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Antibacterial electrospun poly(ε-caprolactone)/ascorbyl palmitate nanofibrous materials

Dilyana Paneva[a], Nevena Manolova[a,*], Mariana Argirova[b], Iliya Rashkov[a,*]

[a] Laboratory of Bioactive Polymers, Institute of Polymers, Bulgarian Academy of Sciences, Acad. G. Bonchev St., Bl. 103A, 1113 Sofia, Bulgaria
[b] Department of Chemistry and Biochemistry, Medical University, V. Aprilov St. 15A, 4002 Plovdiv, Bulgaria

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ABSTRACT

The one-step incorporation of ascorbyl palmitate (AP), a widely used derivative of vitamin C, into nanofibrous mats of poly(ε-caprolactone) (PCL) by electrospinning was demonstrated. The incorporation of AP was attested by IR spectroscopy; the AP content was determined by thermogravimetric analysis (TGA); and the surface composition of the mats: by X-ray photoelectron spectroscopy (XPS). The possibility for deposition of silver nanoparticles onto PCL/AP mats using the ability of AP to reduce silver ions was demonstrated. The silver content was determined by TGA, and the silver nanoparticles were observed by transmission electron microscopy (TEM). The nanoparticles were composed of elemental silver, as verified by XPS analyses. The UV–vis spectrophotometric analyses, study on quenching of the free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and microbiological tests against the pathogenic microorganism *Staphylococcus aureus* showed that AP preserved its stability and its antioxidant and antibacterial activity when incorporated in the nanofibrous mats.

1. Introduction

Electrospinning is regarded as a highly promising method for fabrication of polymer micro- and nanofibrous materials (Lee et al., 2009; Paneva et al., 2009). The method allows one-step incorporation of low molar mass biologically active substances of different nature into these materials. Thereby diverse micro- and nanofibrous materials can be obtained, which are potential candidates for cell and tissue engineering, wound healing, cosmetics, etc. Regardless of the fact that vitamins are a major component of a number of polymer compositions intended for use in medicine, pharmacy, cosmetics and the food industry, until now the investigations on vitamin incorporation in polymer fibrous materials by electrospinning are still scarce. Incorporation of vitamin A and of vitamin E in electrospun fibrous materials has been reported (Chew et al., 2006; Taepaiboon et al., 2007; Puppi et al., 2010).

Ascorbyl palmitate (AP) is an amphipathic derivative of vitamin C that has found wide application as an antioxidant additive in food, pharmaceutical, medical and cosmetic products (Andersen, 2005; Venkat Ratnam et al., 2006). It has been found that its antioxidant activity is almost equal to that of vitamin C. In addition, it is more stable as compared to its precursor and owing to their hydrophobic palmitate chains the AP molecules penetrate more easily through skin and have a greater ability to protect lipids and other skin components from free radical peroxidation (Venkat Ratnam et al., 2006; Kristl et al., 2003). Until now AP has been incorporated in microemulsions (Kristl et al., 2003), bilayer vesicles (Kristl et al., 2003; Gopinath et al., 2004), or nanoparticles (Yoksan et al., 2010). To the best of our knowledge no data on the incorporation of AP in micro- and nanofibrous materials by electrospinning are available in the literature. Regardless of its higher stability AP is sensitive to oxidation thus the development of novel polymer materials able to incorporate AP while preserving its antioxidant properties is of interest.

The amphipathic nature of AP accounts for its compatibility with polymers which are soluble in organic solvents. Poly(ε-caprolactone) (PCL) is a biocompatible and hydrolytically degradable polyester, which is regarded as an extremely promising candidate for the design of different polymer products that can find diverse applications. Particular attention is paid to its applicability in the biomedical field. In addition, it can be easily electrospun into micro- and nanofibrous materials (Woodruff and Hutmacher, 2010; Stoilova et al., 2006, 2007; Paneva et al., 2008).

The present study aims at the preparation and characterization of nanofibrous materials from PCL containing AP by electrospinning. The deposition of silver nanoparticles onto PCL/AP mats by reduction of silver ions under the action of the vitamin has been examined, as well.

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* Corresponding authors. Tel.: +359 02 9793468; fax: +359 02 8700309.
E-mail addresses: manolova@polymer.bas.bg (N. Manolova), rashkov@polymer.bas.bg (I. Rashkov).

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2. Experimental

2.1. Materials

Ascorbyl palmitate (AP), methanol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical were purchased from Sigma–Aldrich, and used as received. Poly(ε-caprolactone) (PCL, CAPA 6800) was supplied by Solvay Interox, UK ($\bar{M}_n = 69,000$ g/mol; $\bar{M}_W/\bar{M}_n = 1.74$). Dichloromethane (DCM), dimethylformamide (DMF), isopropanol and silver nitrate ($AgNO_3$) were purchased from Fluka, and used as received. A *Staphylococcus aureus* strain (*S. aureus*; NBIMCC3359) was purchased from the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC), Bulgaria. The growth medium for *S. aureus* was meat-peptone broth supplied by the National Centre for Infectious and Parasitic Diseases (NCIPD), Bulgaria.

2.2. Preparation of nanofibrous materials from PCL and AP

PCL was electrospun from its solution in a mixed solvent of DCM/DMF at a volume ratio of 9/1. In order to assess the impact of the PCL concentration on the morphology of the obtained fibers at AP content of 30 wt.% the polymer concentration was varied from 10 to 13 and 17% (w/v). For the preparation of PCL/AP nanofibrous mats at a PCL concentration of 10% (w/v) solutions containing 9, 16 and 30 wt.% of the vitamin in respect to total solids were used. For instance, for the preparation of a spinning solution containing 30 wt.% AP at PCL concentration of 10% (w/v), 2 g of PCL were dissolved in 18 ml of DCM, and 1 g of AP was dissolved in 2 ml of DMF. After the complete dissolution of the polymer and the vitamin their solutions were mixed. The electrospinning set-up consisted of a syringe (5 ml) equipped with a 20-gauge stainless steel blunt nozzle. The needle was connected to a high voltage power supply generating positive DC voltage. The solutions were delivered at a controlled feed rate of 3.3 ml/h. The fibers were collected on a grounded rotating drum collector with a rotating velocity of 75 rpm. The nozzle tip to collector distance was 15 cm. The electrospinning was performed at a constant applied voltage of 25 kV.

The dynamic viscosity of the spinning solutions was measured by a Brookfield LVT viscometer, supplied with an adaptor for small samples, a spindle and a SC 4-18/13 R camera, at 25 ± 0.1 °C. The electrical resistance of the spinning solutions was measured in an electrolytic cell equipped with rectangular sheet platinum electrodes having a surface area of 0.6 cm^2 and disposed at a distance of 2.0 cm. During the measurements short electric pulses of opposite directions were applied to the Pt electrodes in order to avoid accumulation of ionic charges and polarization effects in the vicinity of the electrode surface. This allowed solution resistance in the range of 20–2000 kΩ to be measured with an accuracy of ±3%. Calibration of the electrolytic cell was performed using a standard solution of KCl and the constant of the cell (K_{cell}) was determined. The conductivity of the spinning solutions (σ, μS/cm) was calculated from the following equation:

$$\sigma = \frac{1}{\rho} = \frac{1}{K_{\text{cell}}R} \qquad (1)$$

where ρ is the specific resistance of the solution ($\mu\Omega$ cm), R – electrical resistance of the solution ($\mu\Omega$).

2.3. Deposition of silver nanoparticles onto PCL/AP mats

PCL or PCL/AP mats containing 9, 16 or 30 wt.% AP (0.1 g), were immersed in 20 ml of water/isopropanol mixture at a volume ratio of 4/1 for 15 min. Subsequently each mat was immersed in 100 ml of $AgNO_3$ aqueous solution (1.2 mmol/l) for 6 h. The mats were washed with deionized water and vacuum-dried to constant weight.

2.4. Characterization of the fibrous materials

The jet motion during the electrospinning process was imaged by a digital camera (Sony, Japan). The morphology of the electrospun materials was analyzed by scanning electron microscopy (SEM). For that purpose, the mats were vacuum-coated with gold and analyzed by a JEOL JSM-5010 or Philips SEM 515 scanning electron microscope. The fiber morphology was evaluated in terms of the criteria for complex evaluation of electrospun mats reported elsewhere (Spasova et al., 2006) using *Image J* software (Rasband, 2006) by measuring at least 30 fibers from each SEM image. Transmission electron microscopy (TEM) observations were carried out with a Philips CM 100 operating at a voltage of 100 kV. TEM images were recorded with a Gatan 673 CCD camera and transferred to a computer equipped with the Kontron KS 100 software.

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic analyses were performed using an IRAffinity-1 spectrophotometer (Shimadzu Co., Japan) equipped with a MIRacle ATR (diamond crystal, depth of penetration of the IR beam into the sample about 2 μm) accessory (PIKE Technologies, USA). The spectra were recorded from 4000 cm^{-1} to 600 cm^{-1} with a spectral resolution of 4 cm^{-1} using a DLATGS detector equipped with a temperature controller. The PCL fiber spectrum was corrected for H_2O and CO_2 using IRsolution internal software. For the sake of more precise distinguishing of the AP characteristic bands the spectra of the AP-loaded PCL fibrous materials were recorded by using a PCL mat as a background utilizing IR solution internal software.

Thermogravimetric analyses (TGA) were performed using TGA Q500 V20.10 Build 36 equipment under nitrogen (flow rate of 60 ml/min) at a heating rate of 10 °C/min from 25 to 800 °C. The data obtained from the TGA analyses were used for determination of the AP content of the PCL/AP materials according to a procedure described in detail in Yoksan et al. (2010), as well as for determination of the amount of the elemental silver deposited onto the PCL/AP mats. The surface elemental composition of the fibers was determined by XPS. The XPS measurements were carried out in the UHV chamber of an ESCALAB-MkII (VG Scientific) electron spectrometer using Mg K_α excitation with a total instrumental resolution of 1 eV. Energy calibration was performed, taking the C 1s line at 285 eV as a reference. The water contact angles of electrospun mats were measured by a DSA 10 MK2 (Krüss) apparatus at 20 ± 0.1 °C. The mats were attached to a glass slide and a drop of deionized water (15 μL) was deposited on the samples. The contact angles were calculated by image processing of a sessile drop using the Drop Shape Analysis Software (Krüss). The data are average values from 10 measurements.

UV–vis spectra of AP methanol extracts were recorded using a UV–vis spectrometer DU 800 (Beckman Coulter) over a wavelength range of 200–500 nm ($\lambda_{\max} = 268$ nm).

The antioxidant activity of PCL/AP mats was evaluated *in vitro* using DPPH as a free radical. First, AP was extracted from the mats (weight – 2 mg) in methanol (1 ml) at room temperature for 20 min. 2 ml of DPPH (60 μM solution in methanol) were pipetted in a cell and 10-μL aliquots of the polymeric extracts were consecutively added to the reagent. Absorbance at 517 nm was read after each aliquot was added and the readings were plotted versus the volume of the extract added. The linear response range of the radical discoloration was used for calculations. The intercept of the linear equation $A_{517\,\text{nm}} = f(\text{volume extract})$ was taken as an initial absorbance and the antioxidant activity was expressed as microliters of methanol extract capable of reducing the absorption to a corresponding concentration of the free radical of 50%. Three inde-

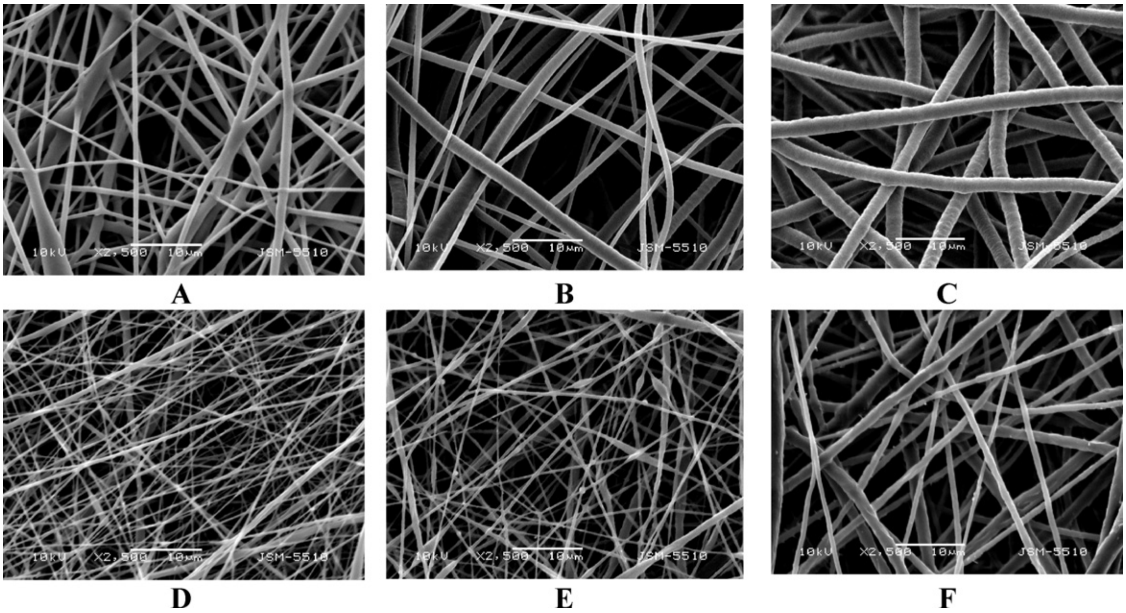


Fig. 1. SEM micrographs of PCL (A–C) and PCL/AP(30 wt.%) (D–E) fibers obtained at PCL concentration: 10 (A and D); 13 (B and E) or 17% (w/v) (C and F). Magnification: 2500×.

pendent analyses were carried out per sample starting from the extraction step.

The antibacterial activity of the nanofibrous materials was assessed against the Gram-positive pathogenic microorganism *S. aureus* by a viable cell-counting method. Upon appropriate dilution with 0.9% saline solution, a culture of ca. 2.4×10^6 cells/ml was prepared and used for the antibacterial tests. The tested nanofibrous samples were round in shape with a diameter of 10 mm and a weight of 6 mg. The bacterial cells suspension (1 ml) was incubated in the presence of the material samples at 37 °C. At a specified time (0, 5, 10 and 20 min), 0.1 ml of bacteria culture was added to 0.9 ml of sterilized 0.9% saline solutions, and several decimal dilutions were made. The surviving microorganisms were counted by the spread-plate method. At various exposure times 0.1 ml portions were taken and spread on nutrient agar. The plates were incubated overnight at 37 °C, and the colonies were counted. The counting was performed in triplicate for each experiment. The number of viable cells was determined as colony forming units (CFU).

3. Results and discussion

AP is insoluble in chloroform and dichloromethane which are good solvents for PCL. AP is soluble in dimethylformamide (DMF) while the latter is a poor solvent for PCL. In order to prepare spinning solutions containing AP and PCL, in the present study DCM/DMF (9/1, v/v) mixture has been used as a common solvent. Fibrous materials from PCL with an AP content of 9, 16 or 30 wt.% in respect to total solids, have been successfully prepared by electrospinning. Further these materials will be designated as: PCL/AP(9%), PCL/AP(16%) and PCL/AP(30%), respectively. In order to find out the PCL concentration at which nanofibers with the smallest average diameters are obtained investigations have been conducted at PCL concentrations of 10, 13 or 17% (w/v) and at 0 or 30 wt.% AP content of the spinning solution. SEM micrographs of the obtained fibers are shown in Fig. 1, and the dependence of the values of their mean diameters on the PCL concentration is graphically presented in Fig. 2. The increase in the polymer concentration leads to the preparation of fibers of larger diameters both from PCL solutions and PCL solutions containing AP. This is due to an increase in the viscosity of the spinning solutions with the increase in the polymer concentration. Since fibers of the smallest diameters are obtained at PCL concentration of 10% (w/v), this polymer concentration has been selected for preparation of PCL/AP(9%), PCL/AP(16%) and PCL/AP(30%) nanofibrous materials. As seen in Fig. 2, in contrast to the fibers obtained in the absence of AP at the three investigated PCL concentrations, the mean diameters of the PCL/AP(30%) fibers are about 2 times smaller. In Fig. 3 SEM micrographs of PCL/AP(9%), PCL/AP(16%) and PCL/AP(30%) nanofibers obtained at PCL concentration of 10% (w/v) are shown. The dependence of the mean fiber diameter on the AP content is shown, as well (Fig. 3D). As seen in Fig. 3D, the higher the AP content, the smaller the values of the mean fiber diameters. The measured dynamic viscosity of the spinning solutions of different AP content is 110 ± 10 cP. Thus, the decrease in the mean fiber diameters with the increase in the vitamin content is not due to a change in the viscosity of the spinning solutions. A similar decrease in the diameters of polylactide (Zeng et al., 2003) or polystyrene (Lin et al., 2004) fibers with no change in the viscosity of the spinning solutions has been observed when electrospinning these polymers in the presence of a

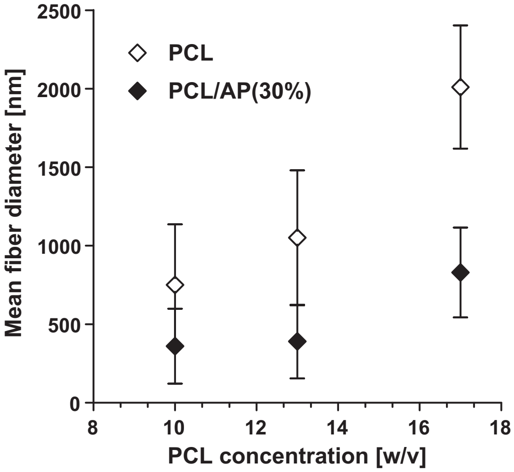


Fig. 2. Dependence of the mean diameters of PCL or PCL/AP(30%) fibers on the PCL concentration.

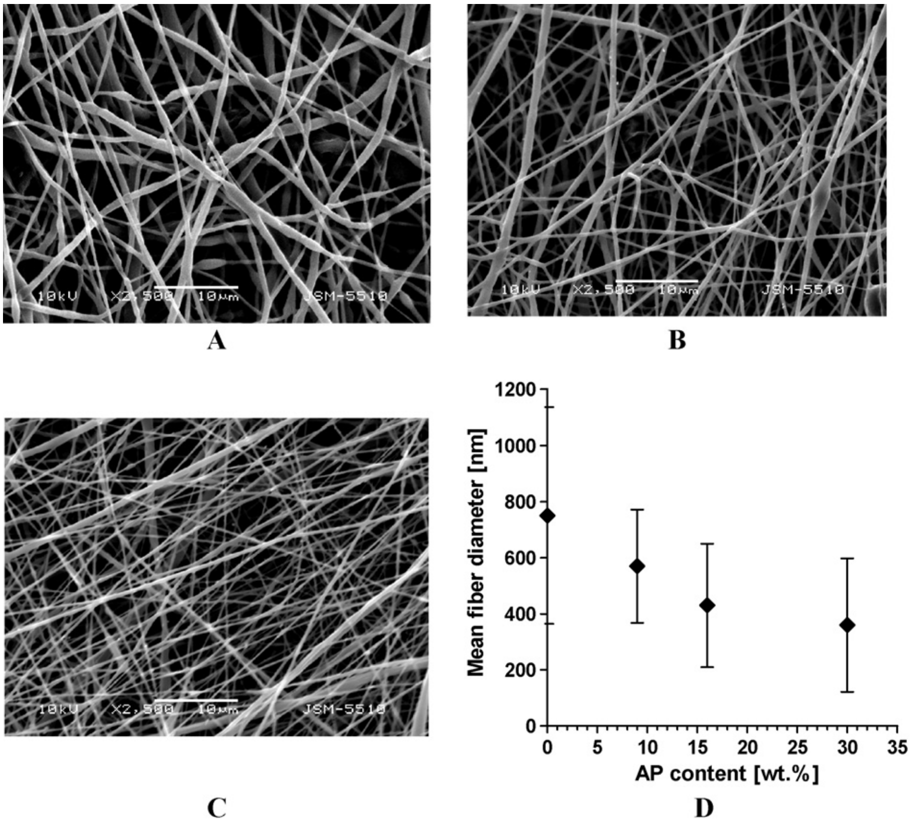
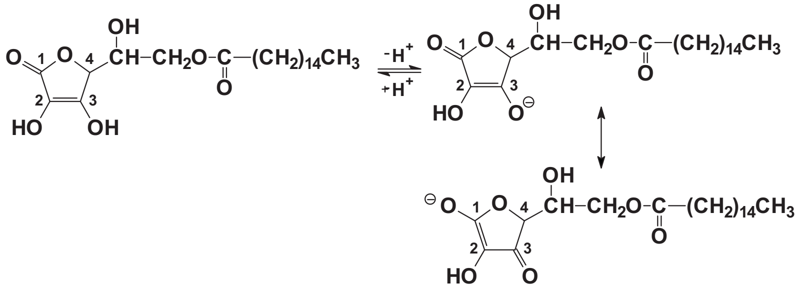


Fig. 3. SEM micrographs of PCL/AP(9%) (A), PCL/AP(16%) (B) and PCL/AP(30%) (C) nanofibers; magnification: 2500×. Dependence of the mean fiber diameter on the AP content (D). PCL concentration: 10% (w/v).

cationic or anionic surfactant. As known (Jaiswal et al., 2005; Palma et al., 2002; Costas-Costas et al., 2005), AP possesses all characteristic properties of an anionic surfactant: a hydrophobic substituent with a sufficiently long alkane chain (an ester-bonded palmitate chain) and an ascorbic acid residue which can be dissociated to an ionic form (Scheme 1). Since the AP ionization is a prerequisite for an increase in the conductivity of the PCL spinning solutions, and hence: for a decrease in the fiber diameters, the conductivity of the spinning solutions used in the present study has been measured. It has been found that the presence of AP leads to a certain increase in the conductivity of the solutions: from 7.0 μS/cm for the PCL solutions, to 8.0, 9.0 and 11.0 μS/cm, for PCL/AP(9%), PCL/AP(16%) and PCL/AP(30%) solutions, respectively. Since the increase in the conductivity is comparatively small, an explanation for the formation of fibers of smaller diameters has been searched for in the behavior of the jet during the electrospinning of PCL and the PCL/AP systems. In Fig. 4 digital photographs of the jet during electrospinning of PCL and PCL/AP(30%) solutions are shown. As seen, in the case of PCL the linear fragment of the jet is 13 mm (Fig. 4A). The incorporation of 30 wt.% AP leads to a decrease in the length of the linear jet fragment, which is shortened to 3 mm (Fig. 4B). The observed phenomenon is in accordance with the results obtained by Zeng et al. (2003) in the case of electrospinning a polylactide solution in the presence of a cationic or anionic surfactant. Similarly to the latter case the decrease in the average diameters of the PCL/AP fibers can be attributed to a shortening of the linear fragment of the jet and an increase in the jet instability as a result of an increase in the conductivity and a decrease in the surface tension of the spinning solutions. In addition, a certain AP/PCL interaction, e.g. hydrophobic/hydrophobic one, is not excluded.

In Fig. 5 IR spectra of AP powder, PCL and PCL/AP(9%) nanofibrous mats in the range from 1850 to 1525 cm^{-1} are juxtaposed. It should be noted that for the sake of better distinguishing of the characteristic bands of the vitamin incorporated in the fibrous materials when recording the spectra of the PCL/AP mats nonwoven PCL textile was used as a background. As seen, in contrast to



Scheme 1. Ascorbyl palmitate and resonance stabilization of its deprotonated form.

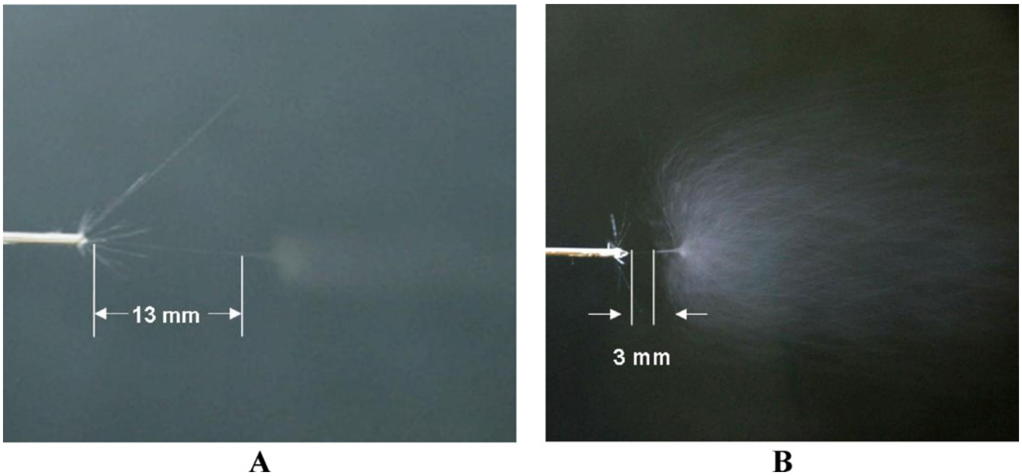


Fig. 4. Digital photograph of the jets of PCL (A) and PCL/AP(30%) (B) spinning solutions, PCL concentration: 10% (w/v).

the PCL fibers, in the spectrum of the mat containing 9 wt.% vitamin, characteristic bands at 1660 (stretch vibration of AP C=C group) and 1695 cm^{-1} (stretch vibration of AP lactone C=O group forming intermolecular hydrogen bonds) and a shoulder at 1755 cm^{-1} (stretch vibration of AP lactone C=O group forming intramolecular hydrogen bonds) are detected. The appearance of these new characteristic bands is due to the presence of AP in the fibers. With the increase in the vitamin content the intensity of the bands at 1660 and 1695 cm^{-1} increases, and the shoulder which is observed at 1755 cm^{-1} at low AP content is replaced by a well defined band at vitamin content of 30 wt.%. The results obtained from the IR spectroscopic analysis are indicative of the successful incorporation of AP in the PCL mats by electrospinning.

Thermogravimetric analyses (TGA) were performed in order to evaluate the amount of AP in the nanofibrous materials. The TGA traces obtained for AP and for PCL fibers were in accordance with literature data on AP and PCL (Yoksan et al., 2010; Unger et al., 2010). The degradation of AP started at 240 °C and that of PCL – beyond 390 °C. In Fig. 6 the thermograms with their first derivatives recorded for PCL and PCL/AP nanofibrous mats are shown. As seen in Fig. 6B, no change in the thermal stability of PCL is induced by the presence of AP (the temperature intervals of their thermal degradation are not altered). Therefore, the TGA thermograms of the mats may be used for determination of the content of AP, incorporated in the nanofibrous materials. With the increase of the AP content in the fibers, the area of the peak corresponding to AP degradation increases and that of the peak for PCL degradation – decreases (Fig. 6B). From the performed TGA analyses it has been found that the amount of AP in the fibers is equal to that in the initial solution, i.e. the electrospinning of the PCL/AP system is an effective method for one-step preparation of nanofibrous materials containing the vitamin in the targeted concentration.

One of the outstanding advantages of the materials obtained by electrospinning is their high surface area. The surface composition has been determined by X-ray photoelectron spectroscopy (XPS). The determined percentages of carbon and oxygen on the surface of the fibers: 79.00 at.% C and 21.00 at.% O for PCL/AP(9%) fibers and 80.60 at.% C and 19.40 at.% O for PCL/AP(30%) fibers are in good agreement with the theoretical values [75.00 at.% C and 24.90 at.% O for PCL/AP(9%) fibers and 75.20 at.% C and 24.80 at.% O for PCL/AP(30%) fibers]. Fig. 7 shows the C 1s spectra of PCL/AP(9%) and PCL/AP(30%) fibrous mats. For the sake of comparison the C 1s spectra of AP powder and PCL fibers are presented, as well. In the case of PCL fibers the ratio between the atomic percentages of the carbon atoms participating in $\underline{C}$–O–C/$\underline{C}$–OH and $\rangle\underline{C}$=O bonds is close to 1/1. In the case of AP the peak area for C-atoms, engaged in $\underline{C}$–O–C/$\underline{C}$–OH bonds is larger as compared to the area of the peak for C-atoms, participating in $\rangle\underline{C}$=O bonds. The presence of AP in an amount of 9 wt.% in the feed leads to a certain increase in the atomic percentages of the carbon atoms participating in $\underline{C}$–O–C/$\underline{C}$–OH of

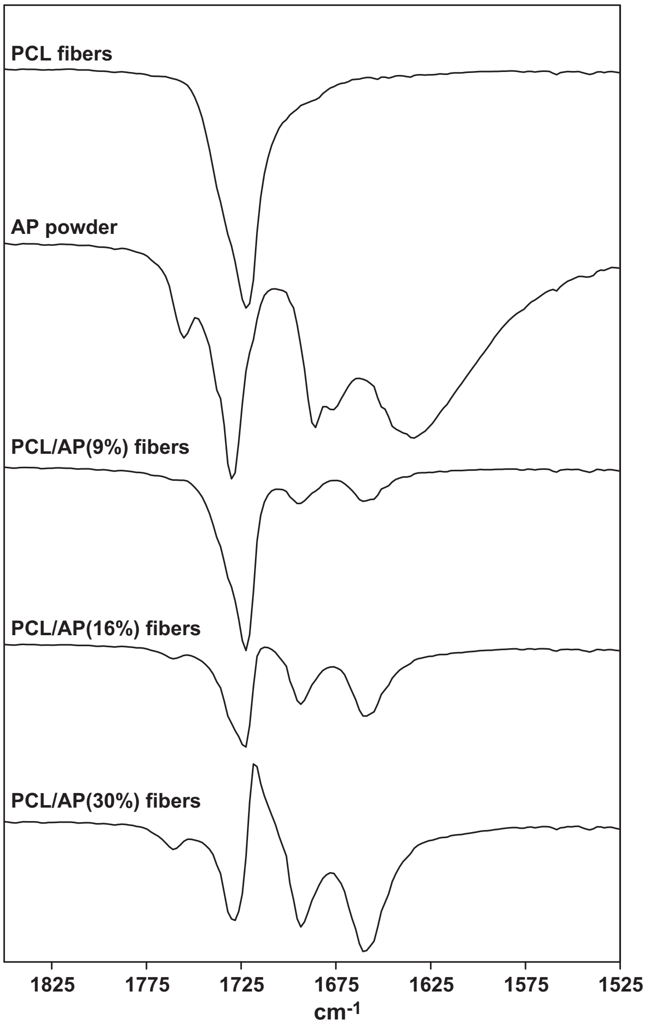


Fig. 5. Juxtaposition of the IR spectra of AP powder, PCL, PCL/AP(9%), PCL/AP(16%) and PCL/AP(30%) mats. The IR spectra of the AP-loaded fibers have been recorded using a PCL mat as a background.

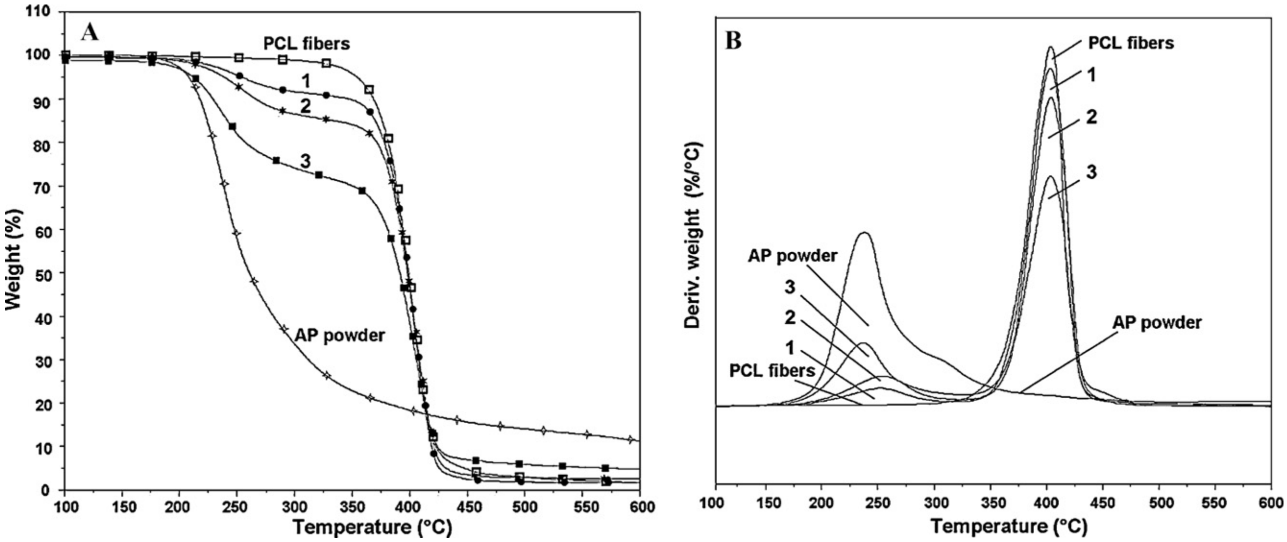
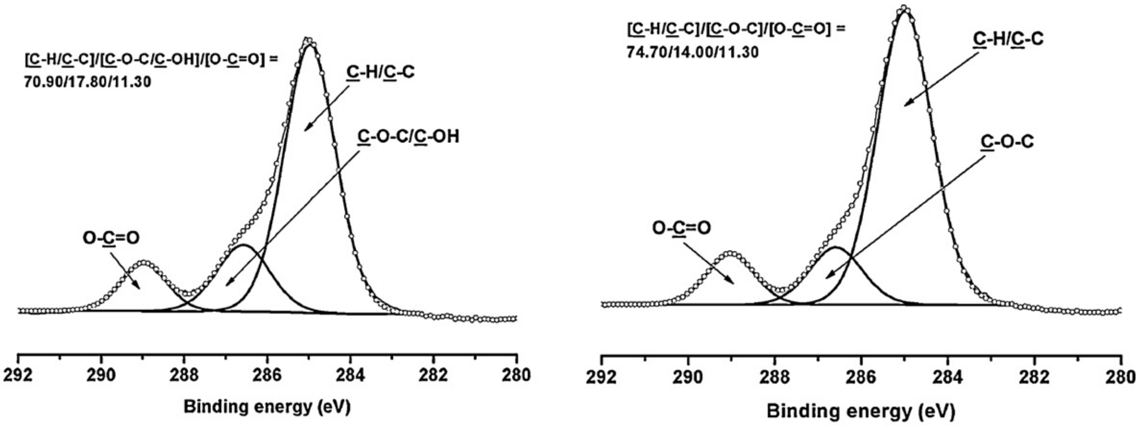
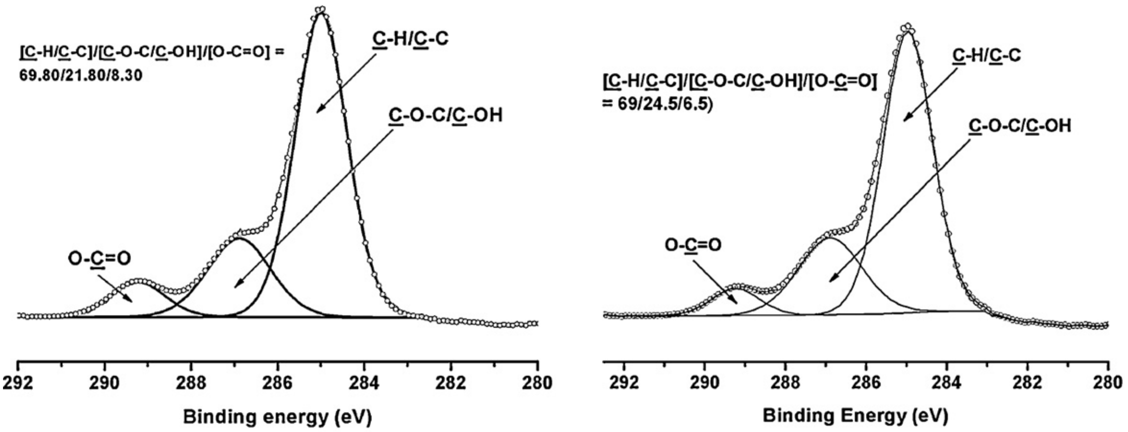


Fig. 6. Thermograms (A) and their first derivatives (B) of AP-loaded PCL nanofibrous materials: PCL/AP(9%) (1), PCL/AP(16%) (2) and PCL/AP(30%) (3), obtained at PCL concentration of 10% (w/v). For the sake of comparison the thermograms of AP powder and PCL fibers and their first derivatives are presented, as well.

the vitamin structure. With the increase of the AP content up to 30 wt.% the peak area for the C-atoms engaged in $\underline{\mathrm{C}}$–O–C/$\underline{\mathrm{C}}$–OH bonds further increases. The results obtained from the C 1s spectra have been confirmed by the recorded O 1s spectra (Fig. 8). Similarly, the presence of 9 wt.% AP leads to a certain increase in the peak area of the O-atoms, participating in C–$\underline{\mathrm{O}}$–C/C–$\underline{\mathrm{O}}$H bonds, and at 30 wt.% the [C–$\underline{\mathrm{O}}$–C/C–$\underline{\mathrm{O}}$H]/[O–C═$\underline{\mathrm{O}}$] ratio becomes 70.50/29.50, i.e. the increase of the vitamin content in the spinning solution leads



PCL/AP(9%) fibers **PCL fibers**



PCL/AP(30%) fibers **AP powder**

Fig. 7. C 1s spectra of PCL/AP(9%) and PCL/AP(30%) fibers. For the sake of comparison the C 1s spectra of AP powder and PCL fibers are presented, as well.

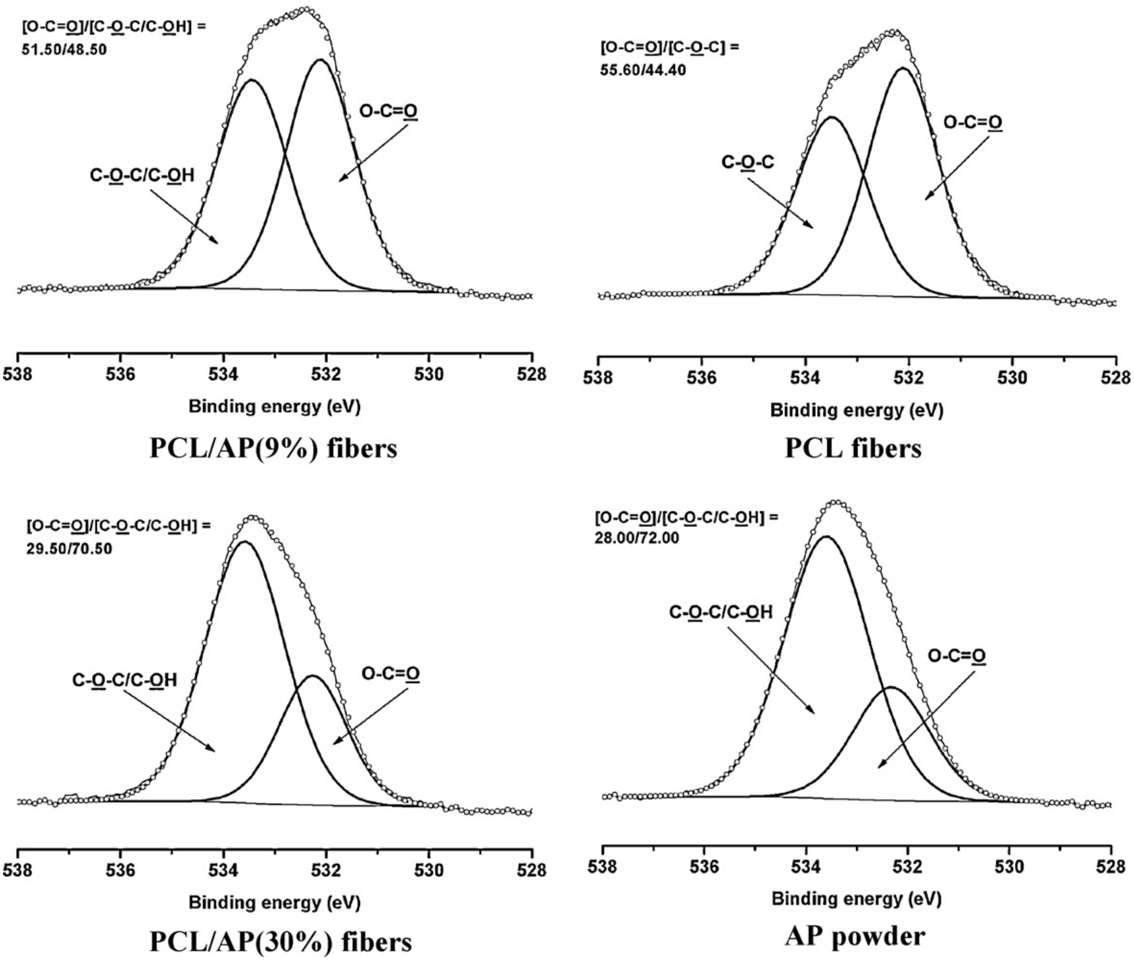


Fig. 8. O 1s spectra of PCL/AP(9%) and PCL/AP(30%) fibers. For the sake of comparison the O 1s spectra of AP powder and PCL fibers are presented, as well.

to the preparation of fibrous materials with a higher AP content on the surface. The performed analyses for water contact angle determination have demonstrated that the incorporation of AP in the structure of the fibers does not change significantly the hydrophobic behavior of the PCL fibrous materials. The water contact angle value of the PCL mat is 122 ± 2.5°. The incorporation of AP in the fibrous materials leads to decrease in the contact angle and the determined average value of the contact angle for the PCL/AP(9%), PCL/AP(16%) and PCL/AP(30%) mats does not depend on the vitamin content and is equal to 110 ± 4.5°.

The presence of AP on the surface of the PCL/AP nanofibrous mats predetermines the rise of some new properties of these nanofibrous mats. It is known that AP mildly reduces silver or

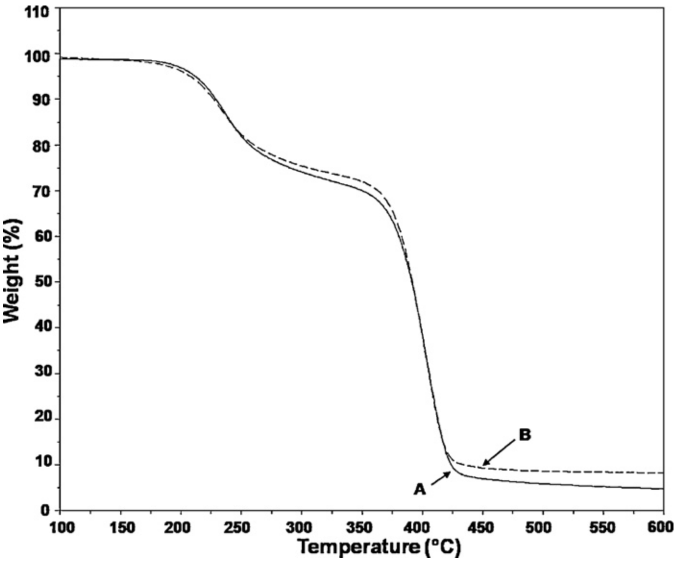


Fig. 9. TGA thermogram of PCL/AP(30%) nanofibrous material before (A) and after (B) a 6-h stay in an aqueous solution of AgNO$_3$.

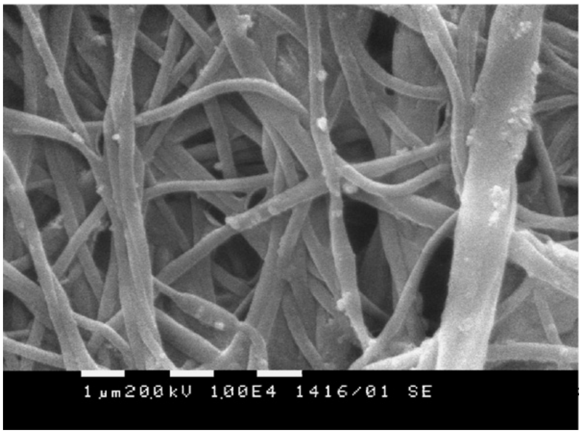


Fig. 10. SEM micrograph of PCL/AP(30%) fibers after a 6-h stay in an aqueous solution of AgNO$_3$; magnification: 10,000×.

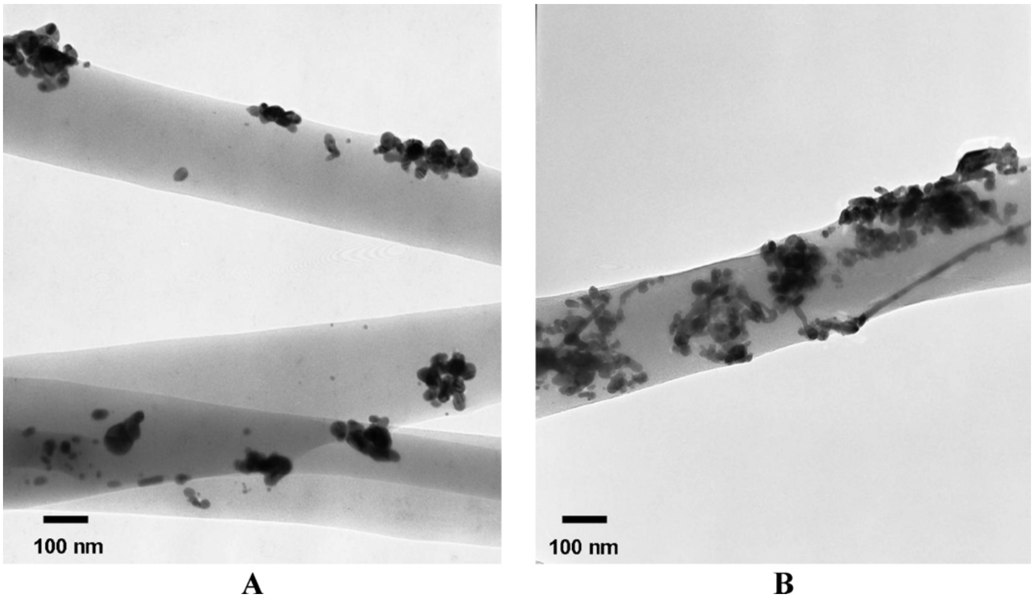


Fig. 11. TEM micrographs of individual PCL/AP(9%) (A) and PCL/AP(30%) (B) fibers after a 6-h stay in an aqueous solution of $AgNO_3$; magnification: 50,000×.

gold ions to elemental silver or gold (Li et al., 2011; Vemula et al., 2007). For this reason in the present study the possibility for deposition of silver nanoparticles onto PCL/AP mats by a 6-h stay in an aqueous solution of $AgNO_3$ has been investigated. In order to impart certain water wettability to the samples they have been immersed in water/isopropanol mixture at a volume ratio of 4/1. In contrast to the PCL mat, the AP-loaded mats have been colored black after a 15-min contact with an aqueous solution of $AgNO_3$. This is indicative of the fact that reduction of the silver ions to elemental silver takes place under the action of AP incorporated in the fibers. The amount of elemental silver deposited onto the PCL/AP mats has been determined by TGA from the thermally non-degraded inorganic component residue at a temperature of 600 °C after subtraction of the ash content (Fig. 9). It has been found that the silver content of the PCL/AP mats is ca. 4 wt.%. Fig. 10 shows a SEM micrograph of PCL/AP(30%) fibers after a 6-h stay in an aqueous solution of $AgNO_3$. Deposits of elemental silver are observed on the surface of the fibers. In order to estimate the fine structure of these deposits the fibers have been observed by TEM (Fig. 11). As seen, most frequently aggregates are observed, which are composed of nanoparticles with an average diameter of ca. 30 nm. With the increase in the AP content of the PCL/AP fibers the number of the aggregates on the fiber surface increases. Nucleation induced by the incorporated AP takes place on the fiber surface. Some nucleation in the homogeneous medium cannot be excluded; however it might be neglected since AP practically is not released from the fibers as evidenced by the TGA analyses discussed below. The obtained results are consistent with those reported by Demir et al. (2008) concerning the deposition of silver nanoparticles onto electrospun poly(glycidyl methacrylate) with hydrazine (a silver ion reducing agent) immobilized onto the fibers. The formation of aggregates of silver nanoparticles and not of individual nanoparticles has also been evidenced by the performed XPS analyses (Fig. 12) by the recorded peaks at 368 and 374 eV. They correspond to Ag 3d5/2 and Ag 3d3/2, respectively, of bulk elemental silver (Kaushik, 1991; Bao et al., 1996). Furthermore, the recorded binding energies values for Ag 3d5/2 indicate that under the used conditions silver oxide is not formed (Kaushik, 1991).

UV spectrophotometric analyses have been performed in order to examine the stability of AP incorporated in the mats. The absorbance peak of AP solutions in methanol is at 268 nm. The UV spectra of methanol extracts of the fibrous mats show that the vitamin is quickly released from the PCL mats. For 20 min 75 wt.% of the incorporated AP are released from the mats independently from the mats composition. In addition, it has been found that the methanol extracts absorbance value of 268 nm as well as its intensity are not altered even after a 4 months storage of the mats in the air and in the dark. This indicates that AP, incorporated in the PCL mats by electrospinning does not undergo any structural changes even after a continuous storage period.

The UV spectrophotometric investigations conducted at 268 nm using methanol extracts of AP obtained from PCL/AP mats onto which silver nanoparticles has been deposited have shown that the amount of the released AP is 30, 40 and 70 wt.% for PCL/AP(9%), PCL/AP(16%) and PCL/AP(30%) fibrous mats, respectively. As determined from the TGA analysis, the loss of AP from the fibers does not exceed 1 wt.%, i.e. AP is not released from the fibrous material during the reduction of the silver ions to elemental silver. It is known that the reducing ability of ascorbic acid with respect to metal ions is due to its oxidation to dehydroascorbic acid (Deutsch, 2000). The latter has very small absorbance above 220 nm. Thus, the lower AP

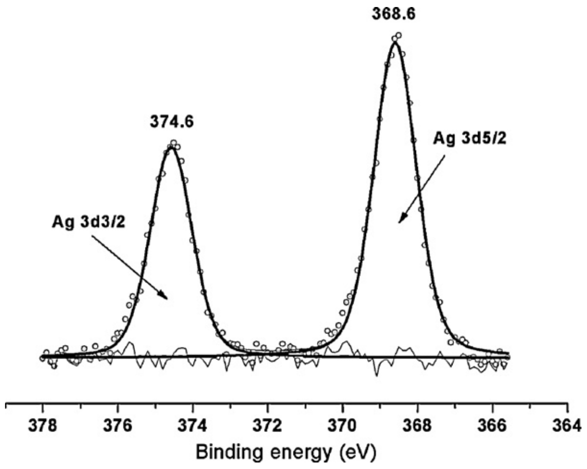


Fig. 12. Ag 3d spectrum of PCL/AP(30%) fibrous mat after a 6-h stay in an aqueous solution of $AgNO_3$.

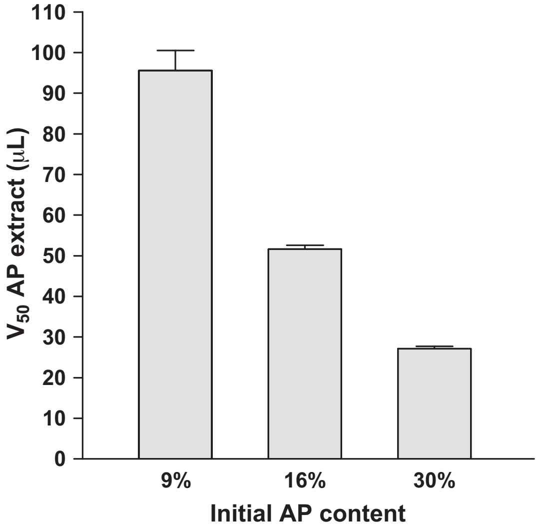


Fig. 13. Dependence of the V_{50} value of methanol extracts of AP with regard to DPPH on the AP content of the nanofibrous mats. DPPH concentration – 60 μmol/l. The data represent the mean values of three independent experiments ±SD.

content of the mats with deposited silver nanoparticles as compared to the neat PCL/AP fibrous materials can be attributed to the presence of a palmitate ester of dehydroascorbic acid, formed during the reduction of silver ions under the action of AP. The results obtained from the UV analyses are in good agreement with the calculated amount of the vitamin participating in the reduction. Taking into account that for the reduction of 2 mol of silver ions 1 mol of AP is needed (Li et al., 2011), as well as considering the silver content of the mats determined by TGA, it has been calculated that under the used conditions the amount of the vitamin participating in the reduction decreases with the increase in the AP content of the mats, and for the PCL/AP(9%), PCL/AP(16%) and PCL/AP(30%) fibrous materials it is 60, 35 and 25 wt.%, respectively. The decrease in the amount of the reacted AP with the increase in the vitamin content of the mats can be attributed to the greater amount of AP on the surface of these mats, as indicated by the XPS analyses. The presence of AP on the surface probably leads to faster initiation of the process of generation of sites for silver nanoparticle formation which serve as nucleation sites for the formation of further nanoparticles. This statement has been substantiated by the observation of a greater number of nanoparticles aggregates in the case of a PCL/AP(30%) fibers as compared to a PCL/AP(9%) ones (Fig. 11).

It is known that the discoloration of methanol solutions of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is one of the fastest and most efficient methods for determination of the antioxidant activity of ascorbic acid (Brand-Williams et al., 1995). In order to prove that AP incorporated in mats by electrospinning preserves its antioxidant activity in the present study methanol extracts of the vitamin from PCL/AP(9%), PCL/AP(16%) and PCL/AP(30%) mats have been used. Aliquots of the obtained AP extracts have been added gradually to DPPH methanol solution until attaining the extract volume (V_{50}), at which the intensity of the DPPH absorbance at 517 nm is reduced by 50%. It has been found that a methanol extract of fibrous PCL material non-containing AP does not exert an effect on the DPPH absorption, i.e. the fibrous PCL materials do not contain components which exhibit antioxidant behavior. Conversely, AP extracted from PCL/AP mats exhibits a well-expressed antioxidant activity against DPPH. It has been found that the amount of AP from the extracts which is necessary to reduce the intensity of the DPPH absorption by 50% regardless of the fiber composition is 20 nmol. Since for the extraction of AP PCL/AP(9%), PCL/AP(16%) and PCL/AP(30%) mats of equal weight have been used, 20 nmol of AP are contained in methanol extracts of different volume. The higher the AP content in the mat sample, the smaller the volume of the extract containing 20 nmol of AP, i.e. the V_{50} value for the respective extract will be smaller. As seen from Fig. 13 and in complete accordance with our assumptions, with the increase of the AP content in the mats the volume of the extract necessary for reduction of 50% of DPPH, decreases.

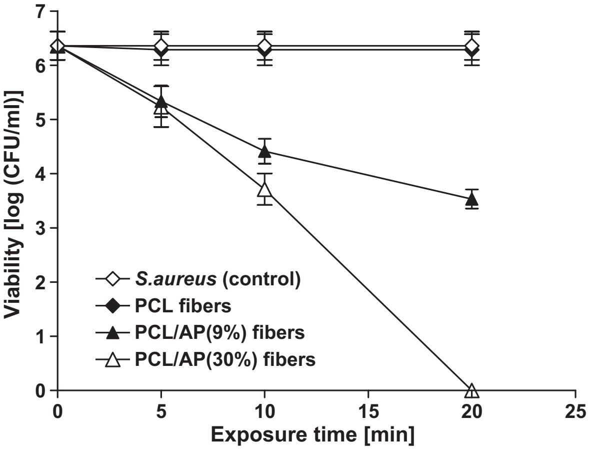


Fig. 14. Log of viable *S. aureus* cells versus the time of exposure to PCL, PCL/AP(9%) and PCL/AP(30%) fibrous materials.

Apart from the antioxidant activity similarly to its precursor – ascorbic acid, AP exhibits antibacterial activity, as well (Tabak et al., 2003). For this reason in the present study the behavior of the PCL, PCL/AP(9%) and PCL/AP(30%) mats in contact with the pathogenic microorganism *S. aureus* has also been investigated. The PCL samples non-containing AP do not inhibit *S. aureus* growth, which is indicated by the fact that the dependence of the number of the viable cells on the exposure time is similar to that of the control (bacterial suspension, Fig. 14). Upon contact with the PCL/AP mats the number of the viable cells substantially decreases and at the 20th min of contact with the PCL/AP (30%) mats there are practically no viable cells. This is an indication that the incorporation of AP imparts antibacterial activity to the PCL mats. The PCL/AP mats, onto which silver nanoparticles have been deposited, exhibit antioxidant and antibacterial activity similar to that of the pristine PCL/AP mats.

4. Conclusions

For the first time the successful incorporation of AP in PCL nanofibrous materials by electrospinning has been demonstrated with the purpose of developing a new carrier, which prevents the vitamin from oxidative destruction. The incorporated AP preserves its stability even after a 4-months storage of the mats in the air. The presence of AP imparts new properties to the PCL mats, such as enabling the deposition of metal nanoparticles by reduction of their salts under the action of AP. The antioxidant and antibacterial properties of the novel PCL/AP nanofibrous materials render them promising candidates for application in medicine and cosmetics.

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