and polyethylene oxide (PEO) composite loaded with curcumin (CU) was accomplished in this study by using electrospinning. These composite nanofibers were crosslinked using trifluoroacetic acid (TFA) mechanically characterized along with the morphological properties of the composite nanofiber mesh. We were successful in manufacturing the composite nanofibers with a wide range of CU concentrations ranging from 10 to 40 wt%. Firstly, dissolved in a saturated water/CU solution it was added to SA/PEO blending, homogenized and electrospun. Mechanical properties were affected by both CU addition and the cross-linking process, resulting in a higher ultimate tensile stress (MPa) (from 4.3 ± 2 to 15.1 ± 2 at 10% CU) and Young modulus (GPa) (0.0076 ± 0.003 , 0.044 ± 0.003 before and after TFA). CU was successfully encapsulated in the SA nanofibers and excellent mechanical properties were obtained. By using a biocompatible TFA crosslinking and the natural properties of alginate this nanofiber composite could potentially be used for filtering, environmental pollution control, food packaging and for tissue engineering.

1. Introduction

Electrospinning (ES) of sodium alginate (SA) allows the production of nanofiber meshes and scaffolds with immense advantages such as a high surface area ideal for drug release and heavy metals adsorption [1]. Hence, ES of SA can be an excellent option for drug delivery and water filtering applications. Although challenging [2,3], electrospinning of SA has been extensively studied in the past. Due to the SA low gelling point, a high molecular weight copolymer like polyethylene oxide (PEO) [3] is added to the solution to form suitable chain entanglements, normally along with surfactants [3].

Another challenge when electrospinning SA is the spontaneous formation of 3D structures instead of a flat surface, due to higher availability of anions from SA at high relative humidity (RH), which can be an issue for most applications [4]. Therefore, a controlled

atmosphere using air conditioning is necessary, increasing costs, especially for high scale production.

In order to increase resistance of SA to water or aqueous body fluids, Hajiali et al. [5] suggested a biocompatible method that consisted in soaking the electrospun SA mats on trifluoroacetic acid (TFA), enabling better control of the degradability rate of the mats and overcoming drawbacks of previous methods [6,7], however its mechanical properties hasn't been determined up to date. Also these researchers did not report curcumin alginate composite nanofibres.

Curcumin (CU), an extract of the *Curcuma Longa*, is claimed to have efficacy in relation to a range of diseases including from anti-cancer to anti-oxidant and anti-arthritic behavior as well as having anti-microbial efficacy [8,9].

This study is focused on developing the manufacturing technique for a biocompatible nanofiber system loaded with CU followed by the characterization of the nanofiber morphology and mechanical testing. Tensile properties of TFA crosslinking are analyzed for the first time while an economical method is reported for electrospinning a homogeneous SA nanofiber under high humidity conditions. This nanofiber composite could be of great interest for applications such as filtering system for environmental pollution control, food packaging and tissue engineering.

2. Experimental section

a. Materials

SA (<40 kDa), PEO (1.000 kDa), Pluronic F-127 (PL), TFA, Phosphate buffer saline solution (PBS) were purchased from Sigma Aldrich. CU with a formulation of 200 mg of nanocurcumin and 2 mg of Bioperin was acquired from Triso.

2.2. Preparation of solution for electrospinning

Two separate solutions of 8 wt% of SA and 4 wt% of PEO were prepared solubilized in deionized water, during 4 h and further mixed together in the proportions 25:9.5 (g SA: g PEO). Subsequently, Pluronic F-127 was added at 2 wt% of the whole solution. Final dry mat resulted in an 84 wt% of SA. To prepare the CU solutions, a saturated solution of CU and water was added to a SA and PEO solution of higher concentration, ending with the same SA/ PEO/solvent specified before. Final loadings were 10, 20, 30 and 40 wt% CU (wt% CU/wt% polymer) to explore the limits of the encapsulated agent.

b. Electrospinning of curcumin loaded SA

The needle to collector distance was kept at 15 cm, using a ES machine (LE-50 FLUIDNATEKTM eSpinning tool, Bioinicia) and the rotator drum at 700 rpm. The applied voltage was 15–23 kV while the feed rate stayed at 0.3–1 mL/h. All fibers were obtained at room temperature (21–25 °C) and 40–50 %RH. The ES time was 3 h, and they were peeled off directly from the drum.

c. Cross-linking of the mats

The cross-linking method used in this project is described by Hajjali et al. [5]. Squares of 9 cm² were cut and immersed into the TFA for 24 h and left for drying during further 24 h inside the fumehood.

d. Characterization of fibers

A XL30 (FEI) Environmental Scanning Electron Microscope (ESEM) was used to study fiber morphology, diameter size and directionality. Images were processed with ImageJ and DiameterJ. Data was treated with the fitting software Fityk when the diameter distributions were not normal

e. Mechanical testing

Strips of 26 × 12 mm² of the electrospun mats were tested using a Deben MT200 microtensile machine with a 5 N load cell, 1 mm/min speed and a separation grip of 10 mm. The specimen thicknesses were obtained with a Dektak stylus profilometer. The tests were done at room temperature and at least five specimens were prepared per type of sample.

f. Statistical analysis

Data was analyzed with R, using an ANOVA test and a TukeyHSD post-hoc treatment, considering $p < 0.05$ as statistically different and results were reported as average (M) ± standard deviation (SD).

3. Results and discussion

a. Fibers morphology

The final electrospun mat containing 84 wt% of SA is shown on Fig. 1A together with the crosslinked version (100% alginate). Attending at Fig. 1B, the nanostructure is seamlessly maintained showing wavy fibers in contrast. Furthermore, the diameter decreased 15% (from 250 to 212 nm), almost matching the original PEO content (16%). It is worth noting that only CU encapsulated by PEO is lost during cross-linking, as CU is not soluble in acid environments [10]. Because of the high humidity, 3D structures built up on the static collector in accordance to previous works [4]. Hence the spinning collector was used at minimum speed, so an acceptable flatness of the mat was achieved. Due to the rotating movement, fibers are dragged on the tangential speed direction, helping to coat the cylinder, instead of contributing to agglomeration.

On Fig. 2, SEM images of the different CU loaded mats are displayed together. The final mats are shown as well on the insets. On Fig. 2A, fibers can integrate 10% of CU with no apparent defects while for 20% CU (Fig. 2B) there are few CU particles and a prominent joining of several fibers. With 30% CU (Fig. 2C) the formation of fibers is affected and saturated of CU leading to the formation of beads and presence

of micron-sized CU particles. Finally, nanofiber formation is totally disrupted at 40% CU (Fig. 2D). Indeed, a uniform layer spreads all over with some micron-sized fibers.

b. Mechanical testing

The main mechanical properties of the mats, such as ultimate tensile stress (UTS), elongation at break E_T (%) and Young modulus (E) are summarized on Table 1. Stress/strain curves can be consulted on the Supplementary Information (SI). Comparing samples before and after TFA and no CU loading, its E (GPa) shows a great increase from 0.0076 to 0.044 GPa (Fig. 3), $F(1, 9) = 11.9$, $p < 0.01$, that is, almost six times larger than after the treatment, adding a great increase of strength in the elastic region. This is probably caused by the suppression of the PEO after the cross-linking process which can act as a plasticizer [11], along with the strong crosslinking effect by TFA. The same reason would explain the decrease in elongation at break (-22%). This strong improvement exceeds other attempts in the literature [12,13].

Analyzing the CU loaded samples similar effects are encountered, obtaining increments of UTS and E in accordance with other electrospun mats loaded with CU [14]. In the case of the E, strong increment (~100%) at 10% CU is lost for subsequent loadings until it recovers at 40% CU, suggesting that CU addition can be detrimental at high doses. Results at 40% could be explained due to the loss of the nanostructure of the mat (Fig. 2D), having more effective area to withstand the tensile force. Elongations at break increase at 10% (+4.71%) and 20% (+2.97%) but further increments of CU are negative, having results non-statistically different from non-loaded samples. Finally, comparing the UTS values, small amounts of CU (10% CU) can be beneficial for the tensile strength increasing from 4.34 to 7.05 MPa, but further increments of CU turn out in a detrimental effect, probably due to more concentration of defects, obtaining even smaller UTS at 30% CU (4.30 MPa), while for 40% CU samples there is not a statistical difference regarding nonloaded samples.

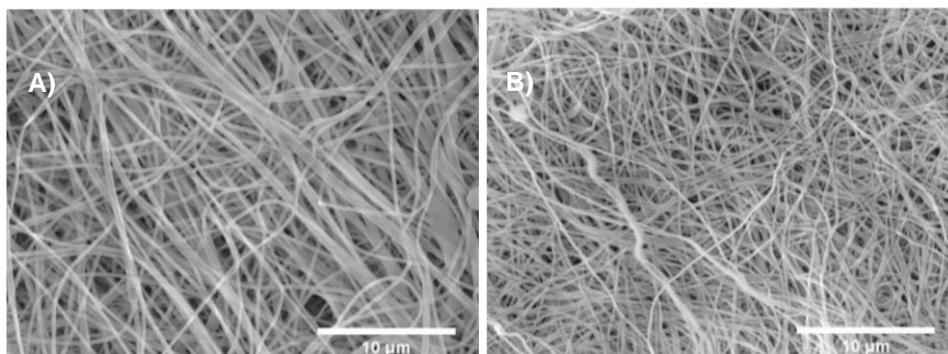


Fig. 1. SEM images. A) SA / PEO nanofibres. B) SA crosslinked with TFA nanofibres.

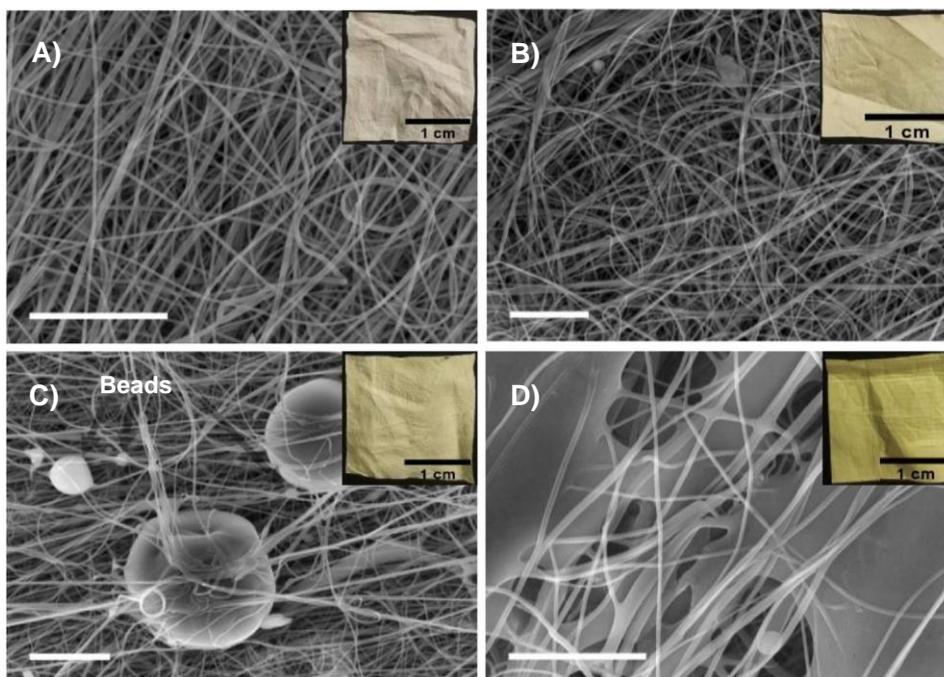


Fig. 2. SEM images SA / PEO mats. A) 10% CU. B) 20% CU. C) 30% CU. D) 40% CU (All scale bars 10 µm).

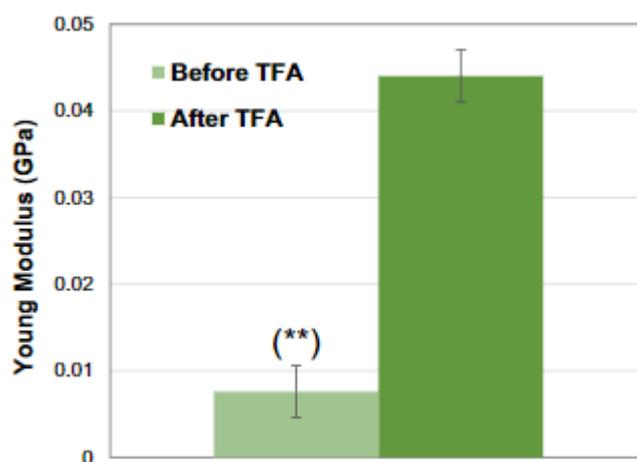


Fig. 3. Young Modulus ($M \pm SD$) of nanofiber samples, before and after crosslinking. $F(1,9) = 11.873$, $p < 0.01$.

Table 1. Mechanical properties of electrospun mats before (left) and after (right) TFA treatment at different CU loadings. (M ± SD)

CU loadin g	Before TFA					After TFA				
	0%	10%	20%	30%	40%	0%	10%	20%-	30%	40%
E (MPa)	7.6± 2.8	15.1± 1.7	11.9± 2.9	7±4	16.8 ±5	44±2. 2.94	32. ±3.4	18.2± 6.7	36±1 3	37.5 ±2
σ_{UTS} (MPa)	4.3± 1.9	7.1±0 .7	4.9±1	4.3± 1.4	5.8± 0.5	5.9±2	9.1±0 .94	7.2±0 .9	7.9±	10±2
E_T (%)	10.1 ±1	10.6± 0.9	10.4± 1.5	9.9± 1.7	8.7± 2.4	7.9±3	7.4±1 .2	9.2±3 .7	6.5± 5	4.5± 2
σ_{0.2%} (MPa)	3.1± 1.4	4.5±0 .6	3.1±0 .8	2.5± 0.7	4±0. 5	2.9±1	5.5±0 .8	4.3±1 .6	4.1± 1	6.2± 1.9
σ_{Y.P} (MPa)	4.3± 1.9	6.5±1 .1	4.2±0 .7	3.7± 1.4	5.8± 0.5	4.9±1. 9	9.1±1	6.8±1 .1	5.5± 2	8.5± 3.2
σ_T (MPa)	4.1± 1.7	6.8±0 .9	4.4±2 .4	4±1. 4	5.2± 0.8	5.4±2	7.6±1 .7	6.7±1 .2	6.8± 3	10±2
E_Y (%)	8.4± 1.8	8.2±2 .3	6.9±2 .6	7.4± 2.3	7.2± 2.5	4.8±3. 2	5.4±1	7.2±3 .1	2.8± 1	3.2± 1.2
E_L (MPa)	2.6± 1.1	3.7±0 .4	5.9±4 .3	2.5± 0.7	3.1± 0.4	2.3±0. 6	4.1±0 .8	2. 9±1.2	3.3± 0.7	4.8± 1.8

σ_{UTS}: Ultimate tensile strength

σ_T: Nominal strength at break

E_T (%): Elongation at break percentage

σ_{0.2%} (MPa): Nominal strength at yield point

σ_{Y.P}: Offset yield strength (0.2%)

E_Y (%): Elongation at yield point percentage

E_L: Elastic limit

E: Young modulus

4. Conclusions

Electrospun nanofibers made of SA-PEO were successfully obtained incorporating CU, confirming TFA as a strong biocompatible cross-linking agent with potential biomedical and filtering applications. Mechanical properties were affected by CU addition and the TFA cross-linking, resulting in higher tensile stress and E at the expense of the elasticity and plasticity of the final mat. The effective manufacturing process to produce curcumin and alginate nanofibres reported in this paper can contribute towards potential applications of such as materials in sustainable food packaging and removal of industrial pollutants from waste water.

5. CRediT authorship contribution statement

Javier Gutierrez Gonzalez: Writing - original draft, Conceptualization, Investigation, Formal analysis, Methodology, Visualization. Esther Garcia-Cela: Formal analysis, Writing - review & editing. Naresh Magan: Writing - review & editing. S. Sameer Rahatekar: Conceptualization, Resources, Writing - review & editing, Supervision.

6. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

7. Acknowledgment

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Supplementary information

Viscosity tests

Viscosity measurements were done to characterize the solution with a Rheometer AR200ex. The parameters used are summed up in the table below.

Viscosity parameters.

Geometry	Type of test	Temperature	Number of tests	Range of shear rate
40mm/1.5° cone and plate	Steady shear	25°	Repeated on at least 2 different samples	0.01-100 (s ⁻¹)

Next, the viscosity measurements of the main solutions are shown below.

SA: Alginate

PEO: Polyethylene oxide

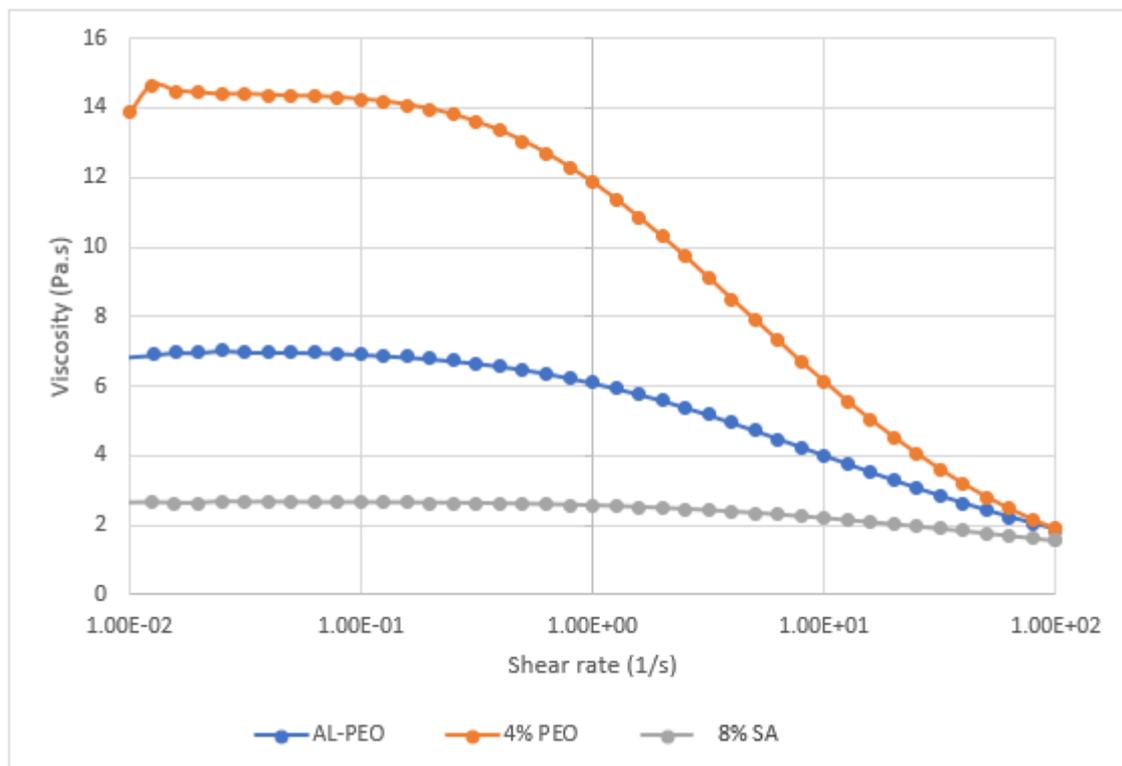


Figure 1: Viscosity of 1000 MW PEO, 8% SA and the final blend.

Tensile Properties

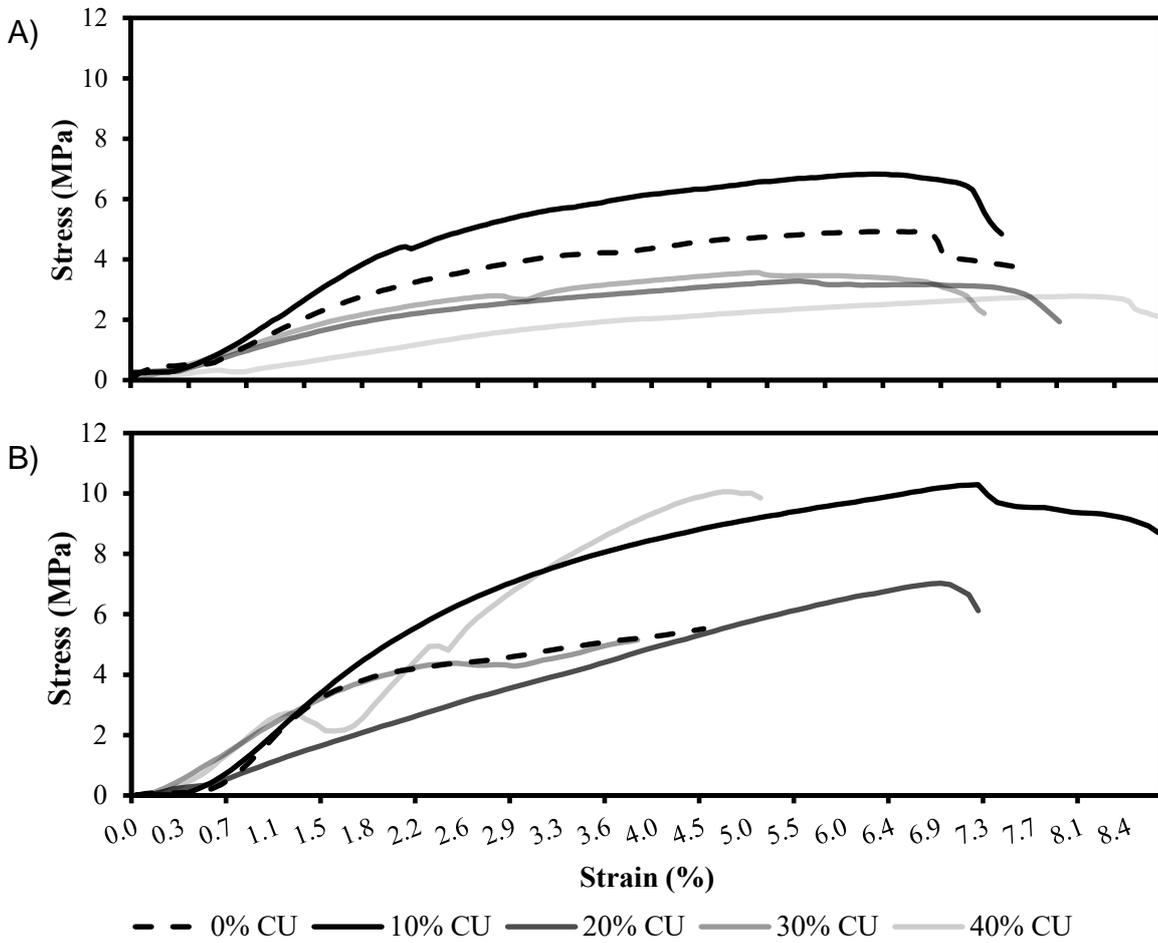


Figure 2. Stress-strain curves of some SA electrospun mats. A) Upper graph represents before TFA treatment. B) Lower graph represents after TFA.