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Novel antimicrobial ultrathin structures of zein/chitosan blends obtained by electrospinning

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ABSTRACT

This paper describes the formulation, morphology and biocide properties of novel antimicrobial electrospun zein based ultrathin fiber structures. From the results, it was found that the electrospun fibers of zein can turn the material into a new strong antimicrobial ultrathin-structured system due to retention of remnant amounts of trifluoroacetic acid as determined by ATR–FTIR spectroscopy. Unfortunately, this system may be considered to yield very aggressive high acidic media due to release of the strong acid, which causes the antimicrobial behavior. Nevertheless, since biocide properties are more desirable at mild acidic conditions, blending zein with the natural antimicrobial chitosan (up 10 wt.%) was found to be the route of choice to yield water insoluble fiber mats, with efficient biocide properties, attributed in this case not to the acid but to the chitosan component. Differential scanning calorimetry thermograms indicated that despite the fact that both components, as suggested by scanning electron microscopy observation, are not miscible, the presence of the chitosan phase leads to slightly enhanced glass transition temperature for the zein phase. Selective removal of chitosan from the blend indicated that this component is primarily present in beaded regions of the ultrathin fiber mats.

1. Introduction

Blending polymers is known to be a very effective way to produce new multipurpose advanced materials. Over the past few years, there has been a lot of interest in the study of polymer blends; especially focused on biodegradable and sustainable materials (Kaplan, 1998). Competition between food and non-food uses of agricultural crops is currently mainly linked to biofuels and not so much to agro-based materials. Nevertheless agricultural based polymers have the added advantage of being renewable, that is, they can provide unlimited sustainable yearly sources of raw materials for the generation of novel functional biomaterials. In more recent years, much attention has also been paid to the use of high electrostatic potentials for the fabrication of nano- and ultrathin-structured fiber-based networks from different biopolymers by the electrospinning technique (Frenot & Chronakis, 2003; Huang, Zhang, Kotaki, & Ramakrishna, 2003; Li & Xia, 2004; Ramakrishna et al., 2006).

Zein, is a relatively straightforward biopolymer to electrospin (Miyoshi, Toyohara, & Minematsu, 2005; Torres-Giner, Gimenez, & Lagaron, 2008; Yao, Li, & Song, 2007a). This agro-based material has a more hydrophobic character than other proteins as a consequence of the presence of the apolar amino acids of proline and glutamine, which are the main constituents of zein. This provides the material with certain specific interesting properties in terms of water resistance, viscosity or thermal resistance. With its most significant features being excellent solubility in alcohols and biological compatibility, zein is a valuable component which is used in the manufacture of plastics, paper coatings, textiles, adhesives, substitutes for shellac, laminated board and solid color printing films. It has also been used in the pharmaceutical industry to coat capsules, allowing protection, controlled release, and masking of flavors and aromas (Shukla & Cheryan, 2001). Additionally, zein has low toxicity and has also being studied in a broad range of areas, such as the food, pharmaceutical, and biodegradable plastics industry (Corradini, Souto de Medeiros, Carvalho, Curvelo, & Mattoso, 2006). In spite of this, its use as an ultrathin-structured reinforcing element or as a blending component is still largely unexplored. There are, however, some examples in the literature of blends of this material with polysaccharides. Thus, Chanvrier (Chanvrier, Colonna, Della Valle, & Lourdin, 2005) described the properties and the phase morphology of this protein when blended with starch by melt extrusion. The study showed that the polysaccharide and the protein are not miscible systems via heat processing and lack of homogeneity and phase separation was observed throughout composition. Another work (Yao, Li, & Song, 2007b) described the electrospinning of zein/hyaluronic acid (HA) blend fibrous membranes, to which, in order to enhance compatibility between the protein and the polysaccharide, poly(vinyl pyrroli-

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done) (PVP) was introduced into aqueous ethanol solutions of the blend. From these works, it is easily observed that typical polysaccharides are not compatible with this protein whether by melt blending or solvent cast routes.

Chitosan is the second most abundant biological polysaccharide (after cellulose) derived from nature and is obtained by deacetylation of chitin present in for instance crustaceous. This material is the only pseudo-natural cationic polymer and thus, it finds many applications due to its unique properties, such as antibacterial performance under certain specific conditions (Harish Prashanth & Tharanathan, 2007). Nevertheless, fiber formation from chitosan by electrospinning is rather difficult because of its limited solubility and polycationic nature in solution. In this context, only the use of some specific solvents, which are generally based on strong organic acids, with low boiling points and low dielectric constants, are proficient in generating good fiber yielding (Ohkawa, Cha, Kim, Nishida, & Yamamoto, 2004; Torres-Giner, Ocio, & Lagaron, 2008). The concept for the electrospinning of chitosan together with a second polymer has been proposed and developed earlier and using this approach biocompatible electrospun fibrous materials were obtained (Duan, Dong, Yuan, & Yao, 2004; Park, Jeong, Yoo, & Hudson, 2004; Spasova, Manolova, Paneva, & Rashkov, 2004). Previous studies have revealed that chitosan as such is not a biocide agent and that only in gelled or viscous acid solution forms shows optimum performance, i.e. when protonated glucosamine groups are released from the solid phase into the solution (Fernandez-Saiz, Ocio, & Lagaron, 2006). The unique pseudo-natural cationic character of chitosan, provided by means of its fraction of ion pairs ($—COO^-$ $—NH_3^+$) so called "active" groups, have been widely discussed throughout the literature (Rinaudo, 2006). Recent work has also shown that the biocide performance of electrospun chitosan based systems can be increased by some minor retention of strong acid in the fibrilar nano-structure, used as solvent to facilitate the spinning of this difficult biopolymer (Torres-Giner, Ocio et al., 2008). Nevertheless, solvent entrapments or chemical evolutions in the inside of polymer nanofibers are efficiently impeded because of the fast drying process which physically occurs during electrospinning (Selling et al., 2007). As a result, solvents usually remain on the electrospun surfaces at very low levels or even traces which still make the electrospun materials made from aggressive solvents attractive depending on the application and the legislation concerns. The present model study reports on the development of novel antimicrobial materials based on ultrathin-structured zein and in blends of zein with chitosan, which have potential for application in fields such as active and bioactive packaging, in antimicrobial food coatings and in biomedical and pharmaceutical areas.

2. Experimental

2.1. Materials

Zein from corn (grade Z3625, 22–24 kDa) and chitosan of low molecular weight (grade 448869, 50–190 kDa and 75–85% deacetylation) were purchased from Sigma–Aldrich (Madrid, Spain). The ethanol of 96% v/v purity and the acetic acid glacial of 99.7% purity were purchased from Panreac (Barcelona, Spain) and trifluoroacetic acid (TFA) of 99.5% purity was from Across Organics (Geel, Belgium). All products were used as received without further purification.

2.2. Electrospinning

An electrospinning assemble equipped with a variable high voltage 0–30 kV power supply was used. Further details of the basic setup can be found elsewhere (Torres-Giner, Gimenez et al., 2008). All the electrospinning experiments were carried out at 24 °C in a controlled relative humidity chamber of 60% RH. Zein and chitosan biopolymers were first fully dissolved under magnetic stirring in ethanol and TFA at room temperature and 37 °C, respectively, and they were mixed together in an overall solvent proportion of ethanol/TFA of 2:1 (wt/wt). According to the manufacturer, TFA is completely soluble in ethanol up to concentrations of 0.33 ml/ml. The solvent mixture of ethanol/TFA 2:1 (wt/wt), was prepared in the limiting solubility concentration because it does present similar physical properties in terms of dielectric constant and boiling point (27.2 and 76.7 °C) to both aqueous ethanol solution 80 wt.% (33.4 and 76.6 °C) and TFA/DCM 70:30 (wt/wt) (29.0 and 61.1 °C), which are well-known to be most excellent solvents for electrospinning of zein (Torres-Giner, Gimenez et al., 2008) and chitosan (Torres-Giner, Ocio et al., 2008), respectively. Different dissolutions were then prepared to reach the following fractions of zein/chitosan in the final blend: 99/1, 97/3, 95/5 and 90/10 (wt/wt). A total polymer concentration of 25 wt.%, flow-rate of 0.20 ml/h, 14 kV of voltage and tip-to-collector distance of 10 cm was set as electrospinning conditions.

2.3. Attenuated total reflectance

ATR–FTIR spectra were collected at 24 °C and 40% RH coupling the ATR accessory GoldenGate of Specac Ltd. (Orpington, UK) to a Bruker (Rheinstetten, Germany) FTIR Tensor 37 equipment. The spectra were collected in the materials by averaging 10 scans at 4 cm^{-1} resolution.

2.4. Differential scanning calorimetry

DSC of typically 2 mg of the materials was performed in a Perkin-Elmer DSC 7 thermal analysis system at a scanning speed of 10 °C/min. The thermal history applied was a first heating scan from 50 to 150 °C, then cooling back to 50 °C, and subsequent second heating scan from 50 °C up to about 300 °C. Before evaluation, the thermal runs were subtracted analogous runs of an empty pan. The DSC equipment was calibrated using indium as a standard. The glass transition temperature (T_g) was determined from the second heating scan using the "half Cp extrapolated method" provided by the Pyris software routine (Perkin-Elmer, US).

2.5. Biocide tests

After electrospinning, all samples were immediately put into a dessicator at 0% RH before undertaking the antimicrobial tests to avoid losses of the biocide protonated glucosamine species as reported in previous work (Fernandez-Saiz et al., 2006; Torres-Giner, Ocio et al., 2008). Humidity exposure has been reported to deactivate the biocide capacity of chitosan by partial evaporation of acetic acid formed from the carboxylated groups present in the biopolymer after film or fiber forming from acidic solutions. The microorganism used was *Staphylococcus aureus* CECT 86 obtained from the Spanish Type Culture Collection. Antimicrobial activity tests were performed by adding the different types of electrospun mats and controls into 10 ml of Mueller Hinton Broth (MHB) of pH 6.2 containing 10^5 CFU/ml of early mid-log phase culture of *S. aureus* and incubated at 37 °C for 24 h. After the incubation period, appropriate serial dilutions were done and 0.1 ml of each MHB sample was plated on Tryptone Soy Agar (TSA) plates. Finally, after overnight incubation at 37 °C, bacterial colonies were counted. Results, as average of three replicates, were expressed as the number of colony forming units per mL.

2.6. Other techniques

The morphology of the electrospun fibers was examined using scanning electron microscopy (SEM; Hitachi S-4100) after sputtering the samples with a gold–palladium mixture in vacuum. All SEM experiences were carried out at 8.0 kV. Fiber diameters of the electrospun fibers were measured by means of the Adobe Photoshop 7.0 software from the SEM micrographs in their original magnification. The viscosity of the polymer solutions was determined by a rotation viscometer VISCO BASIC PLUS L with a Low Viscosity Adapter (LCP), both from Fungilab S.A. (Sant Feliu de Llobregat, Spain). The pH values were calculated using a multi-parameter analyzer CONSORT C380 from Biotech (Madrid, Spain). The dynamic surface tension of the polymer solution was measured by means of a SITA DynoTester tensiometer from Neurtek Instruments (Eibar, Spain). Since the device works by measuring differential bubble pressures within a bubble's lifetime, three different times were set (50,200 and 1500 ms) and the mean-value is presented. All measurements were made at room temperature.

3. Results and discussion

In order to characterize the molecular composition of the electrospun zein based systems developed, ATR–FTIR spectroscopy was applied (see Fig. 1). The ATR–FTIR spectrum of the zein fibers electrospun from TFA solutions indicates that the chemical structure of the biopolymer seems different compared to its molecular structure when obtained from aqueous ethanol solutions. Thus, as major changes, there is a new group of bands in the range between 1000 and 1200 cm^{-1}, which are assigned to the association of trifluoroacetate ions ($C_2F_3O_2^-$) to certain biopolymer chemical groups such as the amine groups to form the salt chitosonium trifluoroacetate (Torres-Giner, Ocio et al., 2008). This assignment arises from comparison of the spectra of both zein and zein-TFA, with these of TFA and ethanol–FTA solvents. It is also observed that the fibers of zein, chitosan and zein–chitosan blends can retain during its formation and storage some free TFA. The retention of some free TFA in the fibers was purposely sought to increase the antimicrobial activity and it can be detected by the presence of the free TFA band at 1775 cm^{-1} assigned to the C=O stretching. The presence of free acid in the fibers is somewhat smaller in neat zein than in neat chitosan indicating that the carbohydrate is more chemically favored to retain TFA. Introducing chitosan in the zein structure produces a progressive increase in intensity of the band envelop between 1000 and 1200 cm^{-1}, which can be attributed to the presence of chitosan in the blend.

The increasing presence with increasing chitosan content in the bioblend of a band at 1744 cm^{-1}, possibly attributed to a C=O chemistry, which is not seen in the spectrum of chitosan and has a very weak presence in the both spectra of zein, could be assigned to specific interactions between the two biopolymers or to specific confinement of the solvent in the characteristic electrospun zein fiber morphology. Although, the precise assignment of the band is uncertain, it could have been produced by the appearance of new carboxylic acids and esters or more likely due to side interactions between the chemistry of both components resulting in alterations in certain bands of the spectrum. It should also be borne in mind that even though the compatibility between the biopolymers is not anticipated as previously reported for similar systems, blending from a common solvent followed by electrospinning could bring additional interactions between proteins and carbohydrates. This speculation is further supported by observation of small T_g shifts by DSC (see later).

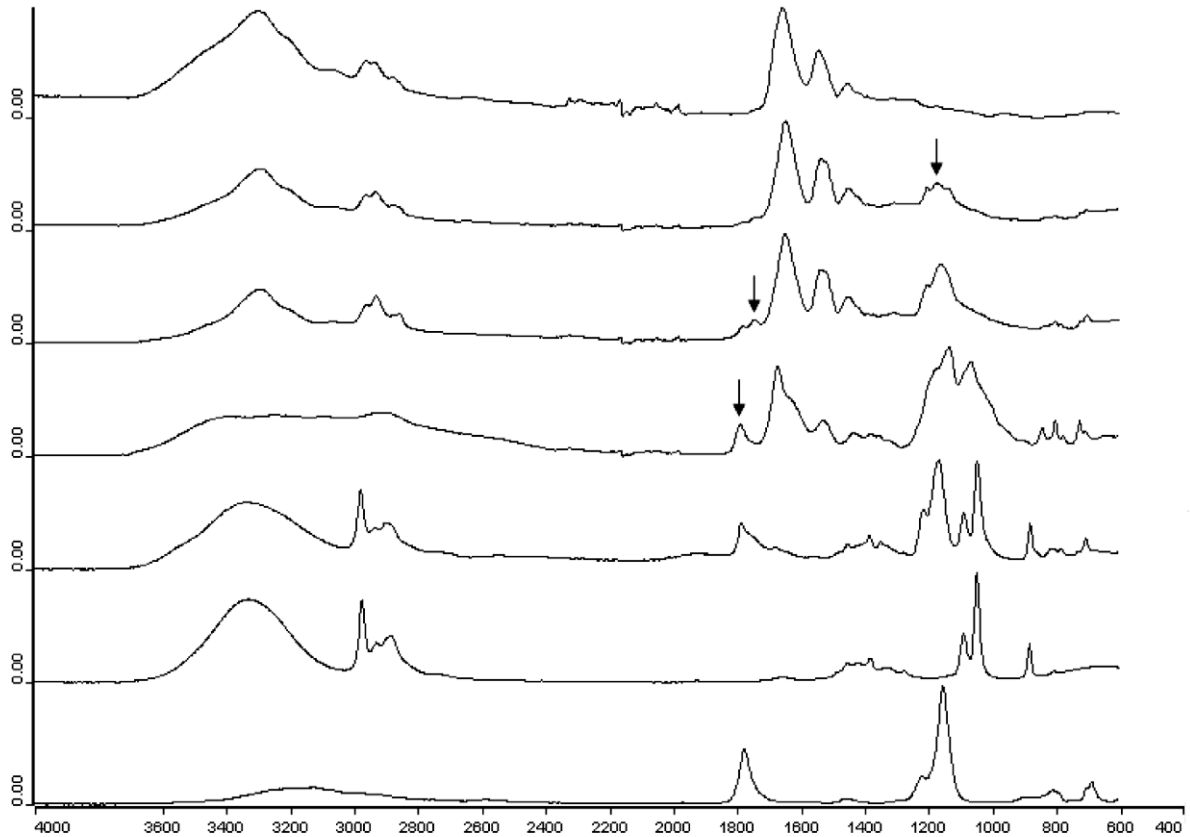

Fig. 1. ATR–FTIR typical spectra of, from top to bottom, zein fibers obtained from 80 wt.% ethanol aqueous solution, zein fibers obtained from ethanol–TFA solution, zein–chitosan 90:10 (wt/wt) fibers obtained from ethanol–TFA solution, chitosan fibers obtained from TFA solution, ethanol–TFA 70:30 (wt/wt) solvent, ethanol solvent and TFA solvent. The arrows indicate relevant bands discussed in the text.

Fig. 2 shows some typical SEM images of the electrospun structures of the various bioblends obtained. Only the bioblends containing below 5 wt.% of chitosan in the formulation generated clear continuous ultrathin fiber morphologies. Pure zein ultrathin fibers, originated from electrospinning of TFA solutions, presented similar ribbon-like fiber morphologies (with average cross-sections of 320.9 ± 92.3 nm) as those reported earlier for the same system electrospun from alcohol solutions (Torres-Giner, Gimenez et al., 2008). When small fractions of chitosan, i.e. 1 wt.% were blended with zein, similar ribbon-like fiber morphologies, but with smaller average diameter (192.3 ± 54.4 nm), were preferentially deposited on the collector. As the ratio of chitosan increased in the bioblend, see 3 and 5 wt.% chitosan loading blends, the shape of the fibers became even smaller (with main cross-sections of 161.7 ± 39.6 and 128.5 ± 26.2 nm, respectively) but also exhibited increasing presence of beaded regions (with mean diameters of 315.6 ± 76.4 and 420.4 ± 86.7 nm, respectively). When the chitosan content was the highest (see the 10 wt.% chitosan content bioblend), the beaded morphology became dominant (with average mean diameter of 598.2 ± 153.5 nm) but these beaded structures were seen to be interconnected by very ultrathin nanofibers (with mean cross-section of 36.1 ± 12.1 nm). Higher contents of chitosan in the blend were trial but were not experimentally feasible due to extensive electrospraying phenomena, solute precipitation and excessive viscosity. The fibers mats presented a smooth surface and higher magnification of the material did not provide evidence of a phase separated morphology. Nevertheless and since the two materials are not expected to be miscible from earlier work (Chanvrier et al., 2005; Yao et al., 2007b), selective dissolution of the chitosan fraction was carried out by washing the fiber mats with dilute aqueous acid solutions of acetic acid (1 wt.%), solvent which earlier tests proved to selectively dissolve only chitosan. Zein and zein fibers were not found to be unambiguously dissolved by the water solution used (Osborne, 1897). Thus, filtration and subsequent drying after dispersion in the solvent system yielded similar solid contents within 1 wt.%. After the washing the fibers, the solvent was allowed to evaporate at room temperature and the samples were immediately observed by SEM (see Fig. 3). This image indicates that a homogenization of the fibrilar morphology and a reduction of the beaded regions is observed, suggesting that, on the one hand, the fibers are water resistant which give them added bonus on moisture contact applications and justifies the selection of zein as matrix, and on the other hand, implies that chitosan seems to be preferentially placed in the beaded regions and hence causes bead formation due to its particular characteristics such as higher surface tension. The washing acid treatment was also found to lead to somewhat thicker or swollen structures, due to probably moisture soaking and solvent-induced fiber coalescence.

As more chitosan was introduced in the blend formulation, the solutions of zein became increasingly more viscous and with higher surface tension (see Table 1). Among other factors, this is thought to be mainly caused by the expected increase in the overall polymer molecular weight (Torres-Giner, Ocio et al., 2008), i.e. the molar mass of the low molecular weight (LMW) chitosan used was reported to vary between 50 and 190 kDa but it was only of 22–24 kDa for zein. Moreover, chitosan concentrated solutions have been measured to lead to high surface tension solutions (78.7 mN/m) due to the biopolymer particular chemistry (Torres-Giner, Ocio et al., 2008). Introducing chitosan, which is known from previous works to be a difficult system to electrospin due to the high viscosity and high surface tension of its solutions, produced in the bioblend a monotonical increase in the viscosity of the polymer solution and a slight increase in surface tension. The formation of beads and beaded fibers is most likely driven by the higher viscosity and surface tension of the bioblend solutions (Fong, Chun, & Reneker, 1999; Yang et al., 2004). Moreover, since it is well-known that adequate fibrous electrospun structures can only be stabilized

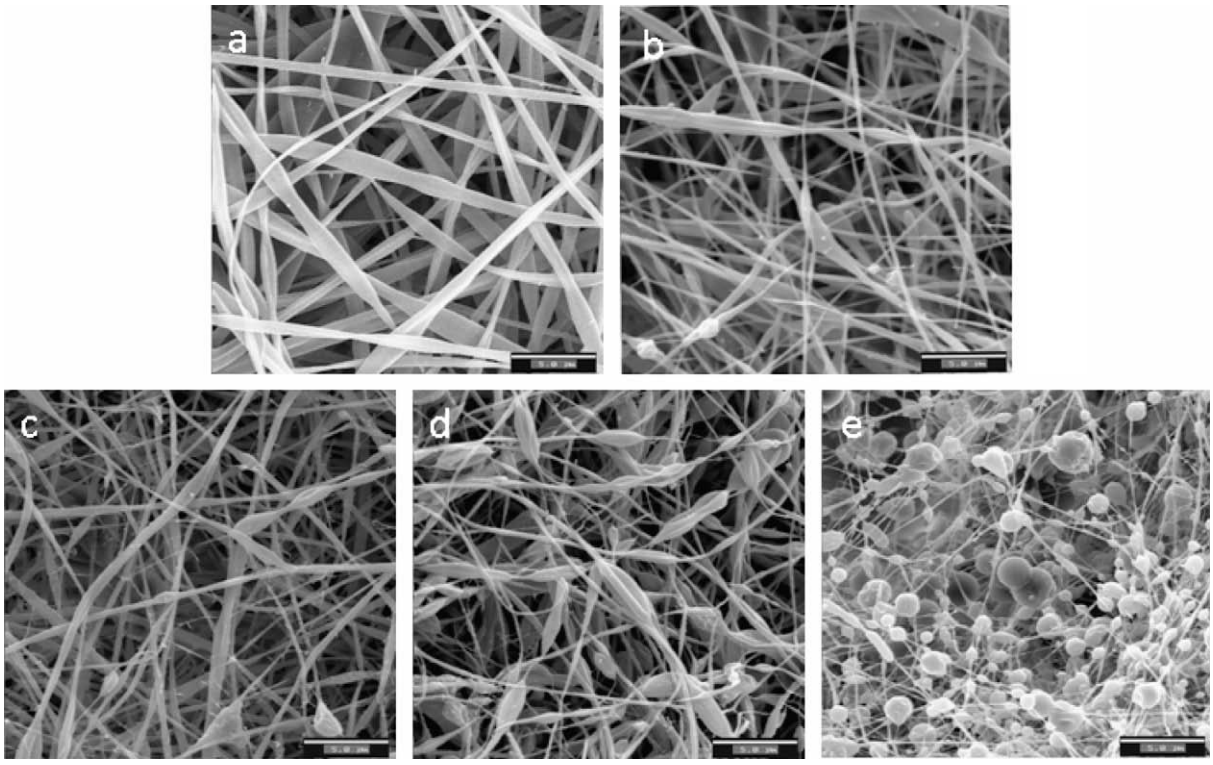

Fig. 2. Typical SEM photographs of electrospun fiber mats obtained from ethanol–TFA solutions of (a) pure zein, (b) 99/1 wt.% zein–chitosan bioblend, (c) 97/3 wt.% zein–chitosan bioblend, (d) 95/5 wt.% zein–chitosan and (e) 90/10 wt.% zein–chitosan. The magnification used was 5k× and scale markers are of 5 μm.

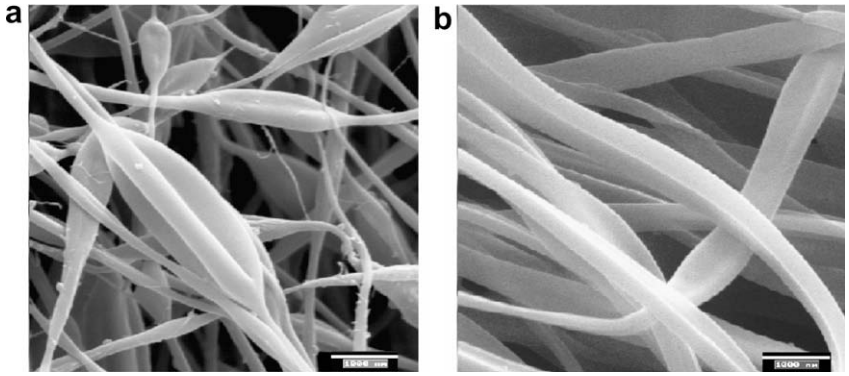

Fig. 3. Typical SEM photographs of electrospun fiber mats obtained from ethanol–TFA solutions of (a) 95/5 wt.% zein–chitosan bioblend and (b) 95/5 wt.% zein–chitosan bioblend washed with aqueous acetic acid solution (1 wt.%). The magnification used was 20k× and scale markers are of 1000 nm.

Table 1
Physical properties of the polymer solutions in ethanol–TFA 2:1 (wt/wt) solvent mixture.

Description	Surface tension (mN/m)	Viscosity (cP)
Pure zein	44.8 ± 5.2	118.5 ± 4.3
Zein/chitosan 99:1	46.9 ± 5.6	245.9 ± 5.5
Zein/chitosan 97:3	53.1 ± 5.9	286.7 ± 6.7
Zein/chitosan 95:5	54.5 ± 6.3	353.8 ± 7.4
Zein/chitosan 90:10	63.1 ± 7.8	475.1 ± 9.1

above a critical concentration and below a critical process viscosity (Fong et al., 1999; Zong et al., 2002), when the viscosity exceeded an upper-limit value (measured around 400 cP) extensive beaded structures were observed in the bioblends reported here.

In terms of thermal properties, although the fibers produced in the current study using TFA presented slightly lower T_g *(157.2 ± 0.6* °C) than zein fibers previously reported using either pure ethanol (158.9 ± 2.1 °C) or ethanol combined with acetic acid (163.3 ± 2.3 °C) solvents (Torres-Giner, Gimenez et al., 2008), the introduction of chitosan in the bioblend formulation slightly but gradually increased the thermal resistance of the zein component, i.e. the T_g increased up to 163.4 ± 1.9 °C for the 10 wt.% chitosan content bioblend. This is likely the result of a stiffening-induced phenomenon caused by intimate contact in the formulation of chitosan fractions with a potentially higher T_g value. In our DSC data, summarized in Table 2, there was no unambiguous indication for a T_g occurrence before the degradation onset, and, although, the determination of the T_g of chitosan is a matter of on-going debate (Neto et al., 2005), some authors have reported T_g values for chitosan of 203 °C (Sakurai, Maegawa, & Takahashi, 2000). Small T_g shifts in blends often indicate interfacial interaction or compatibility between the matrix and the segregated phase component, possibly in this case as a result of electrospinning from a common solution. This interaction could be further substantiated by the observed presence of a new band rising in the FTIR spectra in the blends.

Finally, the biocide properties of the electrospun ultrathin-structured systems were assessed. Table 3 gathers the results of the antimicrobial activity tests carried out against *S. aureus* on the various samples. The microbial solutions generated turbidity to some extent when the fibers were put in contact with the solution, fact that usually implies from previous studies partial dissolution of biocide chitosan protonated glucosamine fractions (Lagaron, Fernandez-Saiz, & Ocio, 2007). Acidity of the bacterial solutions after contact with the fibers seemed to be a very influencing factor controlling bacterial growth (Torres-Giner, Ocio et al., 2008). Thus, controls based on the addition of TFA and having lower acidity (pH < 4) always resulted in bacterial death, while controls with higher acidity (pH > 5) did not have any remarkable effect. In accordance with this, test samples containing 200 mg of fiber mats of zein and of zein/chitosan 95/5 (wt/wt) blend and yielding acid solutions with pH values below 4 resulted in *S. aureus* completed death within 24 h of contact. A 200 mg sample of zein fiber mats obtained by electrospinning from aqueous ethanol solutions, as reported before (Torres-Giner, Gimenez et al., 2008), were also used as control and showed not antimicrobial effect, suggesting that zein itself is not a natural antimicrobial material. Antimicrobial activity is mainly so as a result of the release of trapped TFA from the materials, which is a strong carboxylic acid containing three very electronegative fluorine atoms. Relative to for instance acetic acid, TFA can be 10^5-fold a stronger acid (Eidman & Nichols, 2004). When the biopolymers carrying free TFA (see FTIR spectra in Fig. 1) are put in contact with the bacterial solution, they release acid groups to the solution resulting in a pH drop and in high antimicrobial performance. Nevertheless, at mild acidic conditions provided by using lower amounts of fibers in the tests, the antibacterial action of electrospun fibers of neat chitosan or of zein containing chitosan were found to be much stronger and efficient as biocides than the neat zein fibers. A more important observation is that control TFA solutions titrated to the same pH as for instance neat chitosan fibers were not antimicrobial, hence ruling out the

Table 2
T_g values of zein and zein/chitosan fibers according to their composition.

Description	T_g (°C)
Pure zein	157.2 ± 0.6
Zein/chitosan 99:1	157.8 ± 1.2
Zein/chitosan 97:3	158.5 ± 0.9
Zein/chitosan 95:5	160.9 ± 0.4
Zein/chitosan 90:10	163.4 ± 1.9

Table 3
Quantity of materials used in the biocide tests, resulting bacterial counts after the tests and pH of the materials after immersion in the bacterial solutions. The antimicrobial tests were carried out against *S. aureus* at 37 °C during 24 h.

Description	Quantity (mg)	log (CFU/ml)	pH
Control	–	8.88 ± 0.05	6.3 ± 0.2
Mild TFA solution (control)	–	8.61 ± 0.04	5.4 ± 0.1
Acid TFA solution (control)	–	<1[a]	4.3 ± 0.1
Pure chitosan fibers	25	<1[a]	5.4 ± 0.3
Pure zein fibers	100	7.62 ± 0.04	4.7 ± 0.1
Zein/chitosan 95/5 fibers	100	3.60 ± 0.02	4.6 ± 0.1
Pure zein fibers	200	<1[a]	3.7 ± 0.1
Zein/chitosan 95/5 fibers	200	<1[a]	3.7 ± 0.1
Pure zein fibers from ethanol	200	8.77 ± 0.05	6.0 ± 0.2

[a] No growth was observed.

influence of acidity on the antibacterial performance of the fibers at mild acidic conditions. This is due to the very effective biocide role of the release of protonated glucosamine fractions of chitosan to the bacterial medium from the fibers. It has been recently known that there is a correlation between the antimicrobial character of the chitosan biopolymer and the presence of the carboxylate groups in the polymer structure, which can be easily determined by ATR–FTIR spectroscopy (Fernandez-Saiz et al., 2006). In this rational, the biocide effect could then involve mechanisms such as the penetration of LMW glucosamine fractions in the bacteria cell, binding to deoxyribonucleic acid (DNA), and resulting in subsequent inhibition of ribonucleic acid (RNA) and protein synthesis by the bacteria (Hadwiger, Kendra, Fristensky, & Wagoner, 1985) or the formation of an impervious layer of precipitated chitosan over the cell wall leading to bacterial death (Qin et al., 2006).

4. Conclusion

In this study, it was possible to prepare for the first time bioblends of zein/chitosan fibers by electrospinning. This system produced fibrous materials with different morphologies depending on composition and having diameters in the submicron range. From the results, it was observed that all blends had antimicrobial performance and that this was related in the case of the neat zein to the release of entrapped acid. However, more importantly is the observation that for the case of the bioblends, it was seen that relatively low amounts of chitosan were able to provide an efficient biocide effect which was not related to the TFA acidity but to the natural antimicrobial effect of the biopolymer. Controlled release of protonated glucosamine groups and of entrapped TFA can thus be used to provide antimicrobial properties to zein ultrathin fiber-based structures provided the positive migration of these two systems is allowed from a legislation viewpoint in the selected applications. It is evident that TFA is a very aggressive acid that has been used here as a model system to yield optimum fiber morphology, but it is also known that the electrospinning technique by selecting the right polymer/solvent systems, by controlling the process parameters and/or the post-treatment procedure (such as drying temperature, vacuum, etc.) can lead to a certain control over the remnant solvent in order to tailor its strength as biocide in real applications. Electrospun fibers of these biomaterials could therefore be of great interest in various applications such as active food packaging (whenever the appropriate legislation approves the uses of both chitosan and TFA as food contact materials with specific migration limits) and in pharmaceutical and biomedical applications in for instance tissue engineering, medical implants and body-implant interphases.

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