

SUPPLEMENTARY MATERIALS

1. Synthesis and Characterization of O-Carboxymethyl Chitosan

1.1 Methods

O-carboxymethyl chitosan (O-CMCS) was prepared modifying the method described by [18,29]. The carboxymethylation was performed *via* stirring CS at low molecular weight (LCS, 5 g) in 100 ml of 20% NaOH (w/v) for 15 min. After that, monochloroacetic acid (15 g) was gradually added in the suspension and stirring was continued for 2.5 h at 40°C. The reaction was neutralized with HCl 37% in an ice bath and, subsequently, a solution of 70% methanol was added. The reaction mixture was washed with methanol and dried under reduced pressure for 5 h. Solvent evaporation was performed under reduced pressure by a Heidolph Laborota 4000 instrument (Heidolph Instruments GmbH & Co., Schwabach, Germany). Then, the sample was grounded with a blade mill (A10 IKA-Werke GmbH & Co., Staufen, Germany). Jasco ATR-IR-4100 was used to register the IR spectra applying the compound as powder.

1.2 Results and Discussion

Figure S1 shows the overlapping of FT-IR spectra of CS at low molecular weight (LCS) and the derivate O-carboxymethyl chitosan (O-CMCS). LCS displays two broad bands at 3445 and 3360 cm^{-1} , which are typical of NH_2 and OH stretching, and at 2919 cm^{-1} corresponding to CH stretching of methyl group. Two bands at 1655 cm^{-1} and 1596 cm^{-1} can be attributed to CO of NH-CO stretching and to NH bending, while at 1085 and 1030 cm^{-1} COH stretching bands are shown. On the other part, OCHT spectrum shows two new strong bands at 1580 and 1412 cm^{-1} attributable to symmetric and asymmetric stretching vibration of COO^- salt. Thus, the introduction of carboxymethyl group was confirmed, suggesting that the substitution occurred at C6 position of chitosan. Data displayed here are in agreement with literature [18].

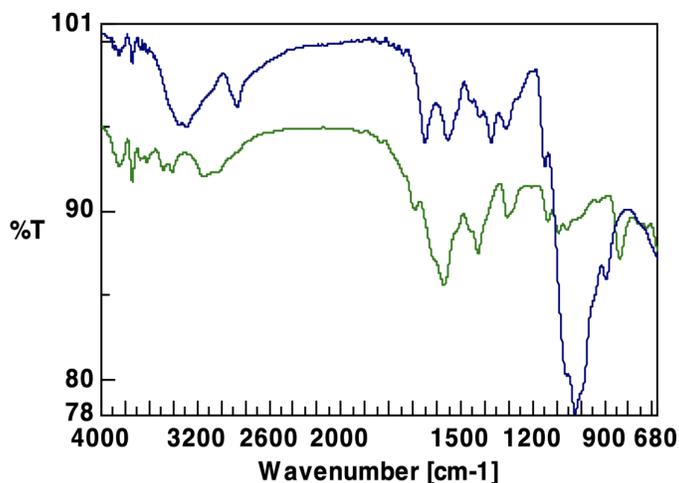


Figure S1. FTIR spectra of LCS (blu line) and its O-carboxymethyl derivative O-CMCS (green line).

2. Characterization of the Polymeric Solutions for Electrospinning

2.1 Methods

All the polymeric solutions (OC1-OC5) and the mixture of c-SLN dispersion and OC3 (c-SLN/OC3) were characterized in terms of rheological properties and conductivity, as reported in [39,40].

2.1.1 Rheological Measurements

Rheological analyses were carried out by means of a rotational rheometer (MCR 102, Anton Paar, Turin, Italy) equipped with a cone plate combination (CP50-1, diameter = 50 mm; angle = 1°) as measuring system.

Sample viscosity was investigated at increasing shear rates in the range 1–1000 s⁻¹ at 33°; three replicates were effected for each polymeric solution. Three replicates were considered for each polymeric solution.

2.1.2 Conductivity Measurements

Sample conductivity was measured with Mettler Toledo™ FiveGo™ F3 conductivity meter apparatus (Fisher Scientific, Milan, Italy). Three replicates were considered for each solution.

2.2 Results and Discussion

In Figure S2, the results of the rheological analyses performed on OC1-OC5 polymeric solutions are reported. As expected, the viscosity value at 1000 s⁻¹ increases on increasing O-CMCS concentration (Figure S2); such shear rate was selected since it simulated the stress to which the polymeric solutions were subjected during the electrospinning process.

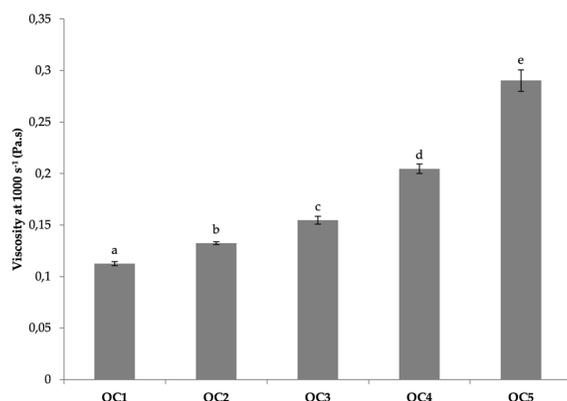


Figure S2. Viscosity values of OC1-OC5 polymeric solutions intended to be electrospun at 1000 s⁻¹. Data are expressed as mean values ± SD (n=3). ANOVA one-way; Sheffè Multiple Comparison (p<0.05): a vs b-e; b vs c-e; c vs d-e; d vs e.

The blending of the c-SLN dispersion with OC3 (c-SLN/OC3), which was selected as the most promising polymeric solution for the production of homogenous nanofibers by electrospinning, did not alter the rheological properties of OC3; as shown in Figure S3, the flow profile of OC3 is comparable to that of c-SLN/OC3.

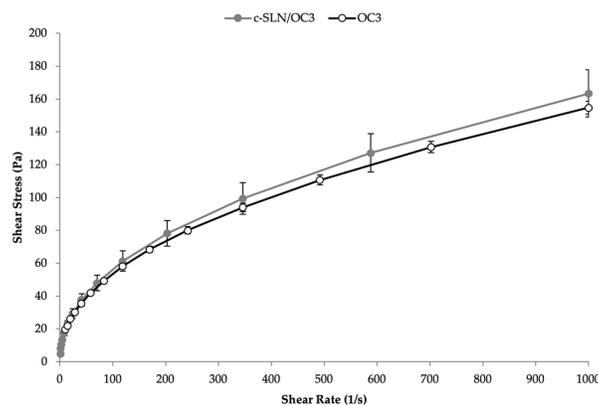


Figure S3. Flow profiles of OC3 and c-SLN/OC3 at increasing shear rates (1-1000 s⁻¹). Data are expressed as mean values ± SD (n=3).

Noteworthy, c-SLN/OC3 was characterized by a conductivity value significantly higher than that of OC3 (2224.33 ± 54.24 μS/cm vs 1661.56 ± 37.89 μS/cm; mean value ± SD, n=3), probably due to the presence of c-SLN, whose surface was positively charged.