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Electrospun polylactide-based materials for curcumin release: Photostability, antimicrobial activity, and anticoagulant effect

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ABSTRACT: New microfibrous materials from polylactide and polyvinylpyrrolidone or polyethylene glycol loaded with the natural polyphenolic compound curcumin have been prepared by one-pot electrospinning. The incorporation of curcumin in the fibers contributes to shielding curcumin from photodestruction and to enhancement of the mechanical properties of the fibers. Moreover, the formation of hydrogen bonds between curcumin and polyvinylpyrrolidone or polyethylene glycol facilitates the drug release. Curcumin release provides for the antibacterial and anticoagulant activity of the curcumin-loaded mats and prevents adhesion and aggregation of platelets onto the surface of the mats. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 42940.

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INTRODUCTION

The modern medicine and pharmacy increasingly turn to the use of natural substances that often are distinguished with remarkable therapeutic activities. One of them is curcumin—a polyphenolic compound also known as diferuloylmethane [1,7-*bis*-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione]) and it is the major biologically active substance found in the roots of *Curcuma longa* plant. Curcumin is characterized by a variety of properties, which determine its versatile biological activity: antibacterial,[1] antifungal,[2] antioxidant,[3] anti-inflammatory,[4] anticoagulant,[5] and antitumor[6] activity. It is not surprising that nowadays it is used as an ingredient in different food supplements or as active substance for drug delivery system formulations. Disadvantages of curcumin are its thermal and photosensitivity and its ability to be easily oxidized at certain pH values. As a result its chemical structure is not preserved due to the occurrence of degradation processes leading to loss of biologically activity and subsequent limitation of curcumin application.[7] The purpose of the incorporation of curcumin (Curc) in a polymer matrix is to avoid these drawbacks. Curcumin is practically insoluble in pure water; its solubility increases in the presence of protic substances. It has been found that in solid dispersions curcumin forms hydrogen bonds with certain polymers, it is amorphous and compatible or partially compatible with such polymers.[8] The preparation of electrospun nonwoven textile containing amorphous curcumin with enhanced photo-, thermal, and mechanical stability is an attractive strategy to obtain materials having a set of desired biological properties.

Electrospun materials are characterized by a large specific surface area, they are lightweight, easy to manipulate and appropriate for application in medicine, pharmacy, medicinal cosmetics, etc.[9,10] Electrospun fibers are a solid dosage form with versatility in terms of the diversity of polymers and agents that can be formulated.[11] Curcumin has been encapsulated in electrospun textile from poly(L-lactic acid) or poly(L-lactic acid)-*co*-poly(ε-caprolactone) for the fabrication of tissue engineering scaffolds and artificial blood vessels with anticoagulant activity.[12–14] Curcumin-loaded fibrous materials based on polylactide,[15] chitosan/poly(L-lactic acid),[16] poly(ε-caprolactone)[17] have been electrospun aiming at preparation of wound dressings. The photostability of curcumin included in micro- and nanofibers has not been estimated so far.

The present contribution was aimed at studying the possibility to prepare new electrospun materials from polylactide (PLA) and polyvinylpyrrolidone (PVP) or poly(ethylene glycol) (PEG) for curcumin delivery. Special efforts were focused on the detailed characterization of the curcumin-loaded mats:

Additional Supporting Information may be found in the online version of this article.

morphology, hydrophilic-hydrophobic characteristics, thermal, and mechanical properties. With a view to outline the potential biomedical application of the obtained mats, the release of the hydrophobic natural product was modulated in terms of the polymer matrix composition and the formation of water soluble complexes between curcumin and PVP (or PEG) based on hydrogen bonds. In order to study the protection of curcumin embedded in the mats from UV-Vis irradiation damage, the photostability of curcumin was spectrophotometrically quantified. The retaining of its biological properties, such as anticoagulant and antibacterial activity, were investigated in contact with human whole blood and against the Gram positive bacteria *Staphylococcus aureus*, respectively.

EXPERIMENTAL

Materials

For the preparation of the fibrous materials polylactide (PLA; Boehringer Ingelheim Chemicals, Germany), (*L/D, L* = 70/30 given by the supplier) with $\overline{M}_w = 165{,}850$ g mol^{-1} and $\overline{M}_w/\overline{M}_n = 2.1$; polyvinylpyrrolidone (PVP K25; Fluka, Switzerland) with $\overline{M}_r = 24{,}000$ g mol^{-1}, polyethylene glycol (PEG; Fluka, Switzerland) with $\overline{M}_r = 1900$ to 2000 g mol^{-1} and curcumin (Curc; Merck, Germany) were used. Dichloromethane (DCM) was supplied by Merck (Germany), dimethyl sulfoxide (DMSO) was purchased from Fluka (Switzerland) and polyethylene glycol sorbitan monolaurate (Tween® 20) from Sigma Aldrich, Germany. For the anticoagulant assays, reactive reagents Néoplastine® CI Plus 10 and C.K. Prest® 5 (Stago, France) were used. *S. aureus* 1337, methicillin-resistant strain (MRSA) from the Collection of the Institute of Microbiology, Bulgarian Academy of Sciences and brain-heart infusion broth (Difco, BD Diagnostic Systems, Sparks, MD) were used for the microbiological tests.

Preparation of the Fibrous Mats

For electrospinning solutions of PLA, PLA/PEG, or PLA/PVP were used with polymer weight ratio in the binary systems of 70/30 or 60/40. The obtained mats were designated as: PLA, PLA_{70}/PEG_{30}, PLA_{70}/PVP_{30}, PLA_{60}/PEG_{40}, and PLA_{60}/PVP_{40}, respectively. The polymers were dissolved in DCM, and curcumin was dissolved in DMSO. The spinning solution was then prepared by mixing the two solutions at total polymer concentration of 9 wt %, curcumin 15 or 30 wt % in respect to the total polymer weight, and solvent weight ratio DCM/DMSO of 75/25. The curcumin containing mats were designated as: $PLA/Curc_{30}$, $PLA_{70}/PEG_{30}/Curc_{30}$, $PLA_{70}/PVP_{30}/Curc_{15}$, $PLA_{70}/PVP_{30}/Curc_{30}$, $PLA_{60}/PEG_{40}/Curc_{30}$, $PLA_{60}/PVP_{40}/Curc_{15}$, and $PLA_{60}/PVP_{40}/Curc_{30}$.

The electrospinning setup consisted of a high-voltage power supply (up to 30 kV); a pump for delivering the spinning solution (NE-300 Just Infusion Syringe Pump; New Era Pump Systems Inc., USA); a syringe supplied with positively charged metal needle (gauge: 20 G × 1½″); and a rotating grounded drum collector (diameter: 5.6 cm). The fibrous materials were obtained at flow rate: 3.0 mL h^{-1}, applied voltage: 17 kV, tip-to-collector distance: 10 cm, and collector rotation speed: 3200 r min^{-1}.

Scanning Electron Microscopy

The morphology of the fibers was evaluated by SEM scanning electron microscope (SEM, Jeol JSM-5510 (Jeol Ltd., Japan)) after vacuum-coating the samples with gold. The fluorescence micrographs were obtained using fluorescence microscope (NU-2; Carl Zeiss, Jena, Germany). The mean fiber diameter was determined using Image J software by measuring at least 20 random fibers per sample. The dynamic viscosity of the spinning solutions was measured by a Brookfield viscometer provided with thermostat Brookfield TC-102 at 25 ± 0.1°C.

FT-IR Spectra

FT-IR spectra were registered using an IRAffinity-1 spectrophotometer (Shimadzu Co., Japan), supplied with a MIRacle ATR device (diamond crystal with depth of penetration of the IR beam of 2 μm, PIKE Technologies) in the range of 600 to 4000 cm^{-1} with resolution of 4 cm^{-1} using a thermally controlled DLATGS detector. All spectra were corrected for H_2O and CO_2 using an IRsolution software program.

Contact Angle Measurements

The water contact angle of the mats (cut in the collector rotation direction) or of the cast films was determined using an Easy Drop DSA20E KRÜSS GmbH apparatus (Germany). Droplets of deionized water (10 μL) were deposited onto the mats/films and the average value of the contact angle was determined based on 10 different measurements for each sample.

Differential Scanning Calorimetry Analyses

Information about the thermal characteristics of the fibrous materials was obtained by differential scanning calorimetry (DSC, Perkin Elmer DSC 8500) in the temperature range of 0 to 200°C and heating rate of 10°C min^{-1} under nitrogen. Thermogravimetric analyses (TGA) were performed using TA Instrument TGA Q5000 apparatus (USA) while heating the samples up to 1000°C.

Mechanical Properties

The mechanical properties of the fibrous materials were assessed using a Zwick/Roell Z 2.5 apparatus (Germany), load cell 2 mV/V, type Xforce *P*, nominal force 2.5 kN, testXpert II. The stretching rate was 25 mm/min, and the initial length between the grips was 40 mm. All testing samples (size of 20 mm × 60 mm and thickness of ca. 200 μm) were cut in such a manner that their length was along the collector rotation direction. The results are presented as average values obtained from at least ten tested specimens of each mat.

Photostability of Curcumin in the Fibers

The photostability of curcumin in the mats was studied by irradiating the fibrous materials ($PLA/Curc_{30}$, $PLA/PEG/Curc_{30}$, and $PLA/PVP/Curc_{30}$; samples with weight of 2 mg) with UV lamp in the wavelength range from 260 to 600 nm (UVASPOT 400/T, Dr. Hönle AG; UV lamp UV 400 F/2, 400 W, Germany). The mats were irradiated for 30 and 120 min (distance between the lamp and the samples—43 cm). The content of curcumin before and after irradiation was quantitatively determined after dissolving the samples in DCM/DMSO (75/25 wt/wt). The obtained solutions were analyzed by UV-Vis spectrophotometer [DU 800 (Beckman Coulter)] at wavelength of 426 nm and the

amount of curcumin was determined. The residual amount of curcumin in the irradiated electrospun materials was calculated as a percentage of the amount of curcumin contained in the non-irradiated mat of the same composition and weight [see eq. (1)]

Residual curcumin after irradiation (%) = (amount of curcumin in the irradiated mat/amount of curcumin in the nonirradiated mat) × 100. eq. 1

Curcumin Release from the Fibrous Materials

The curcumin release profile was studied from the PLA/$Curc_{30}$, $PLA_{60}/PEG_{40}/Curc_{30}$, and $PLA_{60}/PVP_{40}/Curc_{30}$ fibers. The diffusion of the active substance was studied *in vitro* for 24 h at 37°C in acetate buffer (CH_3COONa/CH_3COOH, pH 5.5 and ionic strength of 0.1) containing Tween 20 (acetate buffer/Tween 20 = 95.5/0.5 v/v). Mats with size of 1 cm^2 were immersed in 100 mL acetate buffer/Tween 20 stirred at 150 r min^{-1} with an electromagnetic stirrer. At determined time intervals, aliquots (2 mL) from the buffer solution were withdrawn and their absorbance was recorded at wavelength of 421 nm. Equivalent aliquots volumes acetate buffer/Tween 20 for the respective times) were added in the buffer solution. The amount of the released curcumin over time was calculated referring to a calibration curve (correlation coefficient $R = 0.999$, wavelength of 421 nm). The results are presented as mean values based on three independent curcumin releases measuring for each mat. The bioactive substance content in the mat (PLA/$Curc_{30}$, $PLA_{60}/PEG_{40}/Curc_{30}$, and $PLA_{60}/PVP_{40}/Curc_{30}$) was determined after dissolving the mats (1 cm^2) in 50 mL DCM/DMSO = 75/25 (w/w), after which the absorbance was measured at wavelength of 426 nm.

Anticoagulant Activity

Determination of APTT and PT. The anticoagulant activity of the mats was studied *in vitro* by measuring the activated partial thromboplastin time (APTT) and prothrombin time (PT) with coagulation analyzer apparatus (STA Compact Max, Stago). The PLA/PVP/Curc mats (weight of 4 mg and size of 1 cm^2) were immersed in 400 μL platelet-poor plasma (PPP) for 30 min at 37°C. The PPP was obtained from human whole blood containing sodium citrate (blood/sodium citrate volume ratio of 9/1) centrifuged at 3000 r min^{-1} for 10 min. After the time of contact the fibrous materials were put out and the PPP was analyzed for APTT and PT. For the APTT assay 50 μL of the contacted PPP was mixed with 50 μL APTT reactive reagent (C.K. Prest 5) and incubated at 37°C for 240 s. Then 50 μL $CaCl_2$ (0.025*M*) was added and APTT was determined. For PT assay, 50 μL of the contacted PPP was mixed with 100 μL PT reactive reagent (Néoplastine CI Plus 10) and incubated at 37°C for 240 s whereupon 50 μL $CaCl_2$ was added and PT was recorded. All tests were performed three times for each sample and the average values were used for the interpretation of the results.

Thromboelastography (TEG). Evaluation of the parameters characterizing the blood clot formation and its strength is realized using thromboelastography analysis. The mats (PLA/$Curc_{30}$, $PLA_{60}/PEG_{40}/Curc_{30}$, and $PLA_{60}/PVP_{40}/Curc_{30}$) are immersed in 1000 μL citrated blood for 30 min. After the time of contact, the fibrous materials are removed and the blood sample is transferred in essay tube containing the contact activator kaolin. From the resulting solution 340 μL was taken and placed into a special TEG cup temperate at 37°C. The thromboelastogram profile was obtained using thromboelastograph hemostasis system 5000 (Haemoscope Corporation, Niles, IL) after addition of 20 μL $CaCl_2$ (0.2*M*).

Platelets and Erythrocytes Adhesion on the Fibrous Materials. The platelet adhesion experiments were carried out using platelet-rich plasma (PRP) obtained from centrifuged human whole blood (1000 r min^{-1} for 10 min) containing sodium citrate (blood/sodium citrate volume ratio of 9/1). The PLA/PVP/$Curc_{30}$ mats with size of 1 cm^2 were immersed in 400 µL PRP at 37°C for 30 min. For the erythrocytes adhesion studies, the mats PLA/PVP/$Curc_{30}$ with size of 1 cm^2 were immersed in 500 μL whole human blood for 30 min. After the contact time (with PRP or whole blood) run out the fibrous materials were rinsed thrice with saline (0.9% NaCl solution). Then the mats were immersed in 2.5% glutaraldehyde solution for 3 h followed by a single rinse step with distilled water. The freeze-dried mats were investigated by SEM in an aim to evaluate the blood cell (platelet and erythrocytes) adhesion. In all experiments with PPP, PRP or whole human blood, the blood sample was drawn by venipuncture from healthy volunteers who had not taken any medication for at least 10 days.

Microbiological Assays

The antibacterial activity of the fibrous materials was tested against the pathogenic microorganism *Staphylococcus aureus* (*S. aureus*). The strains were grown aerobically overnight at 37°C. Cells were harvested by centrifugation and were resuspended in sterile PBS. The mats were cut in form of disks (diameter of 1.7 cm and weight of 7 mg) and placed on the bottom of wells of petri dishes (diameter 15 mm) for cell culturing. Each mat was covered with 0.5 mL *S. aureus* bacterial suspension (cell concentration of 7×10^5 cell mL^{-1}). The bacterial growth was studied after light irradiation (1 h at wavelength of 420 nm, distance between the lamp (10 W) and the surface of the petri dishes—10 cm) and without any irradiation. Then aliquots were withdrawn from each suspension and deposited onto petri dishes with solid agar medium. The later were incubated for 24 h at 37°C and the number of the surviving cells was determined as colony forming units (CFUs).

RESULTS AND DISCUSSION

Morphology and Mean Fiber Diameter

The electrospinning of the spinning solutions resulted in the preparation of cylindrical defect-free fibers aligned predominantly along the collector rotation direction (Figure 1). It was found that the presence of PVP in the fibers led to a decrease in their mean diameter: 1800 ± 300 nm for PLA fibers and 1100 ± 200 nm and 1000 ± 250 nm for PLA_{70}/PVP_{30} and PLA_{60}/PVP_{40} fibers, respectively. These results are consistent with the data obtained from the dynamic viscosity measurements conducted with the spinning solutions. It was found that with the increase in the PVP content the dynamic viscosity was considerably decreased 875 ± 25 cP for PLA, 125 ± 5.0 cP for PLA_{70}/PVP_{30}, and 60 ± 0.5 cP for PLA_{60}/PVP_{40}). The addition

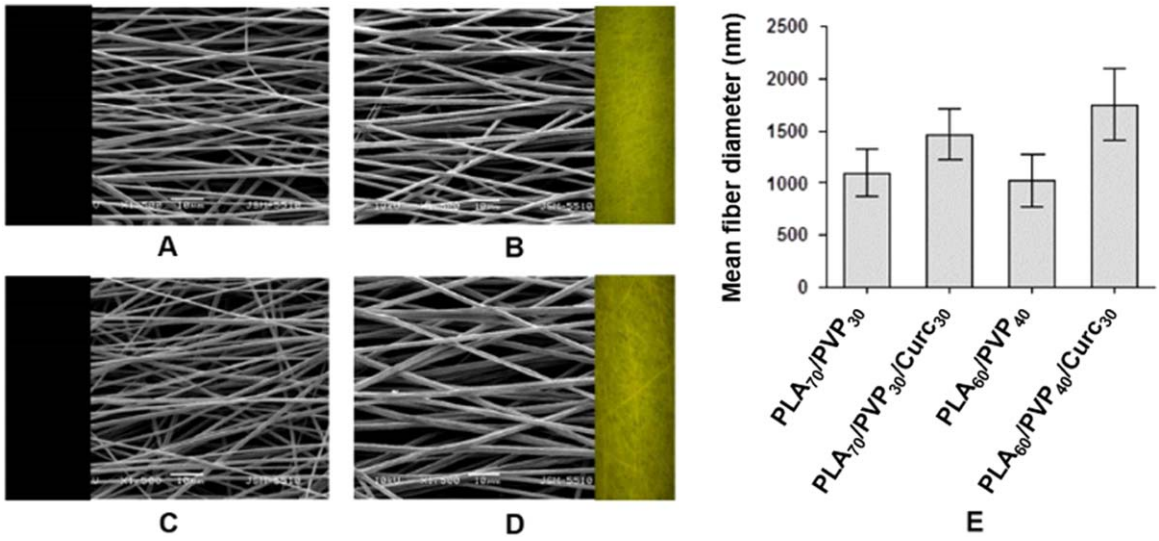

Figure 1. SEM and fluorescence micrographs of fibrous materials based on: PLA_{70}/PVP_{30} (a), $PLA_{70}/PVP_{30}/Curc_{30}$ (b), PLA_{60}/PVP_{40} (c), $PLA_{60}/PVP_{40}/Curc_{30}$ (d); magnification of SEM micrographs: ×1500; magnification of the fluorescence micrographs: ×25. The average fiber diameters are shown in the figure, as well (e). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of curcumin (15 or 30 wt %) to the spinning solutions resulted in the preparation of fibers with larger average diameters [Figure 1(e)]. The impact of curcumin on the fiber diameters is in accordance with the values of the dynamic viscosity of the spinning solutions: 130 ± 3.0 cP for $PLA_{70}/PVP_{30}/Curc_{30}$ and 110 ± 2.0 cP for $PLA_{60}/PVP_{40}/Curc_{30}$. It is known that curcumin displays fluorescence properties, which allowed observation of the mats by fluorescence microscopy. It is evident from Figure 1(a) and Figure 1(c) that the PLA_{70}/PVP_{30} and PLA_{60}/PVP_{40} fibrous materials did not manifest any fluorescence properties which was expected. The incorporation of curcumin in the fibers resulted in recording a fluorescence signal emitted from the mats, which is evidence for the one-step incorporation of the biologically active substance in the fibers by electrospinning [Figure 1(b,d)].

IR-Spectra of the Fibrous Materials

The IR-spectra of curcumin (powder) and $PLA_{60}/PVP_{40}/Curc_{30}$ fibers are shown in Figure 2. For the biologically active substance characteristic bands corresponding to stretching vibrations of C═O and C═C bonds, respectively, were observed at 1506 cm^{-1}, 1151 cm^{-1}, 1026 cm^{-1}. Bending vibrations of C—H (δCH) and C═C (δCC) bonds were recorded at 1427 cm^{-1} and bands characteristic of C—O bonds participating in the formation of an enol bond in the structure of curcumin were observed at 1274 cm^{-1}. Characteristic bands for out-of-plane bending vibrations of the aromatic ring (γAr-H) were observed at 856 cm^{-1} and 808 cm^{-1} and for stretching vibrations of the O-H group at the aromatic ring—at 3508 cm^{-1} [Figure 2(a), inset]. The characteristic bands for PVP were observed at 1647 cm^{-1} for C═O, at 1421 cm^{-1} for the amide bond and at 1286 cm^{-1} for C—N, and those for the polyester—at 1182 cm^{-1}, at 1085 cm^{-1}, and at 1751 cm^{-1}, respectively, corresponding to νCH_3, νC—O—C bonds and δCH_3.

In the IR-spectrum shown in Figure 2(b) some characteristic bands for curcumin in the $PLA_{60}/PVP_{40}/Curc_{30}$ fibers were detected, but the one typical of free hydroxyl groups at the aromatic ring (3508 cm^{-1}) was not observed [Figure 2(b), inset]. This result was attributed to complex formation between PVP and curcumin through hydrogen bonds, which is in accordance with the literature data.[8] The analysis of the $PLA_{60}/PVP_{40}/Curc_{30}$ mats revealed a shift of the characteristic band for PVP assigned to the C═O bond from 1647 cm^{-1} to 1653 cm^{-1}. This can be attributed to intermolecular interactions between C═O groups of PVP and D,L-units of PLA, similarly to the reported data for poly(D,L-lactic acid).[18]

Water Contact Angles of the Electrospun Mats and Films

The water contact angle values of the electrospun mats and those of the films cast from the spinning solutions are presented in Table I. It was found that regardless of the presence of the hydrophilic PVP in the fibers, the mats from PLA_{70}/PVP_{30} and PLA_{60}/PVP_{40} were hydrophobic (120° and 124°, respectively) and the water droplet preserved its spherical shape (Figure 3). Probably, the hydrophobicity in these cases might be attributed to the above-mentioned intermolecular interactions between PLA and PVP. The incorporation of 15 wt % curcumin in the fibers did not exert any significant effect on the value of the water contact angle, which was about 121°.

In our previous study the influence of micro- and nanoarchitecture of the fibrous materials on their capacity to be wetted was examined.[19] In accordance with the data from this study, in this case the values of the water contact angles of the films were also lower compared with those for mats with the same composition (Table I, Figure 3).

Thermal Characteristics of the Fibrous Materials

The thermogram of the PLA fibers displays an endothermic peak for T_g at 64°C, for T_m at 153°C and an exothermic peak for cold crystallization—T_{cc} at 88.5°C (Supporting Information, Figure S1). PVP used in the present study is characterized by a T_g of 156°C. Because of overlapping of the peaks corresponding

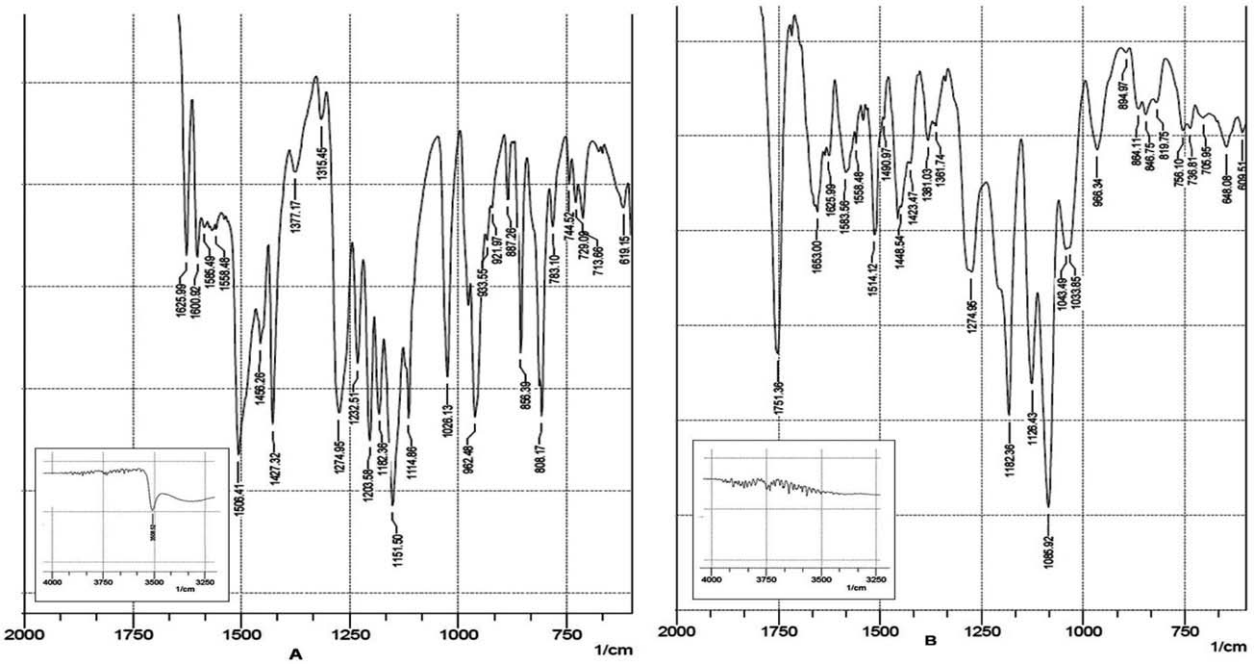

Figure 2. IR-spectra of curcumin (a) and fibrous materials from $PLA_{60}/PVP_{40}/Curc_{30}$ (b) in the range from 2000 to 535 cm^{-1} and from 4000 to 3250 cm^{-1} (insets).

to T_g of PVP and T_m of PLA the calculation of the crystallinity degree of the polyester is rendered difficult. The presence of PVP in the PLA/PVP fibers leads to a decrease in T_g of PLA (59°C for the PLA_{70}/PVP_{30} system and 58°C for PLA_{60}/PVP_{40}). A shift of the endothermic peak for the two polymers towards lower temperatures—150°C, has been recorded, as well. These results are in agreement with the literature data for determination of the thermal characteristics of films obtained from poly(lactic acid)/PVP mixtures.[18]

The incorporation of curcumin in the PLA/PVP mats exerted a greater effect on the T_m value of PLA than on the T_g value. The endothermic peak for the two polymers is shifted to lower temperatures by 5°C (145°C). We assumed that the interaction between curcumin and PVP lowers the T_g of PVP, which in turn leads to a shift in the common peak for PLA and PVP. In the fibers curcumin is found in the amorphous state as evidenced by the absence of an endothermic peak corresponding to a melting point at 175°C (Supporting Information Figure S1). A similar suppression of the formation of crystals from drugs forming complexes with PVP based on hydrogen bonding has been described by other authors, as well.[20]

The thermal stability of the fibrous materials was studied by TGA analysis. In the case of PLA fibers the degradation temperature of the polyester is about 336°C. The incorporation of PVP (T_d ca. 410°C) in the PLA fibers did not result in any significant changes in the thermal stability of the polymers: T_d of the polyester approximately 334°C and 330°C, and that of

Table I. Water Contact Angle Values Measured upon Deposition of a Water Droplet on the Electrospun Mats and Films Cast from the Spinning Solutions

Mat	Water contact angle of mats (°)[a]	Water contact angle of films (°)
PLA_{70}/PVP_{30}	120 ± 2	76 ± 2
$PLA_{70}/PVP_{30}/Curc_{15}$	121 ± 1	66 ± 5
PLA_{60}/PVP_{40}	124 ± 2	80 ± 4
$PLA_{60}/PVP_{40}/Curc_{15}$	121 ± 2	69 ± 4

[a] All mats were cut along the collector rotation direction.

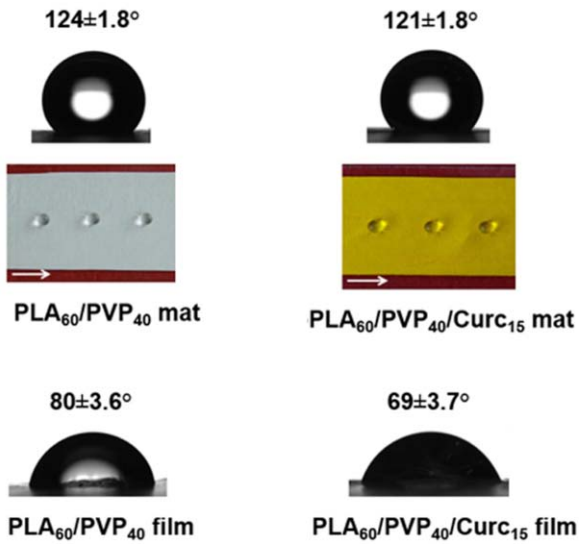

Figure 3. Digital photographs of water droplets on the surface of mats and films; the collector rotation direction is indicated by an arrow. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

PVP—about 413°C, for the PLA_{70}/PVP_{30} and PLA_{60}/PVP_{40} mats, respectively (Supporting Information Figure S2). The incorporation of curcumin in the fibers and the complex formation between the active substance and PVP led to a decrease in the thermal stability of the hydrophilic polymer (394°C for $PLA_{70}/PVP_{30}/Curc_{30}$ and 400°C for $PLA_{60}/PVP_{40}/Curc_{30}$) and to an enhancement of the thermal stability of PLA (338°C for $PLA_{70}/PVP_{30}/Curc_{30}$ and 340°C for $PLA_{60}/PVP_{40}/Curc_{30}$). In the case of the curcumin-loaded fibrous materials up to 90% degradation of the fibers at 1000°C is observed. The 10% residue is most probably due to the presence of aromatic rings in the molecule of curcumin stimulating the process of its thermal carbonization.[21]

Mechanical Characteristics of the Fibrous Materials

It is known from the literature that fibrous materials prepared by electrospinning are characterized by poor mechanical parameters. The latter depend on the diameter of the fibers, their morphology, degree of alignment as well as on the crystallinity degree of the polymers constituting the mat, etc. In one of our previous studies we have proved that the strength of the mats depends on the fiber alignment which can be achieved in various directions or at an angle to the axis of collector rotation. For this reason the manner in which the test bodies are cut is crucial for the standardization of this type of measurements.[19] The values of Young's modulus, the breaking load, and elongation at break of the electrospun mats are presented in Table II.

From the presented stress–strain curves (Figure 4) it is evident that the presence of PVP influences the mechanical properties of the fibrous materials. The presence of 30 wt % PVP led to obtaining of materials with higher values of Young's modulus and breaking load than those of PLA mats (270 and 0.9 MPa, and 68 and 0.4 MPa for PLA_{70}/PVP_{30} and PLA mats, respectively).

It was found that with the increase in the content of the biologically active substance in the PLA/PVP fibers the values of the modulus of elasticity and the breaking load increased (Table II). In the case of PLA_{70}/PVP_{30} fibers with the increase in the strength of the materials a decrease in the degree of deformation was observed: about 9% for the $PLA_{70}/PVP_{30}/Curc_{15}$ fibers and about 3% for $PLA_{70}/PVP_{30}/Curc_{30}$ fibers [Figure 4(b)]. In the case of PLA_{60}/PVP_{40} fibers the incorporation of curcumin in the fibers also led to enhancement of their mechanical characteristics. As seen from Figure 4(c), the mats from PLA_{60}/PVP_{40} fibers similarly to those from PLA_{70}/PVP_{30} fibers were brittle and are characterized by smaller value of maximum elongation at break (not greater than 3%, Table II).

It may be assumed that the improved mechanical properties of the $PLA_{70}/PVP_{30}/Curc$ fibers compared with PLA/PVP ones can be attributed to enhancing the secondary bonding by formation of hydrogen bonds between curcumin and PVP and curcumin and D,L-units of PLA. The obtained results are in agreement with the reported improvement of the mechanical properties of nanofibrous mats based on poly(L-lactic acid)-*co*-poly-(ε-caprolactone) after incorporation of curcumin.[14]

Photostability of Curcumin Embedded in the Mats

As previously noted, curcumin is characterized by high photosensitivity. Upon light irradiation of curcumin solutions in isopropanol (wavelength between 400 and 510 nm for 4 h.) after 15 min degradation of the active substance has been observed, whereat certain cyclic products have been obtained, as well as other low molecular weight aromatic compounds such as vanillin and vanillic acid.[22] It has also been reported that the photostability of curcumin upon exposure to daylight is higher when it is in a dry crystalline state than in solution.[23] In the present study the effect of the duration of light irradiation of the electrospun mats ($260\ nm < \lambda < 600\ nm$) on the photostability of incorporated curcumin was estimated. The results obtained after 30 min irradiation demonstrated that in the $PLA/Curc_{30}$, $PLA_{70}/PVP_{30}/Curc_{30}$ $PLA_{70}/PEG_{30}/Curc_{30}$ and $PLA_{60}/PEG_{40}/Curc_{30}$ fibers curcumin remained unchanged to the greatest extent (more than 95% of its initial content). Only in the case of the $PLA_{60}/PEG_{40}/Curc_{30}$ mat the curcumin content was reduced to 68% (Figure 5). The results obtained after 120 min irradiation indicated that the polymer matrix had exerted a significant effect on curcumin photostability. Curcumin was preserved to the greatest degree in the fibers containing 30 wt % PEG or PVP: the residual content of curcumin in the mat was about 85% and about 76% for $PLA_{70}/PEG_{30}/Curc_{30}$ and $PLA_{70}/PVP_{30}/Curc_{30}$ mats. The polymers used in this study (PLA, PEG, and PVP) can also undergo photooxidation under the action of light with a wavelength above 250 nm.[24–27] It might be assumed that the generation of free radicals in the polymer chains during light irradiation exerts an effect on curcumin photostability; this will be the subject of another study.

Table II. Mechanical Characteristics of the Electrospun Mats

Mat	Young's modulus (MPa)	Breaking load (MPa)	Ultimate deformation (%)
PLA	68 ± 14	0.4 ± 0.1	99.5 ± 7.8
PLA_{70}/PVP_{30}	270 ± 27	0.9 ± 0.1	41.9 ± 3.0
$PLA_{70}/PVP_{30}/Curc_{15}$	390 ± 9	4.0 ± 1.8	8.8 ± 1.9
$PLA_{70}/PVP_{30}/Curc_{30}$	330 ± 26	5.4 ± 1.4	3.1 ± 0.7
PLA_{60}/PVP_{40}	210 ± 17	0.9 ± 0.1	30.4 ± 3.1
$PLA_{60}/PVP_{40}/Curc_{15}$	395 ± 37	7.0 ± 0.2	2.3 ± 0.2
$PLA_{60}/PVP_{40}/Curc_{30}$	405 ± 30	6.0 ± 0.2	1.5 ± 0.2

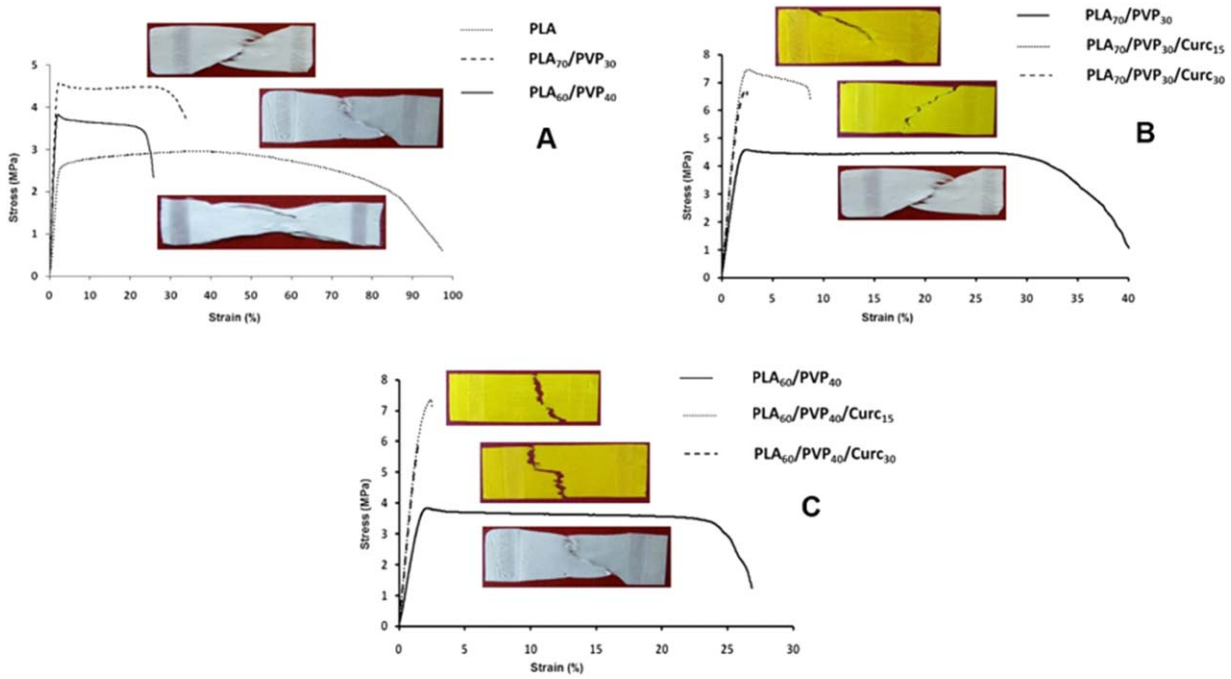

Figure 4. Stress–strain curves of PLA(/PVP) (a), PLA_{70}/PVP_{30}(/Curc) (b), and PLA_{60}/PVP_{40}(/Curc) (c) mats and digital images of the mats. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In Vitro Release of Curcumin

To evaluate the impact of the polymer matrix composition on curcumin release from the fibrous materials the release of curcumin was monitored spectrophotometrically for 24 h in acetate buffer/Tween20 (95.5/0.5 v/v). Tween20 as surfactant and polymers such as PEG and PVP are frequently used as solubilizing agents of poorly soluble drugs to improve their dissolution rate and oral absorption.[28] The release from PLA/$Curc_{30}$, PLA_{60}/PVP_{40}/$Curc_{30}$, and $PLA_{60}/PEG_{40}/Curc_{30}$ mats was studied. From the results presented in Figure 6, it is evident that curcumin was released most slowly and in the smallest amount (ca. 70% for 120 min) from the hydrophobic PLA/$Curc_{30}$ mat even in the presence of surfactant in the dissolution medium. Similar results from release studies of curcumin from PLA fibers have been obtained by other authors where the maximum amount released is about 50% for 120 min.[29]

We found out that curcumin was released more rapidly when the water soluble polymer (PVP or PEG) was included in the fibers. The amount released bioactive substance from the $PLA_{60}/PEG_{40}/Curc_{30}$ fibers during the first stage until a plateau was reached was about 72% for 60 min. Curcumin was released to the greatest extent—about 94%, from the PLA_{60}/PVP_{40}/$Curc_{30}$ mats. Several factors influence curcumin release from the

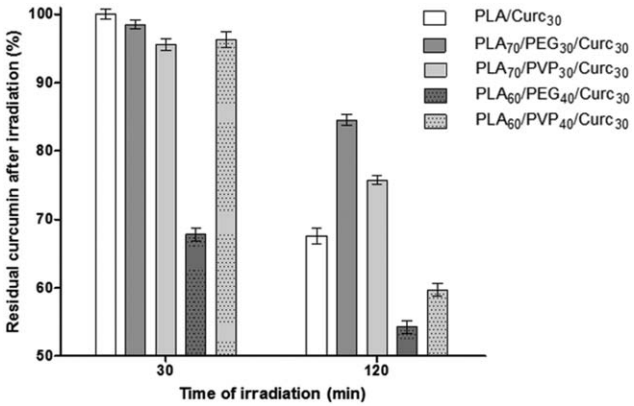

Figure 5. Photostability of curcumin, after 30 and 120 min irradiation of PLA(/$Curc_{30}$), PLA/PEG(/$Curc_{30}$), and PLA/PVP(/$Curc_{30}$) mats.

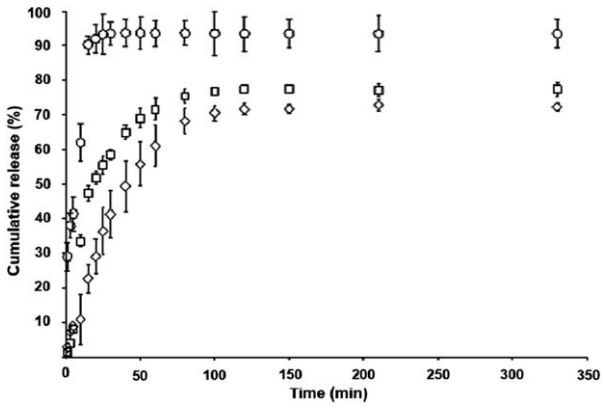

Figure 6. Curcumin release from the PLA/$Curc_{30}$ (◇), PLA_{60}/PEG_{40}/$Curc_{30}$ (□), and $PLA_{60}/PVP_{40}/Curc_{30}$ (○) fibrous materials. The results are presented as average values from three separate measurements with the respective standard deviation; acetate buffer/Tween 20 (95.5/0.5 v/v), pH 5.5, 37°C, ionic strength 0.1.

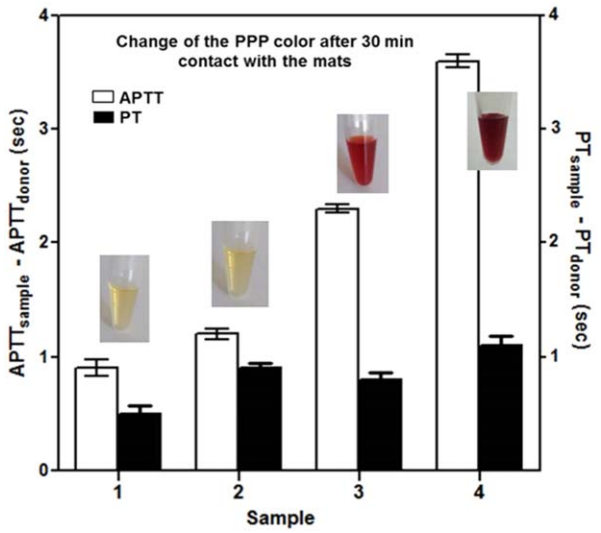

Figure 7. Changes in APTT and PT values expressed as the difference of $APTT_{sample} - APTT_{donor}$ and $PT_{sample} - PT_{donor}$, where $APTT_{donor}$ and PT_{donor} are APTT and PT of the donor (control sample), and $APTT_{sample}$ and PT_{sample} are APTT and PT after 30-min contact of the mats with platelet-poor plasma (PPP); PLA_{70}/PVP_{30} (1), PLA_{60}/PVP_{40} (2), $PLA_{70}/PVP_{30}/Curc_{30}$ (3), and $PLA_{60}/PVP_{40}/Curc_{30}$ (4) mats. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

fibrous materials. In general, the diffusion of a low molecular bioactive substance from micro- and nanofibrous materials is a result of the simultaneous action of (i) the wetting and/or swelling process of the mats which depend on their hydrophilic-hydrophobic characteristics and polymer degree of crystallization, (ii) the crystalline/amorphous state of the drug in the fibers (enhanced release of amorphous drugs), (iii) simple concentration gradient based diffusion of the drug from the fibers, as well as (iv) the diffusion of the water soluble oligomers from the electrospun mats affecting the mats integrity. In addition, the diffusion of the bioactive substance depends also on the fibers morphology (mean fiber diameter and presence of defects or pores). In the present study it was found that curcumin release from the $PLA_{60}/PEG_{40}/Curc_{30}$ and $PLA_{60}/PVP_{40}/Curc_{30}$ mats depended strongly on the materials' hydrophilicity and the interaction between curcumin and PEG or PVP. First, the greater hydrophilicity of the PEG and PVP containing mats as compared with PLA/Curc fibers assists the penetration of the dissolution medium in the mats. This provides conditions water-soluble complex based on hydrogen bonds between curcumin and PEG or PVP to be formed in the immersed mats thus promoting the release of curcumin into the aqueous medium.

Anticoagulant Activity of Curcumin-Loaded Mats

The anticoagulant activity of the fibrous materials was evaluated by determination of the activated partial thromboplastin time (APTT) and prothrombin time (PT) using platelet-poor plasma (PPP). The values of these parameters are expressed as the difference between APTT or PT after 30 min contact of the mats with PPP ($APTT_{sample}$ or PT_{sample}) and APTT or PT of the donor ($APTT_{donor}$ or PT_{donor}): $APTT_{sample} - APTT_{donor}$ and $PT_{sample} - PT_{donor}$ (Figure 7). The values of $APTT_{donor}$ and PT_{donor} were respectively about 30.4 s and 12.5 s. The APTT and PT values after 30-min immersion of the PLA_{70}/PVP_{30} (31.3 s and 12.9 s) and PLA_{60}/PVP_{40} mats (31.6 s and 13.1 s) in PPP did not differ significantly from those of the donor. In the case of curcumin-loaded mats a prolongation of APTT was recorded, by 2.3 s and 3.6 s, respectively, for $PLA_{70}/PVP_{30}/Curc_{30}$ and $PLA_{60}/PVP_{40}/Curc_{30}$ mats, whereas PT was not altered significantly (Figure 7).

The prolongation of APTT is an indication that due to curcumin release the mats display anticoagulant activity and the time for clot formation is longer. PPP changed its color after immersion of the mats. This result was attributed to the diffusion of the anticoagulant agent out from the fibers, with more intensive coloring being observed in the case of mats containing a higher amount of PVP. This suggests that PVP contributes to the more rapid release of curcumin from the fibrous materials, as discussed above in connection with monitoring of curcumin release where the highest amount of released curcumin is observed in the case of the $PLA_{60}/PVP_{40}/Curc_{30}$ fibers.

Thromboelastographic Analysis of Whole Blood after Contact with the Mats

For the first time experiments were performed to study blood clot formation and evaluate the physical characteristics of the clot (degree of formation, strength, and stability) by thromboelastography after contact of the blood samples with the mats. For that purpose $PLA/Curc_{30}$, $PLA_{60}/PEG_{40}/Curc_{30}$ and $PLA_{60}/PVP_{40}/Curc_{30}$ fibers were used.

By means of this method the changes in the viscoelastic properties of the clot can be measured, while recording some major parameters such as: (a) reaction time (R, min) or the time required for clot formation. Generally R is prolonged in the presence of an anticoagulant agent; (b) K-time (min)—the measurement of the rate or kinetics at which a certain level of clot strength is achieved (amplitude of 20 mm in the thromboelastogram profile). Anticoagulant agents prolong K-time; (c) α angle (°) measures the speed at which the fibrin network is formed and crosslinked and provides information about the fibrinogen levels required for clot formation; (d) maximum amplitude (MA, mm) – an indicator of the maximum strength of the clot composed of fibrin network and platelets. The values of R, K, α angle, and MA after 30-min contact of the mats with whole blood are presented in Table III.

From all thromboelastograms obtained after contact of blood with the mats it was evident that R remained practically unchanged—about 2.8 and 2.6 s for $PLA/Curc_{30}$ and $PLA_{60}/PEG_{40}/Curc_{30}$ mats and it was lower for $PLA_{60}/PVP_{40}/Curc_{30}$ (2.0 s). Retardation of clot formation or prolongation of K time under the influence of the anticoagulant agent was recorded for $PLA/Curc_{30}$ (4.2 min) and $PLA_{60}/PEG_{40}/Curc_{30}$ (2.6 min) samples compared with that of the donor (1.8 min). For the $PLA_{60}/PVP_{40}/Curc_{30}$ fibrous material this parameter remained unchanged. In all cases, after contact of the blood with curcumin-loaded mats MA displayed lower values, thus indicating the smaller strength of the formed clot (Table III).

Table III. Values of the Coagulation Parameters after 30-min Contact with PLA/$Curc_{30}$, $PLA_{60}/PEG_{40}/Curc_{30}$, and $PLA_{60}/PVP_{40}/Curc_{30}$ Mats

Sample	*R* (min)	*K* (min)	α (°)	MA (mm)
Normal values	2–8	1–3	55–78	51–69
Donor control	3.5	1.8	64.3	52.9
PLA/$Curc_{30}$	2.8	4.2	50.6	51.5
$PLA_{60}/PEG_{40}/Curc_{30}$	2.6	2.6	58.4	51.5
$PLA_{60}/PVP_{40}/Curc_{30}$	2.0	1.8	67.5	49.2

Adhesion of Platelets and Red Blood Cells onto the Fibrous Materials

An evaluation of the adhesion of platelets and red blood cells onto the fibrous materials was made. Previously we have demonstrated that platelets adhere and aggregate on the surface the fibrous PLA materials.[15] The morphological peculiarities of the cell shape (the presence of prominent pseudopods) and the appearance of a fibrin network are evidence for the occurrence of a process of platelet activation and clot formation. In platelet rich plasma (PRP) with the increase in the PVP content in the fibers the degree of platelet activation and platelet adhesion, as well as that of fibrin network formation decreased [Figure 8(a), PLA_{70}/PVP_{30} and PLA_{60}/PVP_{40} mats). These results are consistent with other data attesting for a decrease in the degree of platelet adhesion in the presence of PVP.[30] On the other hand, curcumin is known for its ability to inhibit platelet aggregation when tests with whole human blood are carried out.[31] In accordance with these data, the incorporation of curcumin in the fibers led to prevention of the adhesion of platelets to the electrospun materials [Figure 8(a), $PLA_{70}/PVP_{30}Curc_{30}$ mat]. The absence of aggregated platelets, as well as of a fibrin network on the surface of these mats corroborated that the biologically active substance exhibited anticoagulant properties and the process of clotting was inhibited. After performing an assessment of the red blood cells adhesion it was concluded that after contact with blood all mats had a characteristic brown-red coloring and the cells had preserved their morphological structure of biconcave discs [Figure 8(b)]. It is known that polymers like dextran, PEG, and PVP (of a low molar mass) are compatible with human blood, but the use of representatives thereof with higher molar mass (dextran 500,000 g mol^{-1}, PEG 35,000 g mol^{-1}, PVP 350,000 g mol^{-1}) can induce red blood cell aggregation.[32] As evident from the SEM micrographs shown in Figure 8, adhered red blood cells were observed, forming aggregates at certain points on the surface of the PLA_{70}/PVP_{30} and PLA_{60}/PVP_{40} fibrous materials [Figure 8(b)]. In the case of a PLA_{60}/PVP_{40} mat the presence of a greater amount of PVP (24,000 g mol^{-1}) in the fibers led to an increase in the degree of cell adhesion and

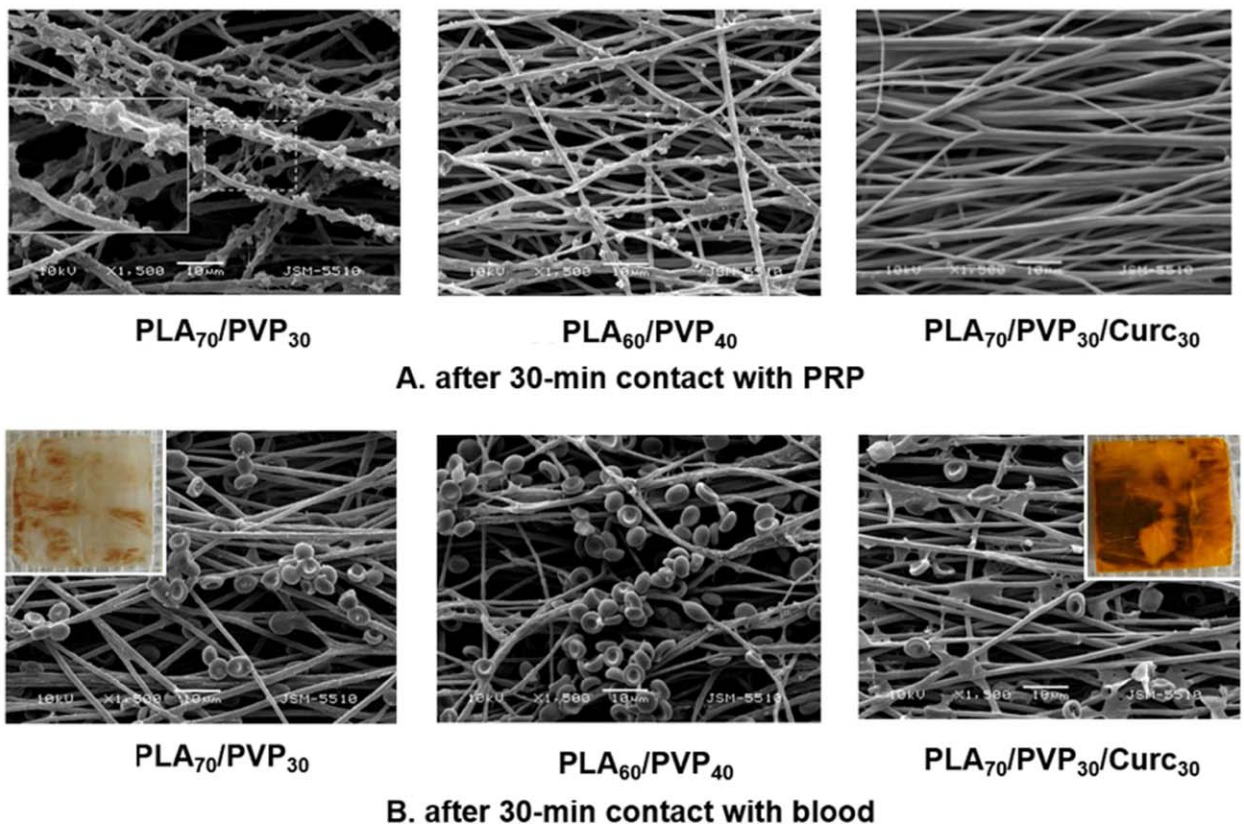

Figure 8. SEM micrographs of PLA_{70}/PVP_{30}, PLA_{60}/PVP_{40}, and $PLA_{70}/PVP_{30}/Curc_{30}$ mats, after 30-min contact with PRP (a) and SEM micrographs and digital photographs (insets) of the mats after 30-min contact with blood (b); magnification: ×1500. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

aggregation as compared with a PLA_{70}/PVP_{30} mat. The incorporation of curcumin in the PLA_{70}/PVP_{30} fibers prevented aggregation, only single nonaggregated red blood cells were observed, the number of adhered cells was smaller.

Antibacterial Activity of the Fibrous Materials Containing Curcumin

For evaluation of the antibacterial activity of the electrospun mats microbiological assays were performed with the pathogenic microorganism *S. aureus*. In contrast to the control sample (PLA_{70}/PVP_{30}) curcumin-loaded mats (after 1 h irradiation) displayed antibacterial activity. The concentration of the living cells after 24-h contact with the mats was reduced by 2 and 3 logarithms for $PLA_{70}/PVP_{30}/Curc_{15}$ and $PLA_{70}/PVP_{30}/Curc_{30}$ fibers, respectively (initial bacterial cells concentration—7×10^6 CFU mL^{-1}). The fibrous materials incubated in the dark (without irradiation) did not exhibit any antibacterial activity (see Supporting Information Figure S3).

CONCLUSIONS

Electrospinning enables the facile preparation of defect-free curcumin-loaded fibers. It provides means to prepare easy-to-handle curcumin-loaded materials, and enables shielding of curcumin from photoirradiation. The presence of polymers which are able to form hydrogen bonds with curcumin, such as PEG and PVP, facilitates the drug release via the formation of water soluble complexes, alters the thermal stability and improves the tensile strength of the mats. The incorporated curcumin is in the amorphous state which is favorable for use as drug dosage forms. It is possible to modulate the curcumin release profile by appropriate selection of the composition of the polymer matrix. In addition, curcumin from the PLA/PVP/Curc mats displays anticoagulant activity and prevents adhesion and aggregation of platelets onto the surface of the mats. After light irradiation, the PLA/PVP/Curc mats manifest antibacterial activity against *S. aureus*. Therefore electrospun PLA/PVP/Curc materials are promising candidates for use in drug-eluting biomedical devices contacting with blood where blood coagulation is not desired and as antibacterial wound dressings.

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