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Polycaprolactone/chitosan blend nanofibres electrospun from an acetic acid/formic acid solvent system

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ABSTRACT

Blend nanofibres composed of chitosan and polycaprolactone (PCL) are highly valuable for biomedical applications since the benefits of natural and synthetic polymers are thus combined. Research towards the stable and reproducible electrospinning of these blends is, however, essential. Therefore, this paper focuses on the novel solvent system 3/7 acetic acid/formic acid for electrospinning PCL/chitosan nanofibres. Addition of chitosan to PCL solutions led to a significant increase in the solution's conductivity because of the polycationic character of chitosan. Moreover, since adding chitosan also considerably raised the viscosity, polymer solutions with a low total polymer concentration could be electrospun. Owing to both effects, the fibre diameter and distribution were significantly lowered with increasing chitosan content. Furthermore, chitosan led to the formation of an ultrafine nanofibrous web formed in between the main fibres. In conclusion, the solvent mixture acetic acid/formic acid is an excellent system for successfully electrospinning blend nanofibres containing PCL and chitosan.

1. Introduction

Chitosan, a natural polysaccharide derived from chitin, shows advantageous characteristics such as biocompatibility, biodegradability, hydrophilicity, non-toxicity and antimicrobial activity. Chitosan is therefore considered as a promising material for biomedical use (Patale & Patravale, 2011; Quiñones, Szopko, Schmidt, & Covas, 2011; Singh et al., 2009). For these medical applications the use of nanofibrous materials is believed to be beneficial thanks to their unique characteristics, these being a high porosity, small pore size and high specific surface area. Nanofibres are thus able to mimic the native extracellular matrix, hence promoting tissue healing (Greiner & Wendorff, 2007). At present, electrospinning is the only well-established technique able to produce nanofibres in a continuous way (Ramakrishna et al., 2005). However, numerous studies report on the difficulties encountered when electrospinning pure chitosan (Cooper, Bhattarai, Kievit, Rossol, & Zhang, 2011; De Vrieze, Westbroek, Van Camp, & Van Langenhove, 2007; Jayakumar, Prabaharan, Nair, & Tamura, 2010; Su et al., 2011). Moreover, chitosan structures are mechanically weak, limiting their practical performance (Wu et al., 2010).

Blending chitosan with a synthetic polymer may provide a superior material that combines the benefits of both, showing a good tissue compatibility and improved mechanical properties. To obtain these blend nanofibres, electrospinning a polymer solution that contains both polymers is the most appropriate production method since complications with electrospinning pure chitosan can thus be avoided. Polycaprolactone (PCL) is an ideal candidate for the synthetic polymer to be mixed with chitosan thanks to its biocompatibility, biodegradability, non-toxicity and good mechanical properties (Van der Schueren, De Schoenmaker, Kalaoglu, & De Clerck, 2011). PCL is also studied for biomedical applications but suffers from hydrophobicity and lack of cell-recognition sites for the support of cell adhesion (Prabhakaran et al., 2008), both of which can be supplied by chitosan. Moreover, it has been stated that PCL shows a good miscibility with various polymers and improves the processability of some polymers (Senda, He, & Inoue, 2001). Hence, blending PCL with chitosan would most likely assist the electrospinning process of chitosan.

The electrospinning of PCL/chitosan nanofibres has only recently been studied (Bhattarai et al., 2009; Cooper, Bhattarai, & Zhang, 2011; Hong & Kim, 2011; Prabhakaran et al., 2008; Shalumon et al., 2010; Yang, Chen, & Wang, 2009). These studies demonstrated the promising potential of PCL/chitosan nanofibres for biomedical applications such as tissue engineering. Further research towards the use of less toxic solvents and reproducible electrospinning is, however, essential to fully exploit their potential. Our previous study proved that the less toxic acetic acid (AA)/formic acid (FA) solvent mixture (Bordes et al., 2010; European

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Pharmacopoeia, 2009) is beneficial for the stable and reproducible electrospinning of pure PCL nanofibres (Van der Schueren et al., 2011). This solvent system may thus also facilitate the electrospinning process of PCL/chitosan blend nanofibres. Therefore, this paper focuses on an in-depth study of the electrospinning of a PCL/chitosan polymer blend out of an AA/FA solvent mixture. The optimum solvent ratio is determined, after which the electrospinning process and resulting fibre morphology are discussed. Infrared spectroscopy is applied to confirm the presence of both polymers.

2. Materials and methods

2.1. Materials

Medium molecular weight chitosan and PCL (M_n 70,000–90,000) were supplied by Sigma–Aldrich. Also the solvents 98 v% FA and 99.8 v% AA were obtained from Sigma–Aldrich.

2.2. Preparation and characterisation of the electrospinning solutions

The electrospinning solutions were prepared by dissolving a certain amount of chitosan and PCL pellets simultaneously in the solvent system. The PCL concentration was expressed in wt% in the solution while the chitosan concentration generally was presented as a ratio of the mass of chitosan to the mass of PCL, expressed in % chitosan (formula (1)). However, for the interpretation of the solution parameters, the chitosan concentration was expressed in wt% in the solution as well (formula (2)).

$$\text{chitosan}\,(\%) = \frac{\text{mass}_{\text{chitosan}}}{\text{mass}_{\text{PCL}}} \quad (1)$$

$$\text{chitosan}\,(\text{wt}\%) = \frac{\text{mass}_{\text{chitosan}}}{\text{mass}_{\text{chitosan}} + \text{mass}_{\text{PCL}} + \text{mass}_{\text{solvents}}} \quad (2)$$

The solutions were magnetically stirred at room temperature for three-and-a-half hours, time needed for complete dissolution. The viscosity of the solutions obtained was measured using a Brookfield viscometer LVDV-II. The conductivity was measured with a CDM210 conductivity meter (Radiometer Analytical).

2.3. Electrospinning of PCL/chitosan nanofibres

During the electrospinning process, the polymer solution was pumped from a 20 ml syringe into a 15.24 cm long needle with an inner diameter of 1.024 mm. A KD Scientific Syringe Pump Series 100 regulated the flow rate of the solution. The voltage was adjusted using a Glassman High Voltage Series EH 30P3 source (voltage range 0–30 kV). The voltage window of stable electrospinning generally was 5 kV, with the stated voltage as upper boundary. Electrospinning was carried out at room temperature ($22 \pm 2\,^{\circ}C$) and a relative humidity of $35 \pm 5\%$. The tip to collector distance was set at 12.5 cm. To electrospin the polymer blend, the flow rate was set at 0.6 ml h^{-1} while this was 1 ml h^{-1} for electrospinning pure PCL.

2.4. Characterisation of the electrospun samples

The morphology of the electrospun structures was examined using a scanning electron microscope (FEI QUANTA 200 F). Prior to the SEM-measurements, the sample was coated with gold using a sputter coater (Balzers Union SCD 030). Fifty diameter measurements on each sample using UTHSCSA ImageTool version 3.0 determined the average fibre diameter.

Fourier Transform (FT) infrared spectra were recorded on a PerkinElmer GX 2000 in the range 370–4000 cm^{-1} with a resolution of 4 cm^{-1} and a data interval of 1 cm^{-1}. 16 scans were performed for each measurement.

Table 1
Viscosity (mPa s) and conductivity (mS/cm) of PCL (8 wt%)/chitosan (10%) polymer solutions at varying AA/FA solvent ratio.

AA/FA solvent ratio	Viscosity (mPa s)	Conductivity (mS/cm)
1/9	5495	0.891
3/7	5453	0.570
5/5	5483	0.320
7/3	2456	0.094

3. Results and discussion

3.1. Optimum solvent ratio

To determine the optimum AA/FA solvent ratio for electrospinning PCL/chitosan nanofibres, a polymer blend containing 8 wt% PCL and 10% chitosan was selected as representative example. Since pure PCL is electrospinnable in the solvent range 10–70 v% AA (Van der Schueren et al., 2011), these boundaries were also chosen for electrospinning the blend. Four different polymer solutions were prepared having an AA/FA solvent ratio of 1/9, 3/7, 5/5 and 7/3. The latter solution did not dissolve completely, while the other blends were homogeneously dissolved. The solvent ratio did not significantly influence the viscosity of the polymer solutions (Table 1) due to the similar viscosities of AA and FA (1.1 mPa s and 1.8 mPa s at 25 °C, respectively) (Supaphol, Mit-uppatham, & Nithitanakul, 2005). Yet the incompletely dissolved 7/3 AA/FA polymer solution showed a lower viscosity due to the lower actual dissolved polymer concentration. The conductivity did alter depending on the solvent ratio with a decrease in conductivity with decreasing FA content (conductivity of 0.891 mS/cm at 1/9 AA/FA to 0.094 mS/cm at 7/3 AA/FA, Table 1). This observation is in line with the significant difference in dielectric constant between both solvents (6.2 for AA and 57.2 for FA at 25 °C) (John, 1998).

Both polymers showed a good stability in the solvent mixture for minimum six hours as was demonstrated by a constant monitored viscosity value during this time period. The solvent system is thus appropriate for the electrospinning study of PCL/chitosan blend nanofibres.

The electrospinning trials performed with the different polymer solutions indicated the feasibility of electrospinning PCL/chitosan blend nanofibres using an AA/FA solvent mixture. Fibre formation was possible for all completely dissolved solutions, namely those having a 1/9, 3/7 and 5/5 AA/FA solvent ratio (SEM images, Fig. 1). However, a stable Taylor cone, essential for obtaining reproducible samples, was only obtained at a 3/7 and 5/5 AA/FA ratio. These solutions were electrospun at 25 kV and 20 kV respectively. Analysis of the resulting fibre diameter showed a diameter of 203 ± 44 nm for 3/7 AA/FA and a diameter of 367 ± 118 for 5/5 AA/FA. This increase in diameter and fibre distribution with increasing AA content is in agreement with the results obtained for pure PCL nanofibres and is related to the decrease in conductivity (Van der Schueren et al., 2011). Because of the smaller fibre diameter and distribution, a solvent ratio of 3/7 AA/FA was selected for further study.

3.2. Determination of electrospinning region of PCL/chitosan polymer solutions

A key prerequisite for obtaining reproducible electrospun samples, is the presence of a stable Taylor cone (Ramakrishna et al., 2005). Thus, to determine the performance of electrospun PCL/chitosan nanofibres, solutions containing different polymer

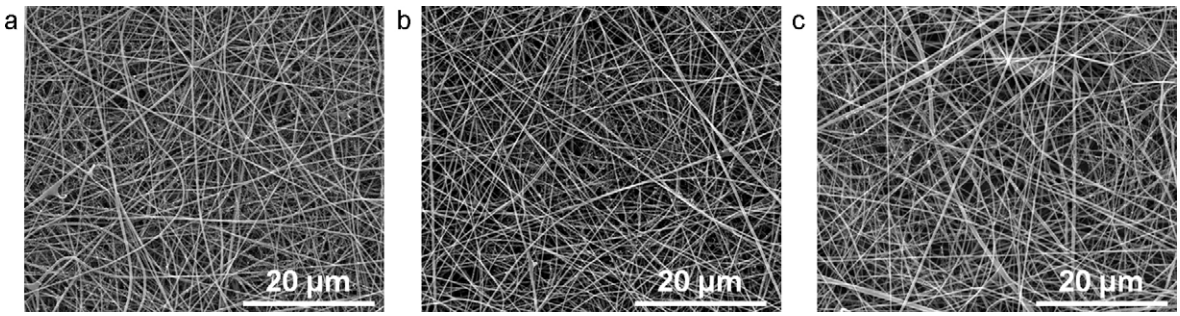

Fig. 1. SEM images of PCL (8 wt%)/chitosan (10%) nanofibres with an AA/FA solvent ratio of 1/9 (a), 3/7 (b) and 5/5 (c).

concentrations (at a 3/7 AA/FA solvent ratio) were attempted to electrospin and were visually judged on the presence of a stable Taylor cone. Table 2 shows both the area in which electrospinning with a stable Taylor cone was possible (white) and the area in which electrospinning was possible, though without the presence of a stable Taylor cone (grey). The latter did result in the formation of nanofibres but irregularities in the nanofibrous structure such as drops and local thickening of the fibre (observed in SEM images) were present due to instabilities in the process. In this analysis, the amount of PCL was restricted to 14 wt% to allow for a substantial proportion of chitosan to PCL in the nanofibres. Noteworthy is that only 1% chitosan could be added to a 14 wt% PCL solution while already 20% chitosan could be added to a 6 wt% PCL solution. Thus, lowering the PCL concentration allowed for an increase in the amount of chitosan, while maintaining a stable process. In addition, the chitosan content has to be chosen within a certain range, determined by the PCL concentration (Table 2).

To further the understanding of the boundary conditions obtained in Table 2, the solution's parameters are to be looked at. Therefore, the viscosity and conductivity of the electrospinning solutions were characterised. As expected, the viscosity increased both with increasing PCL and with increasing chitosan concentration as shown in Fig. 2a. In addition, when analysing the electrospinnability of the blend solutions, the viscosity proved to play a dominant role, in line with literature (Huang, Zhang, Kotaki, & Ramakrishna, 2003; Ramakrishna et al., 2005). All studied solutions having a viscosity lower than 1250 mPa s and higher than 8000 mPa s failed to result in a stable Taylor cone, as schematically visualised in Fig. 2a. Because of the strong rise in viscosity with increasing chitosan content, polymer blend solutions with a low total polymer mass could be electrospun in a stable way. While minimum 14 wt% polymer was required with pure PCL, 6.6 wt% total polymer concentration was sufficient when chitosan was added (Table 2). The electrospinnable solutions demanded a higher applied voltage with increasing viscosity and thus with increasing PCL and chitosan content (Table 2).

As depicted in Fig. 2b, the conductivity is not significantly influenced by the PCL concentration in agreement with previous results on pure PCL (Van der Schueren et al., 2011). However, adding chitosan clearly increased the conductivity because of its polycationic nature. For 10 wt% PCL, the conductivity rises from 0.081 mS/cm for 1% chitosan to 0.627 mS/cm for 10% chitosan. It is, however, important to notice that the conductivity is not a determining factor in the reproducible electrospinning of the polymer blend, in contrast to the viscosity. Solutions electrospinnable with a stable Taylor cone indeed showed conductivities over the full range, while others with comparable conductivities were not electrospinnable (Fig. 2b). In addition, in case of electrospinnability, the increase in conductivity with increasing chitosan concentration raised the applied voltage necessary for electrospinning the PCL/chitosan solutions (Table 2). For 10 wt% PCL, the voltage increased from 17 kV for 2% chitosan to 25 kV for 5% chitosan.

It can thus be stated that PCL/chitosan blend nanofibres can be successfully obtained using a 3/7 AA/FA solvent mixture. The polymer concentration area showing a stable Taylor cone while electrospinning proved to depend mainly on the solution's viscosity. Next, the influence of the above discussed parameters on the resulting fibre morphology is discussed.

Table 2
Electrospinnability of PCL/chitosan blend solutions. White area: fibre formation with stable Taylor cone, grey area: fibre formation without stable Taylor cone, black area: no fibre formation. Values are applied voltages for electrospinning, indicated in kV.

Chitosan concentration (%) \ PCL concentration (wt%)	4	6	8	10	12	14
35						
30						
25						
20	20	30				
15	17	25				
10	15	25	25			
5		15	20	25	27.5	
3		12.5	14	25	25	30
2			12.5	17	25	27.5
1					18	16
0						15

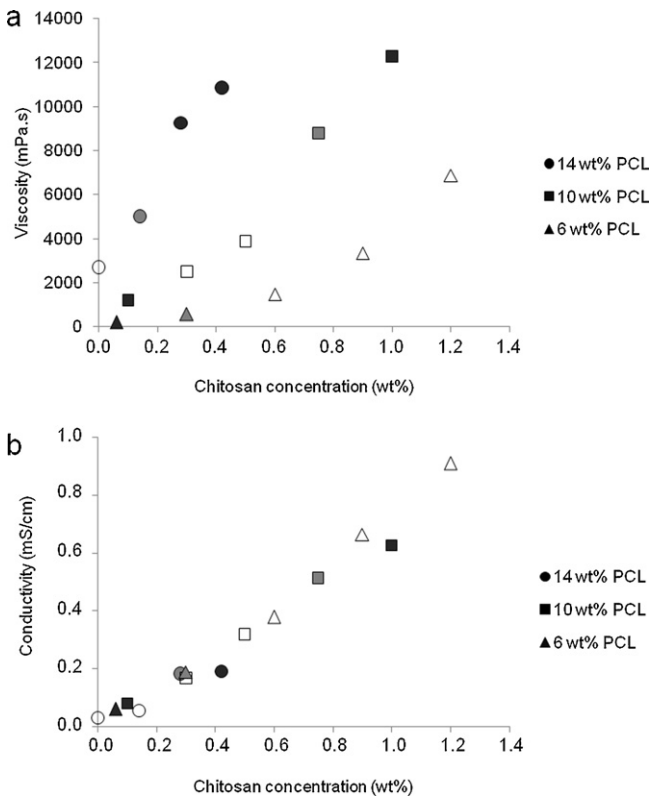

Fig. 2. Viscosity (a) and conductivity (b) as a function of the chitosan concentration for 14, 10 and 6 wt% PCL. White data points: fibre formation with stable Taylor cone, grey data points: fibre formation without stable Taylor cone, black data points: no fibre formation.

3.3. Characterisation of the PCL/chitosan nanofibres

3.3.1. Fibre morphology

The fibre morphology of the PCL/chitosan nanofibres was mainly characterised through an analysis of the average fibre diameter. Fig. 3a shows an overall significant decrease in fibre diameter with increasing chitosan percentage and decreasing PCL concentration. The fibre diameter decreases from 374 ± 110 nm at 14 wt% PCL to 112 ± 23 nm at 6 wt% PCL and 10% chitosan. This substantial decrease in fibre diameter is partially due to the polycationic nature of chitosan which results in an increased conductivity (Fig. 2a) and thus a higher charge density of the polymer jet (Jia et al., 2007). Due to the higher amount of charges carried by the jet, higher elongation forces are imposed causing thinner fibres. Moreover, the addition of chitosan enables the electrospinning of polymer solutions having a low total polymer concentration and this thanks to the strong increase in viscosity when chitosan is added (Section 3.2). The addition of chitosan to PCL thus allows for the production of finer fibres compared to pure PCL nanofibres.

However, at a constant PCL concentration, an overall diameter increase with increasing chitosan concentration is observed (Fig. 3b). At 6 wt% PCL the diameter increases from 114 ± 27 nm at 5% chitosan to 196 ± 47 nm at 20% chitosan. This increase in fibre diameter is attributed to the increase in viscosity when more chitosan was added to the solution, as discussed in Section 3.2. This augmentation in viscosity causes a higher resistance of the jet to the bending instability and a faster solidification of the polymer jet, both leading to thicker fibres (De Vrieze, Westbroek, Van Camp, & De Clerck, 2010; Jacobs, Anandjiwala, & Maaza, 2010). However, the observed trend of diameter increase with increasing chitosan concentration, at a constant PCL concentration, is less distinct compared to the generally found pronounced increase in diameter with increasing polymer concentration (Van der Schueren et al., 2011). This is again related to the polyelectrolyte behaviour of chitosan resulting in an increased conductivity. The combined effect of the increase in viscosity and the substantial rise in conductivity led to a confined diameter increase with increasing chitosan concentration at a constant PCL concentration (Fig. 3b).

The SEM images obtained from the solutions electrospinnable with a stable Taylor cone all showed a uniform, beadless nanofibrous structure (Fig. 4b). In addition, reproducibility was guaranteed as demonstrated by the constant fibre diameter when repeating the electrospinning of a 10 wt% PCL and 10% chitosan polymer solution (Fig. 5). This proves the major potential of the newly developed solvent system. Fig. 4b also clearly demonstrates the decrease in fibre diameter when adding chitosan. In addition, the image shows the presence of an ultrafine nanofibrous web formed in between the main fibres. These ultrafine fibres are not accounted for when analysing the average fibre diameters, but they generally have a diameter of approximately 30 nm. Since this ultrafine web is not present when electrospinning a solution of pure PCL in AA/FA (Fig. 4a), its formation is most likely attributed to chitosan. Adding chitosan to the polymer solution increases the charge density because of its polycationic nature, most likely leading to additional, secondary jet splitting and thus the formation of ultrafine fibres (Nirmala, Il, Navamathavan, El-Newehy, & Kim, 2011). This ultrafine web formation is in line with reported literature on the electrospinning of chitosan, confirming that the presence of chitosan encourages ultrafine web formation (De Vrieze et al., 2007; Geng, Kwon, & Jang, 2005; Nirmala et al., 2011).

3.3.2. Infrared spectroscopy

FT-infrared spectroscopy was applied to confirm the presence of chitosan in the blend nanofibres. The infrared spectrum of the PCL/chitosan nanofibres was to a large extent similar to the spectrum of the pure PCL nanofibres as demonstrated in Fig. 6.

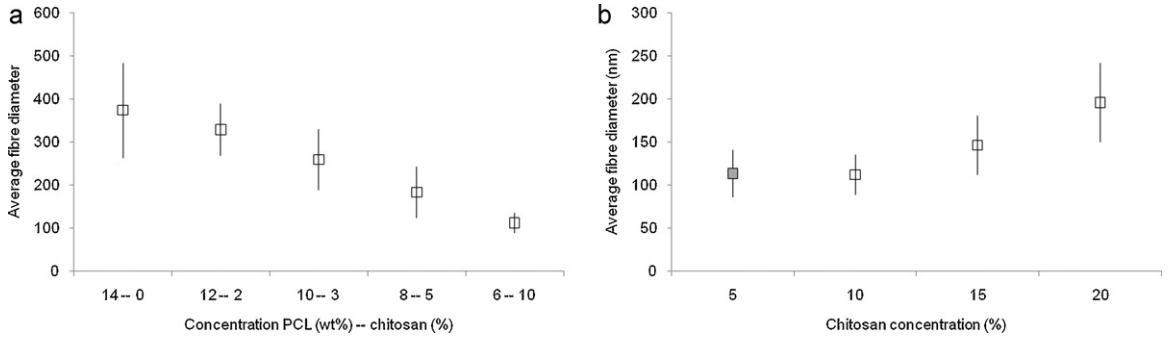

Fig. 3. Average fibre diameter of PCL/chitosan blend nanofibres (a), average fibre diameter of a 6 wt% PCL polymer solution as a function of the chitosan concentration (b). White data points: fibre formation with stable Taylor cone, grey data point: fibre formation without stable Taylor cone.

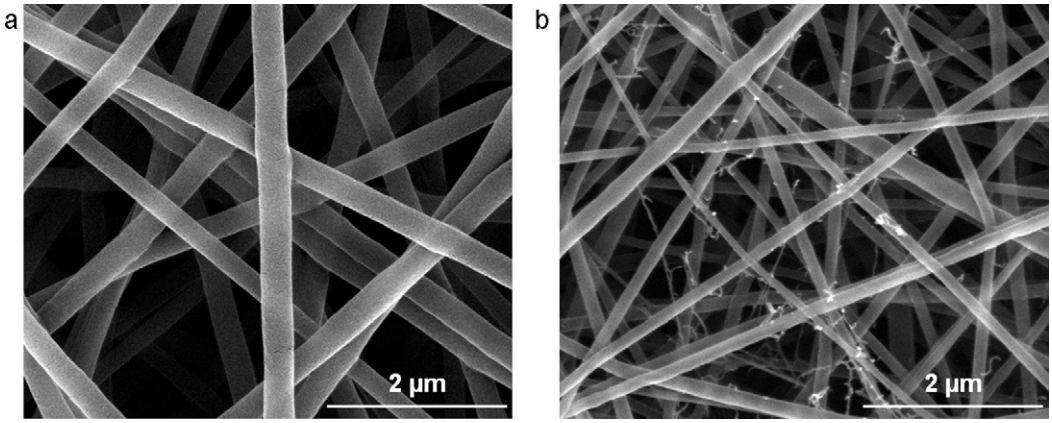

Fig. 4. SEM image of PCL (14 wt%) nanofibres, diameter 374 ± 110 nm (a) and PCL (6 wt%)/chitosan (15%) nanofibres, diameter 154 ± 41 nm (b).

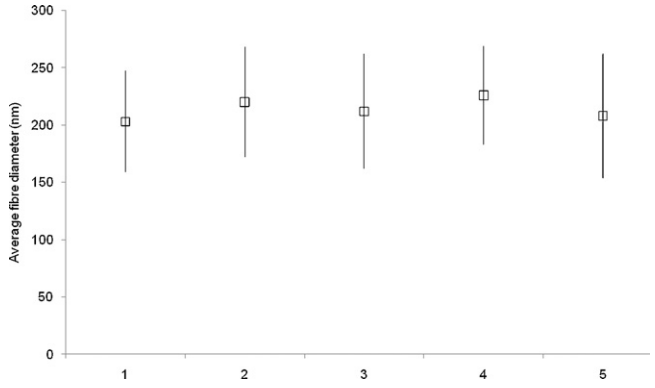

Fig. 5. Average fibre diameter of PCL (8 wt%)/chitosan (10%) nanofibres electrospun at different dates.

Yet a number of additional peaks at 3435 cm^{-1}, 1713 cm^{-1} and 1590 cm^{-1} were observed. The broad peak around 3435 cm^{-1} corresponds to OH and NH stretching of chitosan (Jayasree, Sasidharan, Koyakutty, Nair, & Menon, 2011; Singh et al., 2009) while the peak at 1590 cm^{-1} is attributed to amide II bands of chitosan (Singh et al., 2009). Both peaks thus validate the presence of chitosan in the nanofibrous structure. The peak at 1713 cm^{-1}, however, is not detected in the pure chitosan spectrum. Infrared spectra of PCL/chitosan films showed an analogous peak at 1710 cm^{-1} which was not attributable to PCL or chitosan (Senda et al., 2001). Senda et al. ascribed this peak to hydrogen bond interactions between the carbonyl groups of PCL and chitosan. The additional peak at 1713 cm^{-1} thus suggests that interactions between PCL and chitosan exist in the blend nanofibrous structure.

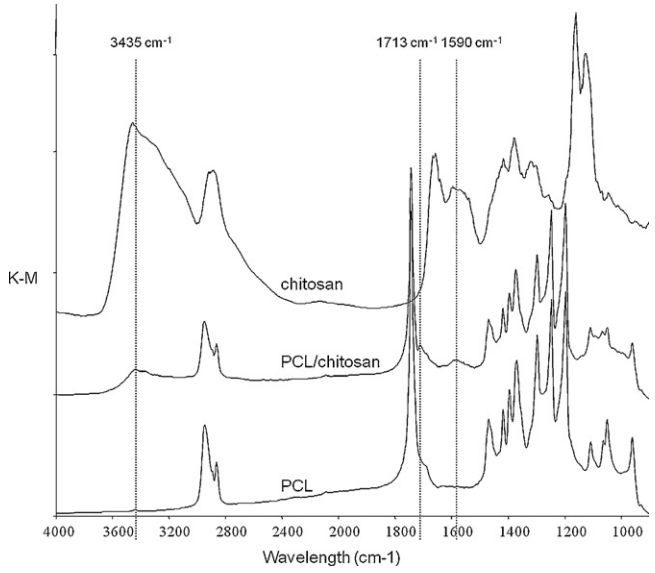

Fig. 6. FT-infrared spectra of PCL (14 wt%) nanofibres, PCL (6 wt%)/chitosan (20%) nanofibres and chitosan powder.

4. Conclusion

In this paper, PCL/chitosan nanofibres obtained via electrospinning polymer solutions containing both polymers were studied. The solvent mixture AA/FA was established as an innovative solvent system resulting in uniform and beadless blend nanofibres. Infrared spectroscopy confirmed the presence of both PCL and chitosan in the blend nanofibrous structure. The solvent ratio 3/7 AA/FA was selected for a more detailed study in order to determine the PCL and chitosan concentrations allowing for reproducible nanofibres. The viscosity of the polymer solution was found to be the main factor influencing the electrospinnability, with only a viscosity range between 1250 mPa s and 8000 mPa s leading to stable electrospinning. The addition of chitosan to PCL increased the charge density of the polymer jet due to its polycationic nature and, moreover, allowed for electrospinning with a low total polymer concentration. Both effects provided a significant diameter decrease. Furthermore, chitosan also caused secondary jet splitting, giving rise to an ultrafine nanofibrous web formed in between the main fibres. In conclusion, the newly developed solvent system AA/FA resulted in reproducible PCL/chitosan blend nanofibres with a small fibre diameter, thus leading to a major breakthrough in the electrospinning of PCL/chitosan polymer blends.

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