International Journal of Pharmaceutics 490 (2015) 32–38

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Polymorphic solidification of Linezolid confined in electrospun PCL fibers for controlled release in topical applications

Loredana Tammaro [a,*], Carmela Saturnino [b], Sharon D'Aniello [b], Giovanni Vigliotta [c], Vittoria Vittoria [a]

[a] Department of Industrial Engineering, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, SA, Italy
[b] Department of Pharmacy and Biomedical Science, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, SA, Italy
[c] Department of Chemistry and Biology, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, SA, Italy

ARTICLE INFO

Article history:
Received 20 August 2014
Received in revised form 19 February 2015
Accepted 24 April 2015
Available online 28 April 2015

Keywords:
Electrospinning
Poly(ε-caprolactone)
Linezolid
Nanofibers
Antimicrobial Activity

ABSTRACT

Poly(ε-caprolactone) (PCL) membranes loaded with Linezolid, chemically *N*-[[(5S)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-*oxo*-5-oxazolidinyl]methyl]acetamide (empirical formula $C_{16}H_{20}FN_3O_4$) have been prepared by electrospinning technique, at different Linezolid concentrations (0.5, 1, 2.5 and 5%, w/w). Structural characterization, morphological analysis and the study of the mechanical properties have been performed on loaded membranes and compared with neat PCL membranes. Linezolid embedded in the membranes is prevalently amorphous, with a low crystallinity showing a different polymorphic form respect to the usual Form I and Form II. The release kinetics of the drug were studied by spectrophotometric analysis (UV–vis). It allowed to discriminate between Linezolid molecules on the surface and encapsulated into the fibers. The antibacterial activity of the electrospun membranes was effective to inhibit *Staphylococcus aureus*. The properties of the loaded membranes and their capability for local delivery of the antibiotic make them good candidates as drug release devices for topical use.

1. Introduction

In recent years infections have become an increasingly important health problem not only for their high diffusion but also for the characteristics of certain pathogens, such as bacteria that have developed resistance to common antibiotics. The reason of this resistance is prevalently due both to the mutation by microorganisms related to the reckless use of antibiotics, and to the increase in the number of immune-compromised individuals. Many researchers are therefore involved in finding new drugs as well as novel routes of administration.

Linezolid is a drug indicated both in lung infections and in complicated skin and soft tissue infections by Gram-positive bacteria (*S. aureus*) (Ballow et al., 2002; Birmingham et al., 2003; Corti et al., 2000). It belongs to oxazolidinones, a class of synthetic antimicrobials that inhibit different strains of multi-resistant organisms presenting a bacteriostatic activity; at certain doses and in connection with certain strains it can become bactericidal. Currently, there are only pharmaceutical oral, in form of tablets, and intravenous formulations of this drug. However the bioavailability of a drug administered orally is low and therefore, especially in the case of infections of the skin, a topical formulation is more appropriate and a local release, lasting for long times, would be desirable.

Moreover in recent years great attention has been paid to the polymorphic behavior of Linezolid. Polymorphism is the ability of a substance to crystallize in different crystal modifications, each of them having the same chemical structure but different arrangements or conformations of the molecules in the crystal lattice. Actually different polymorphs exhibit different physico-chemical properties such as solubility, dissolution rate, bioavailability, and chemical and physical stabilities. Polymorphism therefore has become a topic of great interest in the pharmaceutical industry as it has the potential to significantly affect the properties of both in the active pharmaceutical ingredient (API) and the final dosage form (Hilfiker, 2006).

Many APIs appear in more than one crystalline structure, either because of a different arrangement of molecules in the lattice (packing polymorphism) or because of a different conformation of the molecules in the lattice (conformational polymorphism). Additionally, a precise knowledge of the stability of the crystal forms and their relationship is very critical for API development and formulation in order to avoid undesired surprises in the late stages of development (Brittain, 2002). The discovery of new

* Corresponding author. Tel.: +39 089 964019.
E-mail addresses: ltammaro@unisa.it, loredana.tammaro@enea.it (L. Tammaro).

http://dx.doi.org/10.1016/j.ijpharm.2015.04.070
0378-5173/

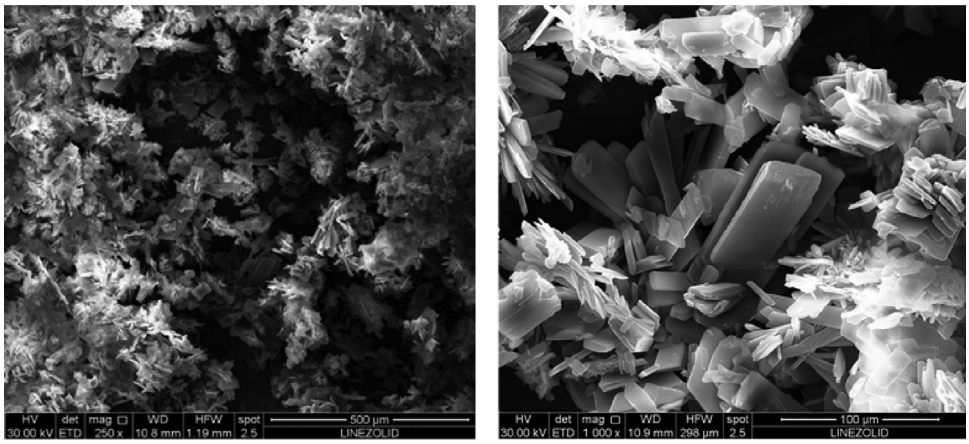

Fig. 1. SEM micrographs of pristine Linezolid powder.

polymorphic forms of a pharmaceutically useful compound provides a new opportunity to improve the performance characteristics of a pharmaceutical product. It enlarges the possibilities to obtain a targeted release profile or other characteristics (Karagiannidou, 2013).

Various solid state forms of Linezolid have been disclosed in the literature: crystalline Form I (Barbachy et al., 1997; Bergen et al., 2002; Bricker et al., 1996; Pearlman, 1999; Pearlman et al., 1998), Form II (Bergen, 2001, 2002, 2003), Form III (Dodda and Pingili, 2005; Sathe et al., 2009), Form IV (Aronhime et al., 2006a), Forms TIII, V, VI, IX, X, XII, XIV, XVII, XVIII (Aronhime et al., 2006b), amorphous form (Aronhime et al., 2006b), hydrated forms (Allegrini et al., 2009; Dodda and Pingili, 2007) and co-crystals (Devarakonda et al., 2009) and in some of them characterization by X-ray powder diffraction and NMR spectroscopy has been reported (Maccaroni et al., 2008; Tanaka and Hirayama, 2008).

Their performance and pharmaceutical profile is currently under study.

The topical preparation of Linezolid, as well as the study of the obtained polymorph, could represent a new route of administration with an excellent bioavailability. The problem to be taken into account in the local drug release is the type of delivery vehicle. The use of biomaterials – both natural and synthetic – is enabling to develop scaffolds specifically designed for many medical applications (Boccaccini and Blaker, 2005; Liu and Ma, 2004; Tsang and Bhatia, 2004; Yang et al., 2001).

The production methods for preparing such devices are wide ranging, and each method has its unique characteristics. The fabrication of reproducible and biocompatible three-dimensional scaffolds resulting in bio-matrix composites useful for different applications, as medical prostheses, drug delivery and tissue template is of particular interest in tissue engineering. In each specific application the ability to shape materials on different length scales, including the nanoscale, is of the utmost importance.

Recently, electrospinning able to produce non-woven membranes of micro and nano-fibers characterized by a high surface to volume ratio, has been demonstrated as a successful means of producing scaffolds having many of the desirable and controllable properties (Cui et al., 2010; Doshi and Reneker, 1995; Teo and Ramakrishna, 2006).

It is applicable to a wide variety of polymers and composite polymers, both natural and synthetic. By this technique polymer micro and nano-fibers are produced from an electrostatically driven jet of polymer solution or melt. The discharged polymer solution jet undergoes a whipping process wherein the solvent evaporates and the highly stretched polymer fiber deposits on a grounded target. A number of experimental parameters control the fiber diameter and morphology.

The present study aims to develop biodegradable membranes, containing Linezolid, able to provide a sustained release of the antibiotic drug. Biodegradable poly(ε-caprolactone) (PCL) membranes loaded with the Linezolid drug were prepared using electrospinning technique. PCL is a non-toxic and tissue-compatible material and can be eventually resorbed in the vital organs (Jiang et al., 2001; Woodruff and Hutmacher, 2010).

Polycaprolactone degrades at a slower rate than other biodegradable polymers, such as polylactide (PLA), polyglycolide (PGA), and their copolymers, and can therefore be used in drug delivery devices, remaining active for long time.

2. Materials and methods

2.1. Materials

The polymer used for the fabrication of biodegradable membranes was the poly(ε-caprolactone) (PCL), with a molecular weight (Mn) of 80,000 Da (Sigma–Aldrich, Italy). Acetone solvent was used as received (Sigma–Aldrich, Italy).

The drug Linezolid (LIN) was extracted from the pharmaceutical forms. The tablet was crushed in a mortar and the obtained powders dispersed in 150 mL of H_2O and 250 mL of ethyl acetate, and kept under stirring for about 1 h at room temperature. Then the two phases were separated by a separating funnel. The organic phase was dried over anhydrous $MgSO_4$ and then concentrated to dryness. A white solid was obtained that turns out to be the active principle. The drug's purity was confirmed by 1H NMR.

2.2. Electrospinning procedure

The spinning solution PCL/drug was prepared by first dissolving 14% (w/w) PCL in acetone and then adding the drug under stirring for 1 h until complete dissolution. A series of PCL/drug blend solutions (PCL/Linezolid = 100/0, 99.5/0.5, 99/1, 97.5/2.5, 95/5, w/w) were prepared by mixing each solution at predetermined ratios.

The spinning mixed solutions were placed into a 5 mL plastic syringe. An electrode lead of a high voltage power supply (HV Power Supply, Gamma High Voltage Research, Ormond, FL) was connected to the needle tip (internal diameter 0.84 mm) of the syringe. A constant positive DC voltage potential was fixed at 25 kV. For collecting of the fibrous mats, aluminum plates of $10 \times 10\ mm^2$ were placed on a grounded aluminum collector. The

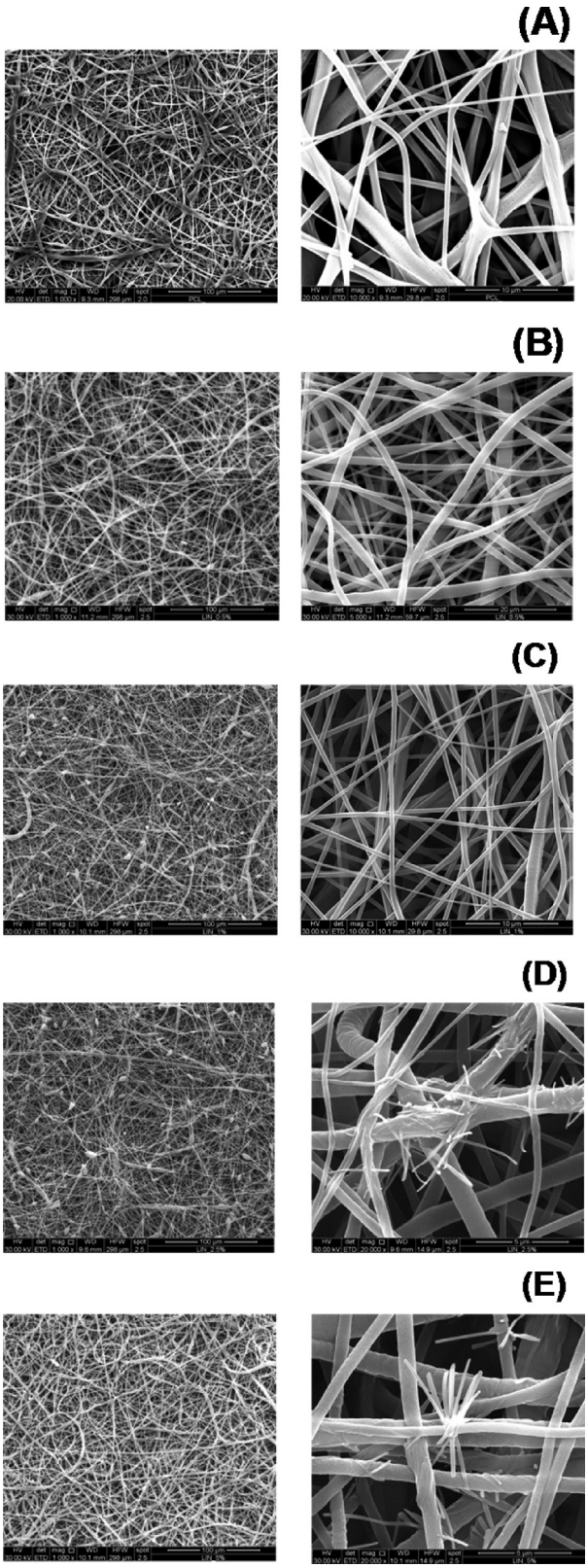

Fig. 2. SEM micrographs of: PCL (A), PCL–LIN0.5 (B), PCL–LIN1 (C), PCL–LIN2.5 (D), PCL–LIN5 (E) fibers.

distance between the needle tip and the collector was 30 cm. A syringe pump (NE-1000 Programmable Single Syringe Pump, New Era Pump Systems Inc., NY) was used to feed the needle with polymer solution at volumetric flow rate of 4 mL/h. The solutions were subjected to electrospinning in a closed chamber. Temperature and relative humidity (RH) inside the chamber were controlled during electrospinning. The collected fibrous membranes were placed in a vacuum oven overnight to fully eliminate solvent residuals, if any. In the following, fibers loaded with Linezolid will be coded as PCL–LIN*x* where *x* is the amount of LIN present in the samples.

Table 1
Fiber diameters extracted from diameter distribution measurements.

Sample	Fiber diameter (nm)
PCL	700 ± 50
PCL–LIN0.5	800 ± 50
PCL–LIN1	450 ± 50
PCL–LIN2.5	500 ± 50
PCL–LIN5	700 ± 50

2.3. Characterization of electrospun fibers

Structural characterization was conducted by X-ray diffraction (XRD) measurements using a Brucker diffractometer (equipped with a continuous scan attachment and a proportional counter) with Ni-filtered Cu Kα radiation (λ = 1.54050 Å), at 40 kV and 40 mA.

The morphology of the electrospun fibers was observed using a scanning electron microscope (SEM; JEOL JSM-T300) after gold coating. The average diameter and the diameter distribution were obtained by analyzing SEM images using a commercial image analysis program (SigmaScan Pro 5).

Mechanical properties of the fibers were measured using an Instron dynamometer (Model 4301), with a load cell of 0.1 kN at room temperature. Rectangular shaped samples with a dimension 20×10 mm were prepared and subjected to the analysis. A crosshead speed of 10 mm/min was used for all the specimens.

2.4. Drug release test in vitro

The presence of drug released in solution was evaluated by ultraviolet spectroscopy (UV–vis) (Model UV-2401, SHIMADZU) measuring the absorbance at 250 nm. The tests were performed using rectangular specimen of 16 cm^2 and same thickness (90 μm), placed into 25 mL of physiological saline solution, stirred at room temperature and 100 rpm in an orbital shaker (VDRL MOD. 711+, AsalS.r.l.). The release medium was withdrawn at fixed time intervals and replenished with fresh medium.

2.5. Antibacterial activity

Microbiological assays for antibiotic diffusion were performed as already reported (Bonev et al., 2008). *Staphylococcus aureus* was preincubated overnight in LB medium (10 g L^{-1} trypton, 5 g L^{-1} yeast extract, 10 g L^{-1} NaCl) at 37 °C. Successively, 5 mL of autoclaved nutrient agar precooled to 42 °C were inoculated with 0.1 OD_{600} of bacterial suspension and poured in 90 mm diameter Petri dishes. Pieces of membrane of $\sim 1.0 \times 1.2$ cm^2 loaded with 2.5 and 5% Linezolid were laid down on the agar surface, avoiding the formation of air bubbles, and plates were incubated at 37 °C for 24 h. As controls, 3 μL of Linezolid solution (w/v in DMSO) at increasing concentration (3.12, 6.25, 12.5 μg mL^{-1}) were applied on the surface of inoculated agar-medium.

To determine the minimal inhibitory concentration (MIC) and the relative minimum weight releasing the MIC (rMWM) an overnight actively growing culture in LB of *S. aureus* was diluted in fresh medium (LB) at a density of 5.0×10^3 colony forming unity

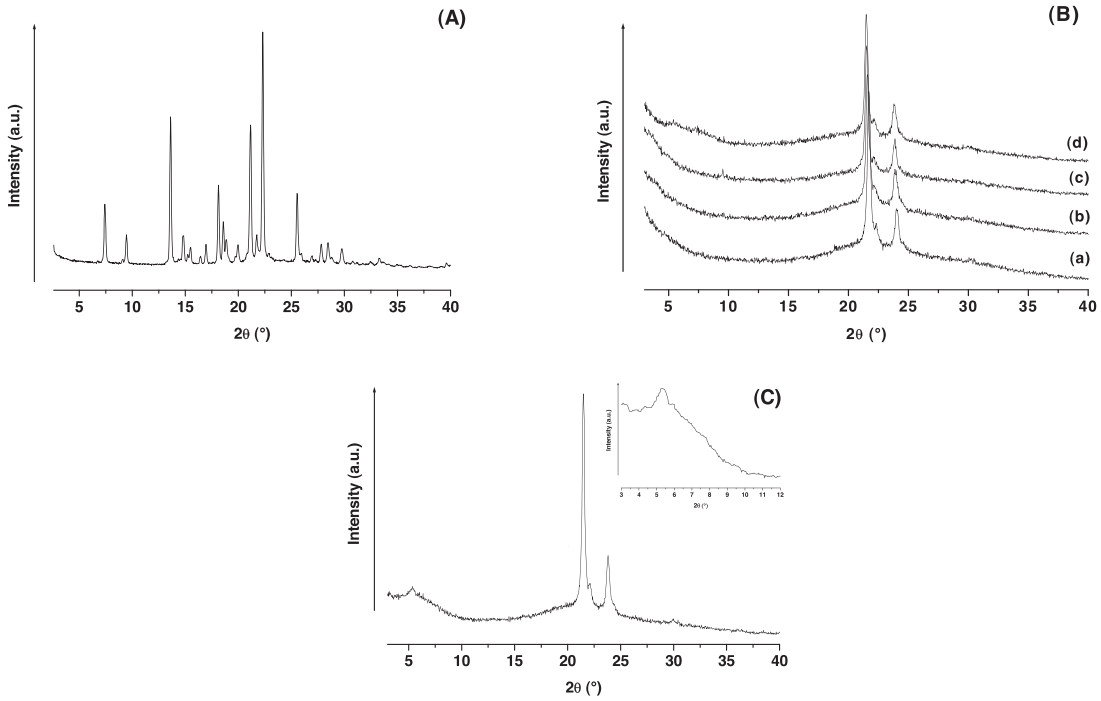

Fig. 3. XRD diffractograms of: pure LIN (A); PCL (a), PCL–LIN0.5 (b), PCL–LIN1 (c) and PCL–LIN2.5 (d) (B); PCL–LIN5 (C).

(CFU) mL^{-1} and incubated respectively, at presence of increasing concentration of Linezolid (0.25, 0.5, 0.75, 1.0, 2.0, and 4.0 $\mu g\,mL^{-1}$) or at increasing weight of PCL-LIN5 (12.5, 25.0, 37.5, 75.0, 150, 250 $\mu g\,mL^{-1}$), 12 h at 37 °C, under constant agitation at 250 rpm. MIC and rMWM were evaluated quantifying bacterial growth by spectrophotometric reading at a wavelength of 600 nm. A sample of bacterial cells grown in LB medium without the addition of any compound was used for the determination of the extent of inhibition. Each experiment was performed in triplicate. Linezolid used for the tests was at the same degree of purity of that present in the membranes. *S. aureus* strain was derived from the collection deposited in the microbiology laboratory directed by G. Vigliotta.

3. Results and discussion

3.1. Morphology

The fabrication of fibrous membranes of PCL containing the Linezolid molecules, aimed to a local controlled release of the drug, is a highly complex procedure. As a matter of the fact the LIN molecules can be incorporated both into the fibers and on the external surface, determining very different kinetics of release. Moreover in the case of incorporation both inside and outside the fibers, the release, in first approximation, will depend on the ratio between internal and external fraction of LIN into the membrane respect to the fibrous structure. Therefore to compare the effect of different LIN concentrations on the local drug delivery, a carefully controlled morphology has to be obtained, and indirect measurement have to be put into action to quantify these fractions. Moreover LIN can crystallize in different polymorphs or in an amorphous form and each structural organization will show its release rate.

The pristine LIN powders were analysed by SEM and Fig. 1 shows the micrographs at two different magnifications. Pure Linezolid is a highly crystalline material with a sheet shape of dimensions ranging between 25–35 μm width, 1–3 μm thick.

Many internal as well as external parameters can influence the electrospinning process and, as a consequence, the fiber morphology. By adopting the chosen final parameters (as reported in the experimental part), fibers loaded with different drug concentrations were successfully fabricated.

The electrospinning of PCL solution (Fig. 2(A)) led to the formation of randomly oriented, defect-free cylindrical fibers with average fiber diameter of 700 nm, while the electrospinning of the bicomponent solutions of LIN and PCL (Fig. 2(B–E)) led to fibers with broader fiber diameter distribution than that of PCL fibers. At low LIN concentration, higher than 0.5%, a tendency to obtain fibers with lower diameter (400–550 nm) could be explained by a better spinnability induced by the LIN molecules. This tendency tends to be reduced at higher LIN concentration (5%), coming back to the value of pristine PCL (700 nm). The values of diameters are reported in Table 1.

In the membranes containing the LIN with concentrations of 2.5% and 5% besides the typical beads, fibrils few microns length and a diameter of about 200 nm arranged randomly along the fibers of the PCL could be observed. This morphology may be induced by the LIN particles that at high concentrations make agglomerates not fully embedded in the fiber, but coating the polymer and appearing as appendices of the fiber itself.

3.2. Structural analysis

The X-ray analysis was performed to investigate the structure of PCL upon electrospinning and the LIN crystalline structure after solidification into the fibrous membranes. As a matter of the fact many different polymorphs, beside an amorphous form of Linezolid, have been described and patented so far. Polymorphism has

Table 2
Mechanical parameters extracted from the stress–strain curves.

Sample	E (MPa)	σ_Y (MPa)	ε_Y (%)	σ_b (MPa)	ε_b (%)
PCL	5.3 ± 1.06	0.39 ± 0.13	8.8 ± 0.5	1.72 ± 0.26	223 ± 48
PCL + LIN 5%	11.7 ± 0.6	1.4 ± 0.22	13.5 ± 1.1	3.48 ± 0.46	152 ± 10

E, elastic modulus; σ_Y, stress at the yield point; ε_Y, deformation at the yield point; σ_b, stress at breaking; ε_b, deformation at breaking.

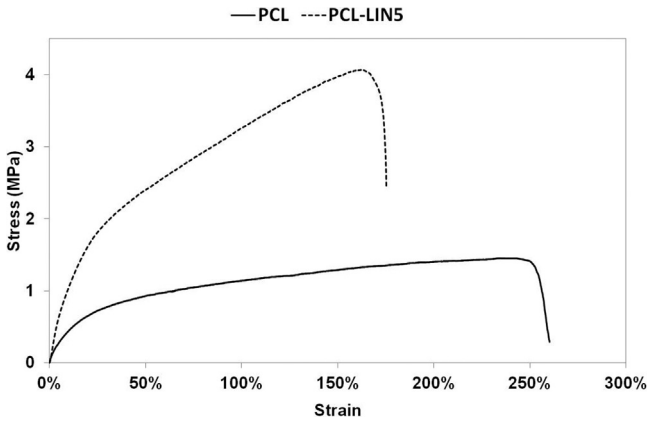

Fig. 4. Stress–strain plots for samples: PCL (a), PCL–LIN5 (b).

the potential to significantly affect the properties of both the active pharmaceutical ingredient and the final dosage form and therefore it is important to clarify the LIN organization into the solid membranes.

The diffractograms of LIN, pristine PCL, and their composite membranes are reported in Fig 3. The pristine electrospun PCL and all the loaded samples show the crystalline structure of PCL well developed with the main diffraction peaks appearing at 21.5° and 23.9° of 2θ. The LIN powders are very crystalline (Fig. 3(A)), showing the most intense diffraction patterns at 7.4°, 13.6°, 18.1°, 21.1°, 22.3°, 25.5° of 2θ. The experimental peaks very closely resemble the diffractogram of Form I described for LIN, that is the most common crystalline form. As for the investigation of the LIN structure in the membranes, it is worth recalling that the LIN fraction is at most 5% so that high peaks are not expected. Moreover unfortunately the Form I most intense peaks between 20° and 26° of 2θ are hidden by the two peaks of PCL at 21.5° and 23.9° of 2θ, and therefore we can base the analysis only upon the main peaks at 7.41°, 9.45°, 13.6°, and 18.1° of 2θ.

Considering the membranes with low content of LIN, that is 0.5% and 1%, none of the LIN crystalline peaks is evident. This can be due to the low concentration of crystals or to a lack of crystallization of LIN molecules into the membranes. Actually the molecules dissolved in the solvent are very diluted and in addition they are separated by the high concentration of the PCL molecules that hinder their approaching during the solvent evaporation. We suggest that LIN, at concentrations of 0.5% and 1% in the electrospun membranes is in the amorphous state, as already found in many systems (Valarezo et al., 2012).

Interestingly in the more concentrated samples with 2.5% and 5% of LIN, although the Form I peaks are completely absent, pointing also in this case to a prevalently amorphous form, a small peak at 5.4° of 2θ appears indicating the partial crystallization of a different polymorph of LIN (see the inset of Fig. 3(C)). It could correspond to Form III or Form X, that both show a peak at 5.4° of 2θ very intense respect to the others. This result is very important because electrospinng would represent a new method to obtain a different polymorph characterized by possibly different properties. Work is in progress to confirm this hypothesis, but in any case we can affirm that the crystallization in confined environments favours a different crystallization route respect to the common Form I.

3.3. Mechanical properties

The mechanical properties of PCL fibers and membranes loaded with the higher amount of drug (PCL–LIN5), as representative of all electrospun samples, were measured and the principal mechanical parameters are reported in Table 2. Generally we observe an improvement, or at least a maintenance, of all the mechanical parameters. A general increment of the elastic modulus is observed in the composites with respect to neat PCL. In particular this parameter increases of about 120% in the sample with 5% of LIN.

The engineering stress–strain curves for the electrospun PCL and PCL–LIN5 fibers under monotonic loading are shown in Fig. 4. Almost linear elastic behaviour was seen until the fiber mats undergo breaking. The maximum tensile strength of the PCL membrane was of 1.72 MPa with an ultimate strain of 22%. The composite samples behave in a similar way, with some differences.

The electrospun PCL–LIN5 reached a maximum tensile strength of 3.50 MPa, with an elongation at break of 15%.

3.4. Drug release studies

Local release of drugs sustained for many days is preferred to systemic administration due to the advantages of these delivery systems, such as: (i) improving the effectiveness of drug and selective targeting; (ii) decreasing the side effects; (iii) reducing the frequency of administration. Therefore following the encapsulation procedures of drug molecules into a biocompatible polymer, it is of utmost importance to investigate the release profiles, depending on the many parameters that it is possible to modulate for the specific applications.

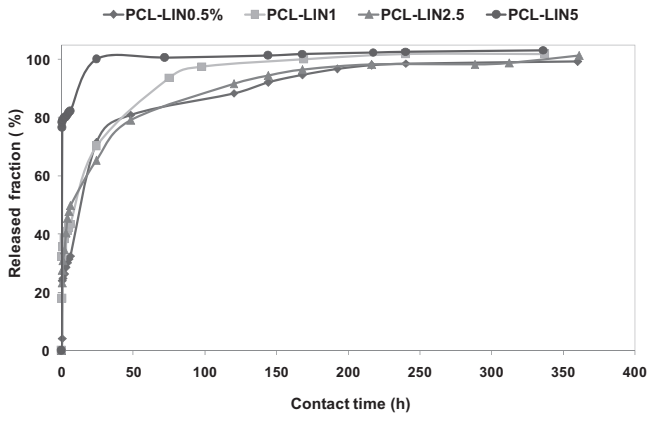

Fig. 5. Released fraction of LIN molecules (wt/wt %) normalized to the expected final concentration.

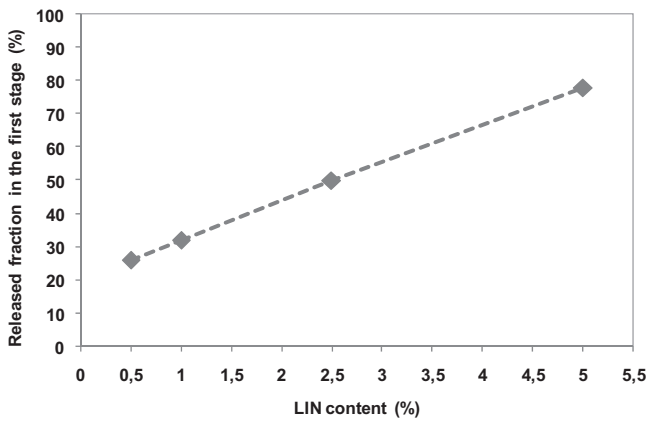

Fig. 6. LIN fraction released from the surface of the fibers, as a function of LIN concentration in the membranes.

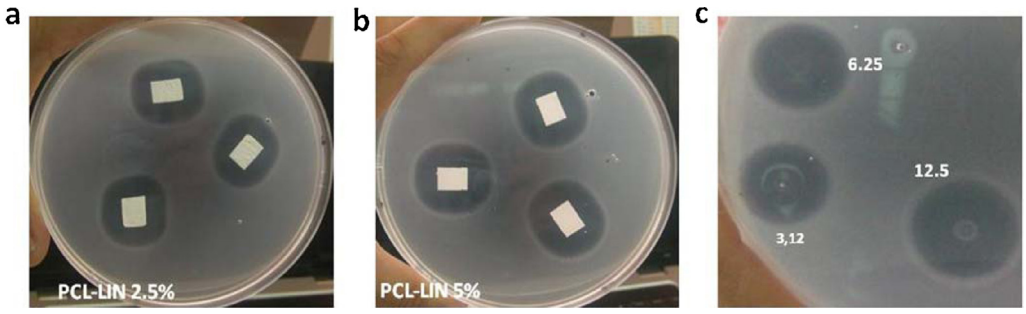

Fig. 7. Microbiological assay of antibiotic diffusion. Three pieces of membrane were laid down on the nutrient agar inoculated with *S. aureus*. a. Membranes with 2.5% Linezolid; b. membranes with 5% Linezolid; c. effects on the growth produced by 3 μL of Linezolid solution (w/v in DMSO) at indicate concentration (μg mL^{-1}). In all pictures the diffusion of Linezolid in the environment is clear from the circular halo indicating growth inhibition.

In Fig. 5 the release of LIN at different concentrations, monitored up to 15 days, is shown, as percentage of the maximum achievable concentration.

As already observed in previous cases (Valarezo et al., 2012) the release curves are very complex, all showing a first release rapid as a burst, followed by slower stages up to the complete exit of the drug. The initial burst, found in many controlled-release systems has been described as caused by a number of mechanisms, including surface desorption, pore diffusion, or the lack of a diffusion front barrier regulating the diffusive process (Sill and von Recum, 2008). In our case the SEM analysis clearly shows the presence of LIN agglomerates on the surface of the fibers, particularly evident in the more concentrated samples (PCL–LIN2.5 and PCL–LIN5). We can therefore suggest that the first stage, that is the burst, is due to LIN molecules, set on the fiber surface. For these molecules the kinetics of release depend on their structural organization, either amorphous or crystallized in one of the possible polymorphs. In this case the crystal dimensions will have influence, too. In Fig. 6 we report the fraction of LIN going out from the surface of the fibers, as a function of LIN concentration in the membranes. We can observe that the fraction of external LIN linearly varies with concentration, extrapolating for zero concentration at 20%. This would suggest that, independently of the total concentration, minimum 20% of LIN molecules is located outside the fibers, with a fraction linearly increasing with the LIN concentration.

The slower release stages are related to the more crystalline phases of LIN molecules and to the fraction encapsulated inside the fibers. For these molecules the kinetics of release mainly depend on the thickness of the fiber walls, the thicker the wall the slower the release. Observing the different LIN concentrations we deduce that the membranes loaded with 5% of LIN have the fibers with thinner walls, in agreement with the larger fiber diameter, as shown in Table 1.

Table 3
Measures of antibacterial activity.

Diffusion tests	
Average area of growth inhibition	
PCL–LIN2.5	7.3 cm^2
PCL–LIN5	8.9 cm^2
Releasing assays (PCL–LIN5)	
MIC	1 μg mL^{-1}
rMWM	25 μg mL^{-1}
Ctot	50 ng μg membrane^{-1}
Ct (12 h)	≥40 ng μg membrane^{-1}
%Ct/Ctot	≥80%

3.5. Antibacterial activity

Antibacterial activity of the membranes loaded with Linezolid was tested against the Gram-positive bacterium *S. aureus*. First we evaluated their ability to inhibit the growth by assays of antibiotic diffusion. Pieces of membrane with higher concentration of Linezolid (PLC–LIN2.5 and PLC–LIN5) were placed in triplicate on agar surface of nutrient medium previously inoculated with *S. aureus* and poured in Petri dishes. After 24 h of incubation at 37 °C the formation of area of growth inhibition was evaluated. As evidenced in Fig. 7 all the membranes produced a circular halo of growth inhibition whose extent was dependent on antibiotic concentration (Fig. 7a–c and Table 3).

Subsequently, to quantify the antibacterial activity we evaluated the minimum weight of membrane releasing a concentration of active antibiotic equivalent to the MIC (minimal inhibitory concentration). We named this measure MWM, namely, minimum weight releasing the MIC. The release data reported above indicate that the total amount of antibiotic released from the membranes also depends on the time, so we used the relative MWM (rMWM), that is the minimum weight releasing the MIC in the considered time. In these determinations only the membrane loaded with 5% Linezolid was analyzed, due to its higher antibacterial activity (see diffusion test) and the high rate of antibiotic release. The results are summarized in Table 3. For *S. aureus* strain the MIC was 1 μg mL^{-1} and the rMWM 25 μg mL^{-1} after 12 h of incubation. Considering the amount of potentially available antibiotic (Ctot) in the PCL–LIN5 and that released (Ct), most of the antibiotic content (≥80%) was released in the first 12 h, in agreement with the data of the release reported above.

4. Conclusions

The experimental parameters to obtain PCL membranes loaded with different concentrations of Linezolid (0.5, 1, 2.5 and 5%) by electrospinning technique have been investigated and stated.

The morphology is very similar both in the pristine PCL and in the loaded membranes, with a tendency to show fibers with lower diameters at intermediate LIN concentrations. In the higher concentrated membranes, crystals of LIN appear on the surface of the fibers. They are characterized by a fibrillar morphology with crystals of different form and much thinner dimensions than the pristine LIN crystals. Structural X-ray analysis shows that Linezolid embedded in the membranes is prevalently amorphous, with a low crystallinity showing a different polymorphic form respect to the usual Form I and Form II. The release kinetics of the drug allowed to discriminate between Linezolid molecules on the surface and encapsulated into the fibers. The exit of the molecules on the surface is rapid, whereas the inside molecules take much longer

times for the complete exit. The antibacterial activity of the electrospun membranes was effective to inhibit *S. aureus*.

The properties of the loaded membranes and their capability for local delivery of the antibiotic make them good candidates as drug release devices for topical use.

References

Allegrini, P., Attolino, E., Razzetti, G., Vladiskovic, C., 2009. Linezolid crystalline hydrate form and linezolid salts. EP 2033960.

Aronhime, J., Koltai, T., Braude, V., Fine, S., Niddam, T., 2006. Crystalline form IV of linezolid. WO 2006004922.

Aronhime, J., Koltai, T., Braude, V., Fine, S., Niddam, T., 2006. Solid forms of linezolid and processes for preparation thereof. US-A1-20060111350.

Ballow, C.H., Jones, R.N., Biedebach, D.J., 2002. A multicenter evaluation of linezolid antimicrobial activity in North America. J. Diagn. Micr. Infec. Dis. 43, 75–83.

Barbachy, M.R., Brickner, S.J., Hutchinson, D.K., 1997. Substituted oxazine and thiazine oxazolidinone antimicrobials. US 5688792.

Bergen, M.S., 2001. Linezolid – crystal form II. WO2001057035.

Bergen, M.S., 2002. Mixing linezolid of an >98% enantomeric purity in a solvent at >80 degrees; separating a crystal (ii) of >99% purity; analysis by the powder X-ray diffraction spectrum/infrared spectrum as a mineral oil mull; bactericides; stability. US 6444813.

Bergen, M.S., 2003. Linezolid – crystal form II. US 6559305.

Birmingham, M.C., Rayner, C.R., Meagher, A.K., Flavin, S.M., Batts, D.H., Schentang, J. J., 2003. Linezolid for the treatment of multidrug-resistant, gram-positive infections: experience from a compassionate-use program. Clin. Infect. Dis. 36, 159–168.

Boccaccini, A.R., Blaker, J.J., 2005. Bioactive composite materials for tissue engineering scaffolds. Exp. Rev. Med. Devices 2, 303–317.

Bonev, B., Hooper, J., Parisot, J., 2008. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. Antimicrob. Chemother. 61, 1295–1301.

Bricker, S.J., Hutchinson, D.K., Barbachyn, M.R., Manninen, P.R., Ulanowicz, D.A., Garmon, S.A., Grega, K.C., Hendges, S.K., Toops, D.S., Ford, C.W., Zurenko, G. E., 1996. Synthesis and antibacterial activity of U-100592 and U-100766, two oxazolidinone antibacterial agents for the potential treatment of multidrug- resistant Gram-positive bacterial infections. J. Med. Chem. 39, 673–679.

Brittain, H.G., 2002. Polymorphism: Pharmaceutical Aspect, Encyclopedia of Pharmaceutical Technology. Marcel Dekker, New York.

Corti, G., Cinelli, R., Paradisi, F., 2000. Clinical and microbiologic efficacy and safety profile of linezolid: a new oxazolidinone antibiotic. Int. J. Antimicrob. Agents 16, 527–530.

Cui, W., Zhou, Y., Chang, J., 2010. Electrospun nanofibrous materials for tissue engineering and drug delivery. Sci. Technol. Adv. Mater. 11 014108 (11pp).

Devarakonda N.S., Thaimattam, R., Muppidi, K.V., Kanniah, L.S., Duggirala, K.N., 2009. Linezolid co-crystals WO 2009140466.

Dodda, M.R., Pingili, K.R., 2005. A novel crystalline form of linezolid. WO2005035530.

Dodda M.R., Pingili K.R., 2007. A novel amorphous form of linezolid. WO2007026369.

Doshi, J., Reneker, D.H., 1995. Electrospinning process and applications of electrospun fibers. J. Electrostat. 35, 151–160.

Hilfiker, R., 2006. Polymorphism in the Pharmaceutical Industry. Wiley-VCH Verlag GmbH & Co., KGaA, Weinheim.

Jiang, S., Ji, X., An, L., Jiang, B., 2001. Crystallization behavior of PCL in hybrid confined environment. Polymer 42, 3901–3907.

Karagiannidou, E.G., 2013. Solid state characterization of linezolid crystal forms. Int. J. Anal. Pharm. Biomed. Sci. 2, 27–36.

Liu, X., Ma, P.X., 2004. Polymeric scaffolds for bone tissue engineering. Ann. Biomed. Eng. 32, 477–486.

Maccaroni, E., Alberti, E., Malpezzi, L., Masciocchi, N., Vladiskovic, C., 2008. Polymorphism of linezolid: a combined single-crystal, powder diffraction and NMR study. Int. J. Pharm. 351, 144–151.

Pearlman, B.A., Perrault, W.R., Barbachyn, M.R., Manninen, P.R., Toops, D.S., Houser D.J., Fleck, T.J., 1998. Process to prepare oxazolidinones. US 5837870.

Pearlman, B.A., 1999. Process to produce oxazolidinones. WO 9924393.

Sathe, D.G., Bhise, N.B., Naidu, A.V., Sawant, K.D., Bhattacharyya, A.S., Naik, T.A., 2009. Process for preparation of (S)-*N*-[[3-[3-Fluoro-4-(4-morpholinyl)phenyl]-2-*oxo*-5-oxazolidinyl]methyl]-acetamide. WO2009063505.

Sill, T.J., von Recum, H.A., 2008. Electrospinning: applications in drug delivery and tissue engineering. Biomaterials 29, 1989–2006.

Tanaka, R., Hirayama, N., 2008. Crystal structure of Linezolid. Anal. Sci. 24, 43–44.

Teo, W.E., Ramakrishna, S., 2006. A review on electrospinning design and nanofibre assemblies. Nanotechnology 17, R89–R106.

Tsang, V.L., Bhatia, S.N., 2004. Three-dimensional tissue fabrication. Adv. Drug Deliver. Rev. 56, 1635–1647.

Valarezo, E., Stanzione, M., Tammaro, L., Cartuche, L., Malagón, O., Vittoria, V., 2012. Preparation, characterization and antibacterial activity of poly(ε-caprolactone) electrospun fibers loaded with amoxicillin for controlled release in biomedical applications. J. Nanosci. Nanotech. 12, 1–10.

Woodruff, M.A., Hutmacher, D.W., 2010. The return of a forgotten polymer – Polycaprolactone in the 21st century. Prog. Polym. Sci. 35, 1217–1256.

Yang, S., Leong, K.F., Du, Z., Chua, C.-K., 2001. The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue Eng. 7, 679–689.