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Research article

Design and characterization of curcumin-loaded electrospun nanofibers based on poly(vinyl alcohol) and sodium alginate

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Abstract. Curcumin (CRC) is a natural active principle with important anti-inflammatory, antioxidant, antibacterial, and antitumor properties but has some limitations, such as poor bioavailability, low water solubility, and rapid metabolism. To preserve CRC's benefits and eliminate its limitations, novel CRC-loaded core-shell electrospun nanofibers were designed. The nanofibers were prepared by co-axial electrospinning method using poly(vinyl alcohol)/CRC as core and poly(vinyl alcohol)/sodium alginate as shell. Polymer coating protects the CRC, increasing its stability. The swelling degree of CRC-loaded nanofibers at pH 5.4 was around 326% higher than at pH 7.4 (297%) due to the repulsions of the anion-anion COO– groups. The release efficiency of CRC at pH 7.4 was 81%, while at pH 5.4 was about 96%. In the first 6 h, there was a slower release of CRC from the nanofibers in both acidic and slightly alkaline environments. Nanofibers showed good hemocompatibility, the values being between 2.36–3.22% after the first 90 min of contact, and after 180 min of treatment, the degree of erythrocyte lysis was between 3.78 and 4.93%. Cell viability assay on V79-4 Chinese hamster fibroblasts demonstrated that treatment with free CRC led to a value of 39% whereas for CRC-loaded nanofibers, the cell viability value increased to 59.66%. The results of the present study indicated that CRC-loaded electrospun nanofibers can have great potential for biomedical applications.

Keywords: *electrospun nanofibers, curcumin, poly(vinyl alcohol), alginate, hemocompatibility*

1. Introduction

Electrospun nanofibers are promising materials which can be successfully used for biomedical applications, such as: tissue engineering, wound healing, osteoporosis treatment and other diseases, including yocardial infarction, cancer therapy, psoriasis [1, 2]. These biomaterials represent an ideal solution for improving the life quality of millions of patients around the world due to their remarkable properties such as high porosity, adjustable size, high surface area, good mechanical properties, excellent biocompatibility with cells and high biodegradability [3]. Among nanofiber's preparation methods, like: self-assembly, freeze-drying, template synthesis, and

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temperature-induced phase separation, electrospinning is a versatile procedure for obtaining fibrous structures with controllable compositions, shapes and morphologies [4, 5]. The nanofibrous structures are more efficient wound healing systems compared to traditional dressings due to their distinguished features, like: size similarity to the diameter of collagen fibrils in the natural extracellular matrix, tailorability, persistent diameter, ability to absorb exudates and provide a moist environment for respiration and cells proliferation [6, 7]. All these qualities, but also the porous nature of these structures can reduce bacterial infection, can ensure high permeability, can protect injured tissue from dehydration and may allow release of drugs or other bioactive molecules in a sustained manner [6]. The low effectiveness of antimicrobial agents on antibiotic-resistant bacterial infections can lead to increased mortality and changes in the ability of microorganisms to resist antibacterial agents, thus contributing to a decrease in their therapeutic effectiveness [8, 9]. In recent years, many attempts to improve the efficiency and to
reduce the duration of wound healing have focused on increasing the antimicrobial activity of topically applied formulations by preventing the incidence of infections, improving tissue regeneration while reducing pain, discomfort, and scarring [10]. However, a lot of improvements still must be done in order to translate these formulations to a clinical stage.

In this context, the general objective of the present study consisted in the preparation, by coaxial electrospinning, of a new type of curcumin (CRC)-loaded nanofibers based on poly(vinyl alcohol) (PVA), sodium alginate (AG) and [4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride] (DMT-MM), the latter used as an activator of the cross-linking reaction.

Curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione) a lipophilic polyphenol is a highly potent bioactive agent found in turmeric with important anti-inflammatory, anti-oxidant, antibacterial and even antitumoral properties [11–13]. Curcumin has been used for the treatment of various inflammatory conditions and chronic diseases. Regarding the topical application of CRC, it has been shown that it has an effective contribution to wound healing mechanisms by acting in different stages, such as the inflammatory, maturation and proliferative ones, thus improving the overall wound healing process [12, 14]. Besides these advantages, there are also some limitations regarding curcumin's therapeutic effectiveness, such as poor bioavailability, low solubility in water and and rapid degradation in physiological buffer by hydrolysis [15, 16]. Another disadvantage occurred at high concentrations of CRC in its topical application which proved to be toxic [17]. In order to preserve the benefits of curcumin, on the one hand, and to eliminate the limitations and disadvantages, on the other hand, new nanofiber-based systems have been designed for its incorporation.

Two polymers were proposed for the preparation of nanofibers, a natural polymer – AG and a synthetic polymer – PVA. Sodium alginate is mostly found in brown algae and is often used in biomedical applications, such as wound healing, drug delivery, and tissue engineering due to its properties of biocompatibility, biodegradability, and lack of toxicity [18, 19]. AG electrospinning has been used in the biomedical field in several applications because it can provide a larger surface area compared to the traditional wet spinning technique [20]. However, there are some limitations due to the lack of molecular entanglement and the rigid structure of AG, which makes difficult to obtain a continuous and uniform fibrous structure from pure alginate solutions by electrospinning [21].

PVA is an efficient spinning aid as it can provide sufficient entanglement with alginate at the molecular level [22–24]. Moreover, PVA is a water-soluble and biodegradable polymer that also has good biocompatibility, being often used in biomedical applications [25]. Due to his amphiphilic structure, it is generally used as a surfactant in nanoparticulate formulations and in hydrogels in order to improve mechanical properties of the obtained films [26]. PVA is also used in combination with AG because it increases the mechanical but also the biodegradable and hydrophilic properties of composite nanofiber membranes and has a significant contribution to obtaining uniform nanofibers [27–29].

Electrospun AG/PVA nanofibrous scaffolds have already been investigated for skin tissue engineering [30], wound dressing [28, 31] and bone regeneration process [32].

According to the studies developed by Jadbabaei *et al*. [30] one of the main weaknesses of sodium alginate is the low spinning capacity. The authors demonstrated that the combination of AG with PVA in a ratio of 1:6.5 led to obtaining uniform nanofibers

with good physical, chemical, mechanical and biological characteristics which can be successfully used for skin tissue engineering. In another study, Wang *et al*. [28], developed nanofibers based on AG and PVA loaded with taxifolin for the treatment of diabetic wound healing. In both studies, PVA was added to improve the electrospinning and mechanical properties of AG.

However, to the best of our knowledge, electrospun nanofibers based on PVA and AG in the presence of DMT-MM as an activator and containing curcumin in the core have not been reported until now.

The originality of this study consists in the use of an activator whose role is to promote the esterification reaction between PVA and AG, leading to the obtaining of better individualized nanofibers with smaller dimensions, higher stability in environments that mimic biological fluids and nontoxicity as compared to classical covalent crosslinking agents. Another novelty aspect is given by the fact that the nanofibers obtained in this study were prepared by co-axial electrospinning with the CRC solution as core and polymer solution as shell, which confers better stability of CRC because it is protected by the polymer shell. CRC-loaded electrospun nanofibers based on PVA and AG with diameters between 140 and 200 nm were obtained, by varying the electrospinning parameters. The obtained nanofibers can have great potential be used for biomedical applications evidenced by their good release capacity of CRC, high hemocompatibility and non-toxicity.

2. Materials and methods

2.1. Materials

Poly(vinyl alcohol) (PVA), 85.5-86.5% hydrolyzed, M_w: 85 000–124 000 g/mol and alginic acid sodium salt (AG), high viscosity were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Curcumin (CRC), 95% (total curcuminoid content), from Turmeric rhizome has been purchased from Alfa Aesar. 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride, an activator which was required for the nanofibers preparation, was acquired from Merck Millipore (Darmstadt, Germany). The biological materials used for *in vitro* cytotoxicity test were: V79-4 (*Cricetulus griseus*) chinese hamster fibroblast cell line (ATCC CCL-93); dulbecco's modified growth medium (DMEM), (PAN-Biotech GmbH, Aidenbach, Germany); fetal bovine serum (FBS) (Euroclone S.p.A., Milan, Italy); 1% antibiotic solution (Capricorn Scientific GmbH, Ebsdorfergrund, Germany); trypsin/ethylenediaminetetraacetic acid (EDTA) (Biowest, Nuaillé, France); MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Merck KGaA, Darmstadt, Germany).

2.2. Preparation of curcumin-loaded nanofibers

Nanofibers based on sodium alginate (AG) and poly (vinyl alcohol) (PVA) were prepared by co-axial electrospinning using a PVA or PVA/curcumin mixture for the core and a solution of PVA and AG for the shell [12]. A Spinbox Electrospinning from Bioinicia (Valencia, Spain) equipment was used for the preparation of the nanofibers. Esterification of the carboxylic groups of AG with the hydroxyl groups of PVA occurred in the presence of DMT-MM, an activating agent (Figure 1).

The parameters considered in this study were the following: the ratio between the two polymers, the amount of activator, the voltage [kV] and the distance

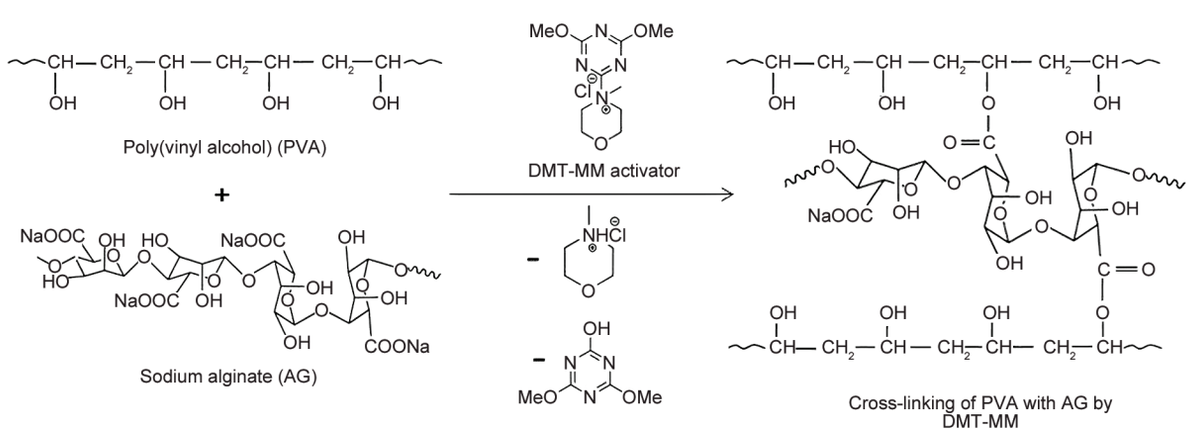

Figure 1. Esterification reaction of PVA with AG in the presence of DMT-MM activator.

between the co-axial emitter and the collector. The 10% PVA solution was prepared by dissolving PVA in distilled water at a temperature of 80–90 °C using a magnetic stirrer until the solution became homogeneous. AG solution, at a concentration of 1.5 wt%, was prepared by dissolving high molar mass AG in distilled water at room temperature. After the complete dissolution of the polymers, the two solutions were mixed at room temperature under continuous magnetic stirring. Separately, the activator solution [4-(4,6-dimethoxy-1,3,5-triazinyl-2)-4-methylmorpholinium chloride] (DMT-MM) (see Table 1) in distilled water (1 ml) was prepared. The activator solution was added over the polymer solution and was kept under continuous magnetic stirring overnight. For CRC-loaded nanofibers (PAAC samples), a volume of 10 ml of 10% PVA solution was mixed with 1 ml of CRC solution according to Table 1. CRC was solubilized in DMSO at a concentration of 10 mg/ml. To obtain the nanofibers, the polymer solutions were introduced into the syringes of the electrospinning apparatus equipped with tubes that were connected to the co-axial emitter provided with a needle connected to a high voltage source. The polymer solutions were passed through a spinning hole (jet) and then drawn using electrostatic energy with a direct current voltage that varied between 25 and 30 kV. The resulting fibers were collected on a collecting drum. The distance between the co-axial emitter and the collector varied between 10–20 cm and the flow rate used for pump 1 (core) and for pump 2 (shell) varied between 0.003–0.01 ml/min (see Table 1).

2.3. Characterization

2.3.1. Structural characteristics

FT-IR analysis of the obtained nanofibers was performed using a Shimadzu IRSpirit spectrometer (Kyoto, Japan) with QATR™-S Single-Reflection ATR Accessory. The measurement wavenumber range was between 400–4000 cm^{-1} (wide-band). The nanofiber samples were tightly clamped on the prism surface before measurement on the sample surface. The incident angle was 45°.

2.3.2. XRD analysis

XRD characterization was performed to study the crystallinity of pure CRC and nanofibers with and without CRC. X-ray diffraction analysis was performed on a Rigaku SmartLab X-ray diffractometer (Tokyo, Japan) in Bragg–Bretano geometry in the angular range 2–60° (2θ), Cu anode (X-ray wavelength of 1.5406 Å) with a scanning step of 0.07º and a recording rate of 5º/min.

Table 1. Experimental program used to obtain nanofibers.

Sample code*	PVA/AG ratio [mg/mg]	AG/DMT-MM ratio [mol/mol]	Curcumin/PVA [mg/mg]	Flow rate for pump 1 – in the middle [ml/min]	Flow rate for pump 2 – outside [ml/min]	Distance between the coaxial emitter and the collector [cm]	Voltage [kV]
P-1	100/0	–	–	0.003	0.003	15	29±1
P-2	100/0	–	–	0.008	0.008	15	29±1
P-3	100/0	–	–	0.008	0.008	20	29±1
P-4	100/0	–	–	0.01	0.01	10	29±1
P-5	100/0	–	–	0.01	0.01	10	25±2
PA-1	90/10	–	–	0.01	0.01	15	29±1
PA-2	80/20	–	–	0.01	0.01	15	29±1
PA-3	70/30	–	–	0.01	0.01	15	29±1
PA-4	50/50	–	–	0.01	0.01	15	29±1
PAA-1	90/10	1/1	–	0.01	0.01	15	30±1
PAA-2	90/10	1/2	–	0.01	0.01	15	30±1
PAA-3	90/10	1/3	–	0.01	0.01	15	30±1
PAA-4	80/20	1/3	–	0.01	0.01	15	30±1
PAAC-1	90/10	1/1	0.05/1	0.01	0.01	15	30±1
PAAC-2	90/10	1/2		0.01	0.01	15	30±1
PAAC-3	90/10	1/3		0.01	0.01	15	30±1

*P: nanofibers based on PVA, PA: nanofibers based on PVA and AG, PAA: nanofibers based on PVA and AG in the presence of activator, PAAC: nanofibers based on PVA, AG and activator prepared by co-axial electrospinning using as a core a CRC/PVA solution.

2.3.3. Morphological characteristics

Morphology, structure and diameter of the nanofibers were investigated by scanning electron microscopy (SEM). The analysis of the nanofibers in a dry state was carried out in duplicate, using an equipment type Quanta 200 scanning electron microscope (Oregon, USA). The mean diameters of the nanofibers were measured and calculated using the ImageJ software that is commonly used for the processing and analysis of microscopy images.

2.3.4. Swelling behavior of nanofibers in aqueous solutions

The swelling degree of the nanofibers was investigated in solutions mimicking biological fluids (PBS, pH 7.4 and 5.4) and was imposed as a result of their potential use as drug carriers. The swelling degree was determined by the gravimetric method. 20 mg of nanofibers were immersed in PBS solutions where they were kept for 7 days (168 h) at a temperature of 37 °C. At predetermined time intervals, the nanofiber membrane was removed from the medium, traces of water were eliminated using filter paper, and the sample was weighed using an analytical balance. The swelling degree Q was calculated using Equation (1):

$$Q\,[\%] = \frac{w - w_0}{w_0} \cdot 100 \qquad (1),$$

where w is the nanofiber membrane in a swollen state; w_0 is the nanofiber membrane in a dry state before being immersed in the medium

2.3.5. Curcumin loading and release studies

100 mg of nanofibers were placed in 5 ml of DMSO and kept under energetic magnetic stirring to solubilize all the amount of CRC loaded in the nanofibers. After 24 h, the supernatant was analyzed by UV–Vis spectroscopy to quantify the amount of CRC released from the nanofibers. Then, the nanofiber membrane was introduced into a fresh DMSO solution and this maneuver was repeated until all CRC amount was released from the nanofibers. The amount of CRC loaded in nanofibers was approximately 0.045 mg CRC/1 mg nanofibers. *In vitro* release of CRC from nanofibers was performed using static Franz diffusion cells (Copley vertical diffusion cell system). An artificial cellulose membrane was used for this test. The membrane separates the donor compartment, which contains the test material, from the receiver compartment filled with 7 ml of collection medium (PBS, pH 7.4 and 5.4 containing 1% Tween 80) at 37±1 °C. The receiver liquid was well stirred throughout the experiment. Sink conditions were respected throughout the experiment. 1 ml of receptor solution was withdrawn and replaced with an equal volume of fresh prethermostated medium. CRC concentration in the receptor medium was determined by UV–Vis spectroscopy (Nanodrop One UV–Vis Spectrophotometer from Thermo Fischer, Massachusetts, USA) at a wavelength of 223 nm. The experiment was performed in triplicate. To calculate the release efficiency of CRC, *Ref* [%], the Equation (2) was used:

$$Ref\,[\%] = \frac{m_r}{m_l} \cdot 100 \qquad (2)$$

where m_r is the amount of CRC released from nanofibers [mg]; m_l is the amount of CRC loaded into nanofibers [mg].

2.3.6. Hemolysis test

The hemolysis test of nanofibers was imposed due to the fact that these membranes might come into contact with blood. *In vitro* evaluation of hemolytic properties is an important analysis of biomaterials that will come into contact with blood. Institutional ethical approval and appropriate informed consent were obtained before performing the hemolysis test. The test was carried out as it was described in the literature by Nica *et al.* [33], Rață *et al*. [34] and Chhatri *et al*. [35]. Human blood from healthy, non-smoking volunteers was used for this test. Nanofiber discs with a diameter of 5 mm were placed in Eppendorf tubes containing 0.5 ml of 0.9% saline and were allowed to equilibrate for 1 h. Then, 0.5 ml of erythrocyte suspension was added to each Eppendorf. The erythrocyte suspension was obtained by adding 25 ml of physiological serum over 5 ml of blood purified by repeated washings with physiological serum. In parallel, positive control samples with 100% lysis (0.5 ml of erythrocyte suspension was added over 0.5 ml of Triton X-100 solution, 2% (w/v)), and negative control samples with 0% lysis (0.5 ml of erythrocyte suspension was added over 0.5 ml of 0.9% saline solution) were prepared. The samples were left in the oven for 90 and 180 min at a temperature of 37 °C. After incubation, the samples were centrifuged and the oxyhemoglobin absorbance was measured spectrophotometrically using a UV-Vis Nanodrop One spectrophotometer.

Samples were analyzed in triplicate, and the erythrocyte lysis degree was calculated according to Equation (3):

$$Haemolysis\ [\%] = \frac{Abs_{sample} - Abs_{negative\ control}}{Abs_{pozitive\ control} - Abs_{negative\ control}} \cdot 100 \quad (3)$$

2.3.7. Cytotoxicity test

Cytotoxic effects evaluation of the simple nanofibers (PAA-1), nanofibers with curcumin (PAAC-1 and PAAC-2) and curcumin (CRC), was performed on V79-4 (*Cricetulus griseus*) fibroblast (ATCC CCL-93), adherent cells. V79-4 cells were cultured in Dulbecco's Modified Growth Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution (penicillin 100 µg/ml and streptomycin 100 IU/ml), maintained in an incubator (Binder, GmbH, Tuttlingen, Germany) at a temperature of 37 °C in a humidified environment with 5% CO_2 [36]. The evaluation of the nanofibers effect on cell viability was carried out by the modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric method [37, 38]. Discs of nanofiber membranes with a diameter of 5 mm and free curcumin were inserted into the plates (24-wells) in the medium of culture, then V79-4 cells were detached with trypsin/EDTA, counted and cultured in 24-well plates ($5 \cdot 10^4$ cells/well), over the mentioned materials. Nanofibers without curcumin (PAA-1), nanofibers with curcumin (PAAC-1, PAAC-2) with a composition of 1 mg polymer/0.048 mg curcumin and free curcumin (in a quantity equivalent to that of nanofibers) were used for the test. After 48 h of treatment, the cells were processed respecting the steps of the MTT cell viability assay, and the absorbance was measured at 570 nm using the Biochrom EZ Read 400 automated microplate reader (Biochrom Ltd. Cambridge, United Kingdom).

Cells morphology

Morphological evaluation of V79-4 cells was assured by a Nikon TS100 inverted microscope supplied with a MshOt MS60 digital camera (Nikon, Tokyo, Japan), using a 10× objective.

Statistical analysis

In vitro experiments were carried out in triplicate, and results were expressed as mean ± standard error (SE). The data were statistically interpreted using Student's t-test. Probability values lower than 0.01 ($p < 0.01$) were considered statistically significant.

3. Results and discussions

3.1. FT-IR spectroscopy

FT-IR spectra of free CRC, nanofibers without CRC (PAA-1) and nanofibers with CRC (PAAC-2) presented in Figure 2 confirmed both the reaction between the AG and PVA and the presence of CRC in the obtained nanofibers. The characteristic peaks of the alginate at 1595 cm^{-1} (C=O bond) and the characteristic peaks of PVA at approximately 3290 cm^{-1} (O–H vibrations) and 1721 cm^{-1} (C=O group) can be observed in the FTIR spectra. The intense peak observed at 3288 cm^{-1} in the spectra of PAA-1 and PAAC-2 samples is attributed to the stretching of the hydroxyl group (OH) [39]. In the spectra of PAA-1 and PAAC-2 nanofibers, a shifting of the peaks from 3290 to 3288 and 3260 cm^{-1} due to hydrogen bonds is observed. The peaks in the 1700–1735 cm^{-1} region and 1250 cm^{-1} corresponding to C=O and C–O groups reveal the presence of ester groups formed between AG and PVA [40]. Also, a decrease in intensity of the peak was observed from 1721 cm^{-1}, and it shifted to 1710 cm^{-1}. All this reveals the interaction between AG and PVA. The appearance in

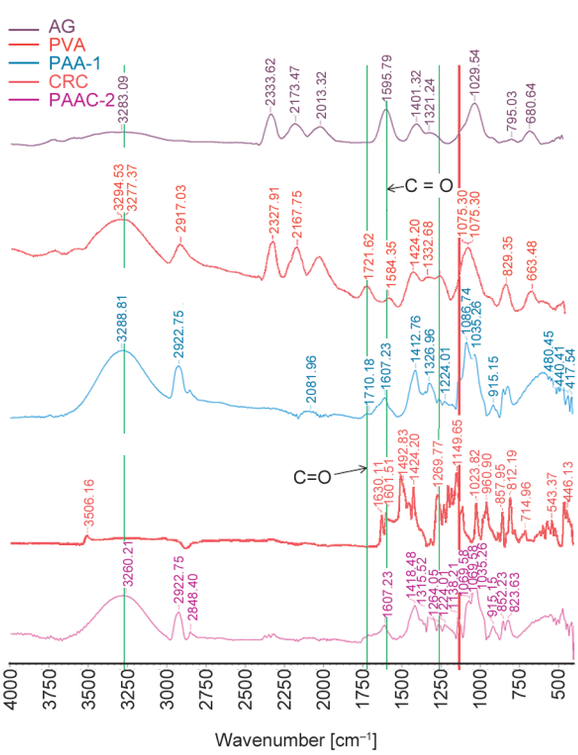

Figure 2. FT-IR spectra for simple CRC, nanofibers without CRC (PAA-1) and nanofibers with CRC (PAAC-2).

the spectrum of the PAAC-2 sample of the peak characteristic of the phenolic group (C–O) at 1269 cm^{-1}, as well as the shift of the peak from 1149 cm^{-1} in the CRC spectrum to 1138 cm^{-1} in the spectrum of the PAAC-2 sample confirms the presence of CRC in PAAC-2 type nanofibers.

3.2. XRD analysis

X-ray diffraction (XRD) is a common method to investigate the crystalline structure of solid materials. The crystallinity degree has an important influence on the material properties such as hardness, melting point, density and transparency. Therefore, XRD was used to analyze the crystalline and purity nature of nanofibers with and without curcumin and of curcumin. XRD spectra of pure CRC, nanofibers without CRC (P-5 and PAA-2) and nanofibers with CRC (PAAC-2) are presented in Figure 3.

Peaks of 2θ at 8.75° (d = 10.098 Å) and 17.5° (d = 5.064 Å) from pure CRC and PAAC-2 nanofibers spectra reveal the successful loading of CRC into the nanofibers. Likewise, it was observed a diminution of the intensity of the pronounced peak of 2θ at 19.5° (d = 4.549 Å) from spectra of P-5, PAA-2 and PAAC-2 nanofiber samples. These results are in accordance with the data from the specialized literature [31]. Moreover, XRD is a usual method to assess the state of active principles that are loaded within drug carriers, and therefore, it was carried out in order to investigate the physical form of CRC loaded into the nanofibers compared to free CRC. In fact, several intense sharp Bragg peaks at 8.75, 14.62, 17.31 and 18.2°, indicating its crystalline nature, can be observed in the spectrum of free CRC. In contrast, only two of these intense and sharp peaks are noticed in the profile of CRC-loaded

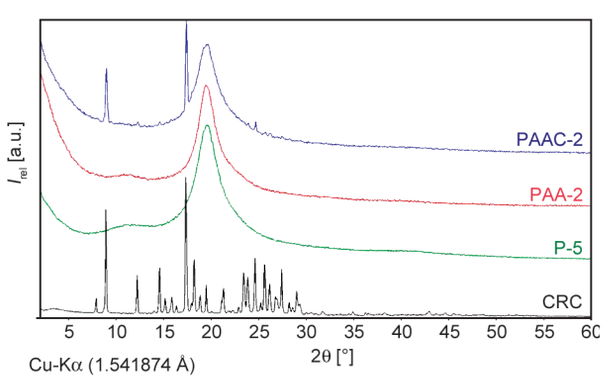

Figure 3. XRD spectra of pure CRC, nanofibers without CRC (P-5 and PAA-2) and nanofibers with CRC (PAAC-2).

nanofibers (PAAC-2 sample), demonstrating a reduction in the overall crystallinity. This suggests that CRC is entrapped within the electrospun nanofibers in a disordered crystalline or amorphous state.

3.3. Morphological characteristics

The morphology, structure, and diameter of obtained nanofibers were investigated by scanning electron microscopy (SEM) and the obtained results are presented in Figures 4–6.

The mean diameter of the nanofibers varied between 140 and 200 nm. To calculate the mean diameter illustrated in Figure 5 and Figure 6, the ImageJ program was used, and a number of 50 nanofibers were taken into account. It can be observed that the control nanofibers, obtained only from PVA (P1–P5), show a relatively uniform distribution without beads, most likely due to the higher viscosity of the polymer solution. The addition of AG to the system (samples PA-1 and PA-2) and the decrease of the amount of PVA led to an increase in the number of beads. This behavior can be due to the reduction of the viscosity of the polymer solution. It is important to notice that after the addition of the activator to the system (samples PAA-1, PAA-2, PAA-3), no beds were observed. After adding the activator, an esterification reaction of the carboxylic groups of AG with the hydroxyl groups of PVA takes place, which leads to a more viscous solution and, therefore, to the disappearance of the beads [41]. For obtaining smooth nanofibers, it was found that the most favorable ratio between PVA/AG was 90/10, wt/wt, and, consequently, for the loading of CRC, this ratio was the starting point for the preparation of PAAC-1 and PAAC-2 samples. In order to optimize the diameter of nanofibers and to avoid sticking the nanofiber structures to each other, different voltages that varied from 25 to 30 kV were applied. When the applied voltage was 25 kV, the electric field strength was not sufficient to properly stretch the jets, which resulted in the nanofibers sticking to each other due to incomplete solvent evaporation. Increasing the applied voltage from 25 to 29–30 kV led to a greater spread of the jets and, consequently, to a better uniformity of the nanofiber diameters. Flow rate and distance between the co-axial emitter and the collector also play an important role in the evolution of the nanofiber's mean diameter (Figure 6). It was found that with the increase in the flow rate, the size of the nanofibers decreased. At a distance between the

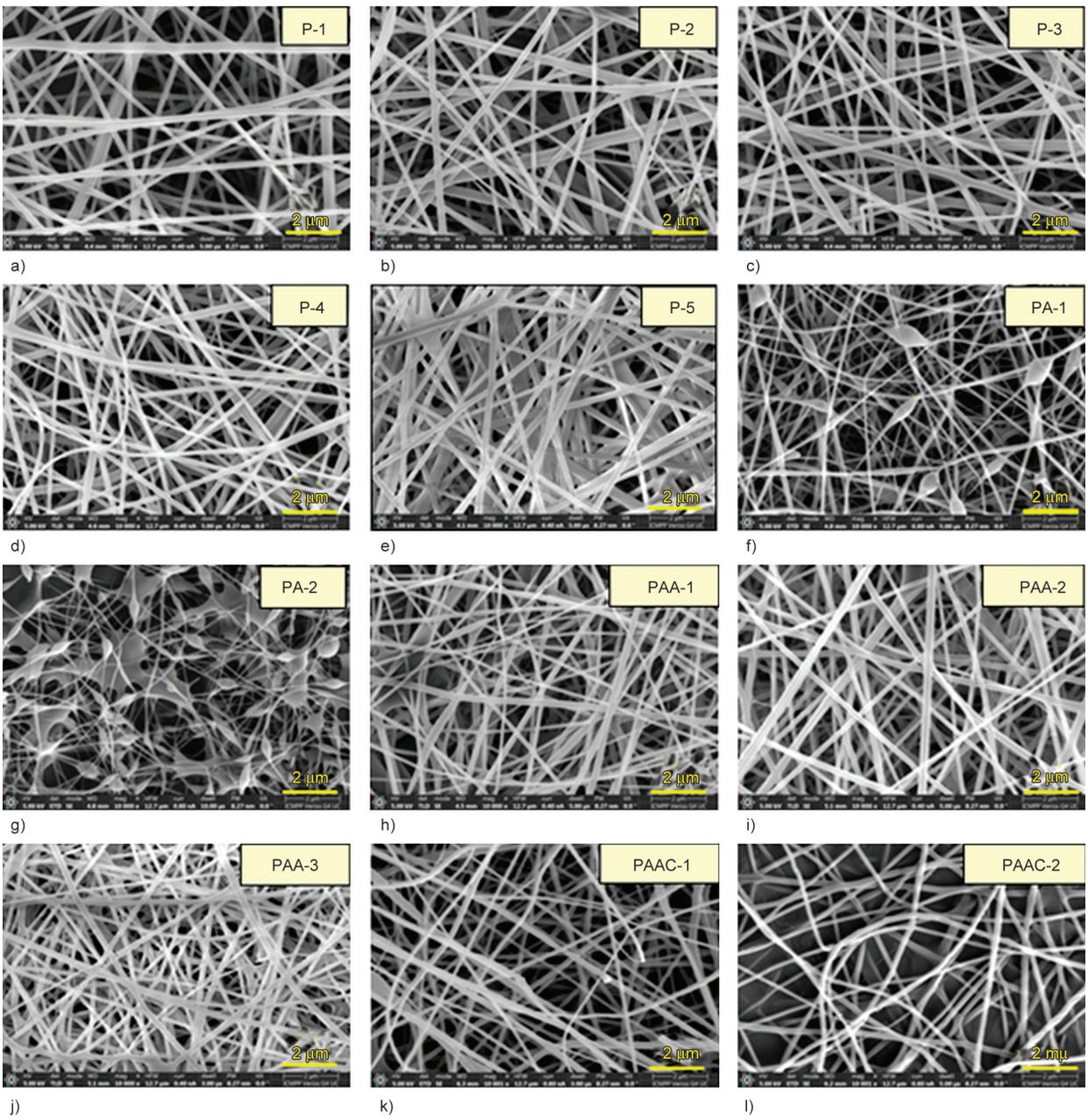

Figure 4. Scanning electron microscopy images of nanofibers. a) P-1, b) P-2, c) P-3, d) P-4, e) P-5, f) PA-1, g) PA-2, h) PAA-1, i) PAA-2, j) PAA3, k) PAAC-1, l) PAAC-2.

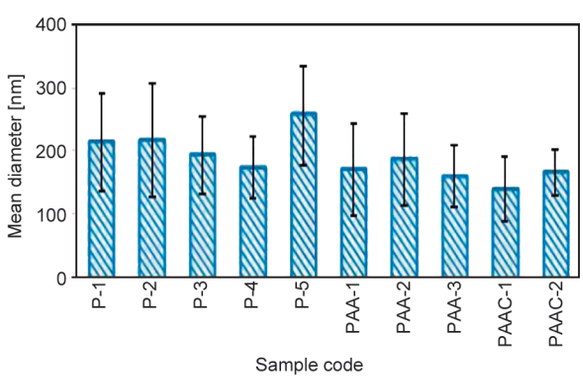

Figure 5. Nanofibers mean diameters depending on the molar ratio between the two polymers.

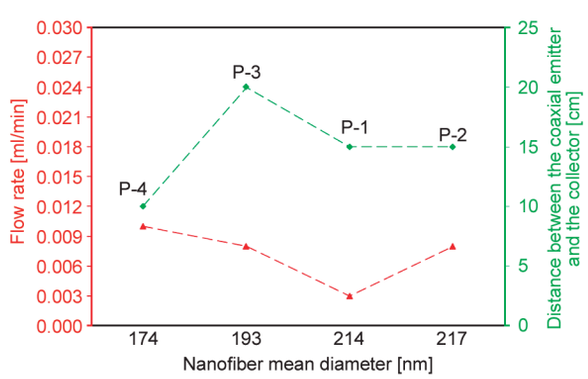

Figure 6. Relationship between the nanofibers' mean diameter and solution flow rate and distance between the coaxial emitter and the collector.

co-axial emitter and the collector of 10 cm, the nanofibers' mean diameter decreased, but a higher degree of nanofibers sticking was found. The results obtained in our study correlate with the results from the specialized literature [42].

3.4. Swelling behavior of nanofibers in aqueous solutions

The swelling degree of the CRC-loaded nanofibers (PAAC-3) was investigated in PBS solutions (pH 7.4 and 5.4). At pH 7.4, the swelling degree was approximately 297%, whereas at pH 5.4, it was approximately 326%. PZC point of PAAC-3 determined by salt addition ($NaNO_3$ – 0.1 M) [43] was approximately 7.1. Figure 7 shows a rapid swelling in the first half hour followed by stabilization up to 180 min. The swelling capacity of nanofibers in pH 5.4 solution is higher than in pH 7.4 solutions due to the repulsions of the anion-anion COO– groups. The meshes of the polymer network are more extensive and, consequently, allow water molecules to enter the polymer network. At pH 7.4, most of the carboxylate groups are protonated, and the lower swelling values of the nanofibers can be attributed to the presence of non-ionic hydrophilic –OH and –COOH groups on the PVA and alginate chains, respectively. This decrease can be attributed to Na+ counter ions that protect carboxylate anions and prevent anion-anion repulsion. Another important aspect is that the –OH groups are mostly in protonated form and present a lower affinity for water due to the less polar character at pH 7.4. These results are in accordance with the data from the literature for the system sodium alginate/poly(vinyl alcohol) hydrogels developed by Shivakumara and Demappa [44].

3.5. Curcumin loading and release studies

In vitro release profiles of CRC from PAAC-3 sample in PBS solution at pH 7.4 and 5.4 are presented in Figure 8. The amount of CRC loaded in nanofibers was approximately 0.045 mg CRC/1 mg nanofibers. Following the obtained results, it was found that the drug release in the PBS solution at pH 7.4 was lower than at pH 5.4. The release efficiency of CRC from nanofibers through the cellulose membrane at pH 7.4 was about 81%, while the release efficiency of CRC from nanofibers at pH 5.4 was about 96%. These results are in agreement with the swelling results presented above. In the first 6 h, there was a slower release of CRC from the nanofibers until equilibrium

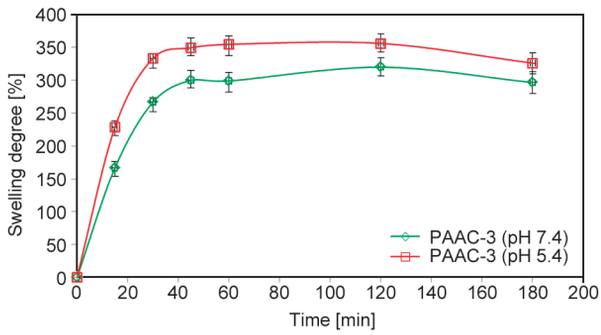

Figure 7. Swelling degree of the PAAC-3 nanofibers in solutions with pH 7.4 and pH 5.4.

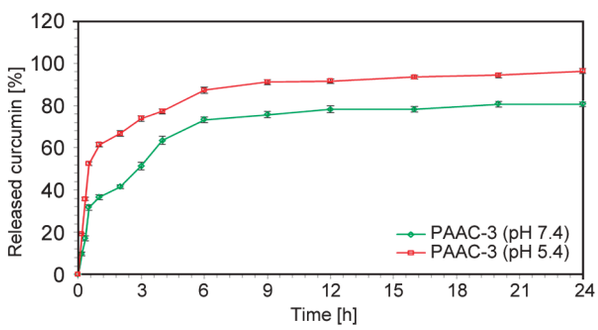

Figure 8. *In vitro* release degree of CRC from PAAC-3 nanofibers in PBS solutions (pH 7.4, pH 5.4).

in both acidic and slightly alkaline environments was reached. After establishing equilibrium, a percentage of approximately 9% of CRC was released in the medium with pH 5.4, and a percentage of approximately 8% of CRC was released in the medium with pH 7.4. The results obtained in this study are consistent with the results from the specialized literature [12, 45, 46]. Asif *et al.* [46], for example, synthesized and characterized curcumin-loaded chitosan nanoparticles to improve curcumin bioavailability. The authors found that 15 h after the beginning of the experiment, the release percentage of curcumin from all tested formulations in 6.8 pH phosphate buffers was found to be between 60 and 83%, and the release of the pure curcumin was about 25%.

3.6. Hemolysis test

The results of the hemolysis test are presented in Figure 9. Five types of nanofibers were analyzed, namely: PVA-based nanofibers (P-1); PVA/AG nanofibers (PA-1); crosslinked nanofibers based on PVA, AG and activator (PAA-2); CRC-loaded nanofibers based on PVA, AG and activator (PAAC-1 and PAAC-2). The obtained results evidenced that all the analyzed samples have good compatibility with the blood, which falls within the normal limits (smaller than 5% compared to the positive control sample),

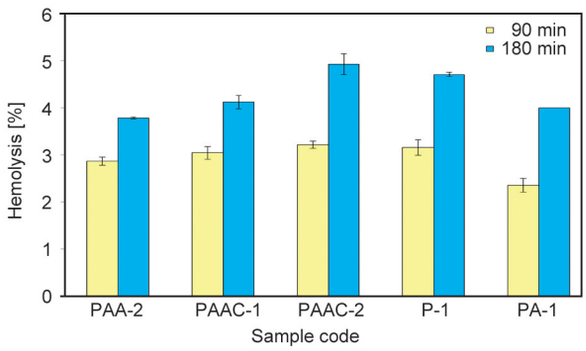

Figure 9. Results of the hemolysis test of nanofibers with and without incorporated CRC.

the values being between 2.36–3.22% after the first 90 min of contact. After 180 min of treatment, the degree of erythrocyte lysis increased to values that varied between 3.78 and 4.93%. As expected, the loading of CRC into nanofibers led to an increase in the degree of erythrocyte lysis. Also, increasing the activator amount in the system led to a slight increase in hemolysis degree. However, it can be noticed that all the nanofibers showed good hemocompatibility.

3.7. Cellular viability test

The objective of the *in vitro* experiments was to evaluate the toxicity degree of some nanofibers with and without CRC (PAA-1, PAAC-1 and PAAC-2) and free CRC for 48 h of treatment on the V79-4 Chinese hamster fibroblasts. Treatment with PAA-1

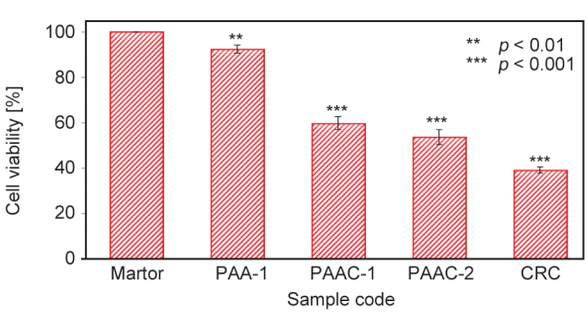

Figure 10. Viability of V79-4 cells after 48 hours of treatment with nanofibers and CRC (Values are expressed as mean ± SE).

nanofibers led to minor decreases (92.35%) in cell viability compared to the untreated control (Figure 10), being practically non-toxic. As can be seen from Figure 9, treatment with free CRC led to a strong cytotoxicity with a value of cell viability of approximately 39%. When CRC was incorporated into the nanofibers, the cell viability values increased to 59.66% for the PAAC-1 sample and to 53.72% for the PAAC-2. The use of nanofibers based on poly-L-lactic acid (PLLA), ε-polycaprolactone (PCL) and polyurethane (PU) determined a differential response in the case of L929 cells (mouse fibroblasts from subcutaneous connective tissue), being more intense after applying the treatment with materials obtained from PCL, reporting cell viability of 55% [47], value close to those obtained in the present study.

3.8. Cell morphology

As shown in Figure 11, unloaded nanofibers (PAA-1) do not affect the cells' morphology, the general aspect being comparable to the control. The main changes are a reduction of cell adherence through the loss of intercellular contact or elongation of the pseudopods. The PAAC-1 treatment caused a significant loss of cells' adhesion to the substrate, and the number of cells detached and floating in the culture medium increased. Moreover, they have reduced dimensions and a spherical appearance. The intense effect was observed after PAAC-2 application; most of the cells were detached from the microwell plate, forming a mass with a gelatinous appearance. The treatment with CRC (0.2 mg/ml) resulted in the formation of intensely colored aggregates with different sizes that blocked the metabolic exchanges of the cells, leading to their death. A recent study revealed that HeLa cells treated with high curcumin concentration induced dramatic morphological changes represented by contracted cells, development of microtubule spikes, membrane blebbing and cell debris, typically aspects of the apoptosis process [48]. On the other hand, optical microscopy images indicate

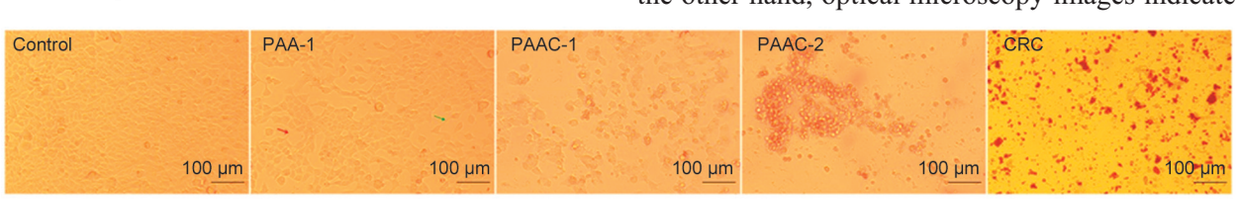

Figure 11. Representative microphotographs regarding V79-4 cells response after loaded and unloaded curcumin-nanofibers exposure; CRC – curcumin; red arrow – loss of intercellular connection; green arrow – pseudopods elongation. a) Control, b) PAA-1, c) PAAC-1, d) PAAC-2, e) CRC.

that L929 fibroblast cells had adequate interaction with SPU (biodegradable amphiphilic-block segmented polyurethanes) electrospun nanofibrous surface and did not show cytotoxicity, but curcumin loading caused a decrease in cell viability depending on the concentration and duration of treatment, reaching values up to 82% in the case of contact between cells and PCE (PEG-PCL-based polyurethane) nanostructures loaded with curcumin for 24 h [49].

4. Conclusions

Curcumin-loaded electrospun nanofibers were prepared by co-axial electrospinning using PVA/CRC as core and PVA and AG as shell. To increase the crosslinking degree of the nanofibers, [4-(4,6-dimethoxy-1,3,5-triazinyl-2)-4-methylmorpholinium chloride] was used as an activator. FT-IR spectra confirmed both the reaction between AG and PVA and the loading of CRC. The XRD analysis showed that adding alginate decreases the crystalline nature of the obtained nanofibers and that CRC is loaded into a more amorphous state. Moreover, SEM micrographs demonstrated that the diameter of the tested nanofibers was smaller than 200 nm. The swelling degree of CRC nanofibers in an alkaline solution (pH 7.4) was approximately 277%, whereas at pH 5.4, it was approximately 329%. The release efficiency of CRC from nanofibers in solution with pH 7.4 was about 89%, while in solution with pH 5.4, it was about 94%. The obtained nanofibers are hemocompatible, and the MTT test evidenced the non-toxicity of unloaded nanofibers, high cytotoxicity in the presence of free CRC, and a reduction in toxicity of CRC-loaded nanofibers. The cell morphology assay illustrated that unloaded nanofibers do not induce cell damage. However, the treatment with CRC-loaded nanofibers was followed by cellular changes, represented by loss of cell adhesion, smaller cell sizes, elongation of the pseudopods, etc. It is noted that the application of PAAC-2 leads to a more intense effect than that of PAAC-1. On the other hand, the treatment with free CRC resulted in significant cell death, especially due to the many colored aggregations that block the metabolic exchanges between the cells. In conclusion, it can be stated that CRC-loaded PVA/AG nanofibers have great potential as a controlled release system and can be used for biomedical applications. The results obtained in this study are promising and challenging and deserve to be continued with more in-depth *in vitro* tests. The optimal system from a physicochemical and biological point of view can be considered in future studies for the realization of *in vivo* animal and even clinical tests.

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