АНТИОКСИДАНТНА АКТИВНОСТ НА НОВИ ЕЛЕКТРОПРЕДЕНИ ВЛАКНЕСТИ МАТЕРИАЛИ, ЗАРЕДЕНИ С КВЕРЦЕТИН

10.53230/tgm.1310-912X.2021.0003.01

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ANTIOXIDANT ACTIVITY OF NOVEL QUERCETIN-LOADED ELECTROSPUN FIBROUS MATERIALS

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ABSTRACT

Fibrous materials based on cellulose acetate and polyethylene glycol containing the natural biologically active compound quercetin were successfully prepared by electrospinning. The obtained fibrous materials were characterized by scanning electron microscopy (SEM), IR spectroscopy, X-ray diffraction analysis (XRD), water contact angle measurements and UV-VIS spectroscopy. The incorporation of polyethylene glycol in the fibrous material resulted in increased hydrophilicity and enhanced release of quercetin from the fibers. Quercetin-containing fibrous mats exhibited high antioxidant activity as estimated by DPPH free radical scavenging method. Therefore, the novel polymeric materials containing quercetin are promising candidates for biomedical and pharmaceutical applications.

Keywords: *quercetin, electrospinning, antioxidant activity, cellulose acetate fibers.*

1. Introduction

In recent years, the health concerns associated with the side effects of synthetic compounds used in cosmetics, medicine, and food industry and the emergence of antibiotic resistance of pathogens have driven research towards the development of novel materials encapsulating plant extracts. It is well known that some plant extracts possess antidiabetic, antihyperlipidemic, antioxidant and anti-inflammatory activities [1]. Quercetin, a bioflavonoid, present in fruits and vegetables, such as tea, apples, onion and berries [2] possesses antioxidant, anticancer, anti-inflammatory, antidiabetic, and neuroprotective properties [3]. However, quercetin is poorly soluble in aqueous media and gastrointestinal fluids and it is degraded by the intestinal flora [4]. It is therefore necessary to develop suitable carriers of quercetin capable to enhance its solubility in water, which will lead to increased bioavailability and thus higher biological activity. An approach to circumvent the low water solubility of quercetin is its incorporation into suitable polymer matrices.

Fibrous materials produced by electrospinning have shown great potential as drug-eluting stents and wound dressing materials [5–8]. The large specific surface area of the electrospun materials and the possibility for modified drug release lead to enhancement of the therapeutic effect of the embedded drugs and reducing their side effects.

The aim of this work was to prepare and characterize fibrous polymer materials containing quercetin (QUE) by electrospinning of cellulose acetate/polyethylene glycol/QUE solutions. The composition of the polymer matrix was selected so as to improve the release of quercetin from the fibers. The antioxidant activity of the obtained novel materials was investigated.

2. Materials and methods

2.1. Materials

Cellulose acetate (CA; Aldrich, St. Louis, MO, USA) with $\overline{M}_n$ = 30,000 g / mol and DS 39.8%, polyethylene glycol (PEG; Fluka, Buchs, Switzerland) with (Mr = 1,900-2,200g / mol), quercetin (QUE, ≥95%; Sigma-Aldrich, St. Louis, MO, USA) and Tween 80 (Acros Organics, Netherlands) were used. Acetone (Sigma-Aldrich Darmstadt, Germany) and ethanol (Sigma-Aldrich, ≥99,8% (GS)) of analytical grade of purity were used. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich (Darmstadt, Germany), was of analytical grade of purity and was used without further purification. The disposable consumables were supplied by Orange Scientific, Braine-l'Alleud, Belgium.

2.2. Preparation of the fibrous materials

In the present study, three types of fibrous materials were obtained by electrospinning: CA, CA/PEG and CA/PEG/QUE. For electrospinning three types of solutions were prepared in acetone/water 80/20 v/v: CA, CA/PEG (80/20 w/w), and CA/PEG (80/20 w/w) with QUE (10 wt% in respect to total polymer weight). The total polymer concentration was 10 wt%.

The electrospinning apparatus used for the preparation the fibers consisted of a pump with adjustable speed and 5 ml syringe (12 mm internal diameter) fitted to a metal needle with a tip (size: 20GX1½″) connected to the positively charged electrode of a high voltage power supply (up to 30 kV). The spinning solutions were placed in the syringe. The electrospinning process was carried out at 21° C, relative humidity – 50 %, constant applied voltage of 25 kV and a tip-to-collector distance of 15 cm to the grounded rotating metal cylindrical collector (1000 rpm). The constant delivery rate of the spinning solutions of 3 ml/h was provided by a pump Syringe Pump NE-300 (New Era Pump Systems, Inc.).

2.3. Characterization

The morphology of the fibrous materials was evaluated by scanning electron microscopy (SEM). SEM analyses were performed on a Jeol JSM-5510 scanning electron microscope (Japan). The samples were vacuum-coated with gold by cathode sputtering using a Jeol JFC-1200 apparatus.

Mean fiber diameter was estimated by Image J software [9] by measuring the diameters of at least 20 random fibers per sample from three different SEM micrographs for a total of 60 measurements. Their morphology was assessed applying the criteria for overall evaluation of electrospun materials [10].

Fourier transform infrared spectra (FTIR) of mats (1 cm^2) were recorded using an IRAffinity-1 spectrophotometer (Shimadzu Co., Japan), supplied with a MIRacle ATR device (diamond crystal; depth of penetration of the IR beam into the sample - approximately 2 μm) (PIKE Technologies, USA) in the range of 600–4000 cm^{-1} with a resolution of 4 cm^{-1}. All spectra were corrected for H_2O and CO_2 using an IRsolution software programme.

X-ray diffraction (XRD) analyses were performed at room temperature using a computer-controlled D8 Bruker Advance ECO powder diffractometer with filtered Cu Kα radiation. Data were collected in the 2θ range from 5° to 60° with a step of 0.02° and counting time of 1 s $step^{-1}$.

Water contact angles of fibrous materials were measured using an Easy Drop DSA20E KRÜSS GmbH apparatus (Germany). Drops of deionized water (10 μl) were deposited on the surface of test specimens (2 cm×7 cm; cut in direction of the collector rotation). The mean contact angle value was determined after averaging at least ten measurements for each sample.

Quercetin release profile was studied in vitro at 37°C in acetate buffer at pH 5.5, constant ionic strength 0.1 (CH_3COONa/CH_3COOH) containing Tween 80 (acetate buffer/Tween 80 = 99.2/0.8 v/v). The tested mats were immersed in 100 mL buffer solution stirred at 150 rpm with an electromagnetic stirrer. Aliquots of the test solution were withdrawn at determined time intervals and their absorbance was recorded at a wavelength of 373 nm. The amount of released quercetin was calculated using a calibration curve (correlation coefficient R = 0.999) for the mats in acetate buffer/Tween 80 (99.2/0.8 v/v), pH = 5.5, constant ionic strength 0.1.

The antioxidant activity of the materials was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. 0.5 mL of QUE in ethanol (5×10^{-3} M) or CA/PEG and CA/PEG/QUE fibrous materials containing QUE - 0.5 mg were immersed in 3 mL of DPPH solution in ethanol (1×10^{-4} M). The as-prepared mixed solutions were kept in dark at 20 °C for 30 min. The antioxidant activity was evaluated by measuring the absorbance of the solutions at 517 nm using a DU 800 UV-vis spectrophotometer (Beckman Coulter), to detect the amount of DPPH radicals remaining in the solution. The antioxidant activity (AA%) was calculated using the following equation:

$$\text{Inhibition, AA,\%} = \left[\frac{(A_{DPPH} - A_{sample})}{A_{DPPH}}\right] \text{x } 100$$

where A_{sample}- absorption at 517 nm for DPPH solution after the addition of the solution containing QUE or fibrous materials, ADPPH - absorption at 517 nm for DPPH solution. All experiments were performed in triplicate.

3. Results and discussion

In the present study following fibrous materials were prepared by electrospinning: CA, CA/PEG and CA/PEG/QUE mat. The obtained mats are schematically represented in ***Figure 1***.

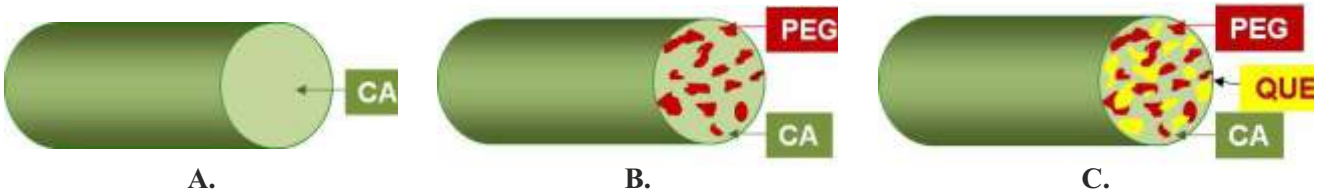
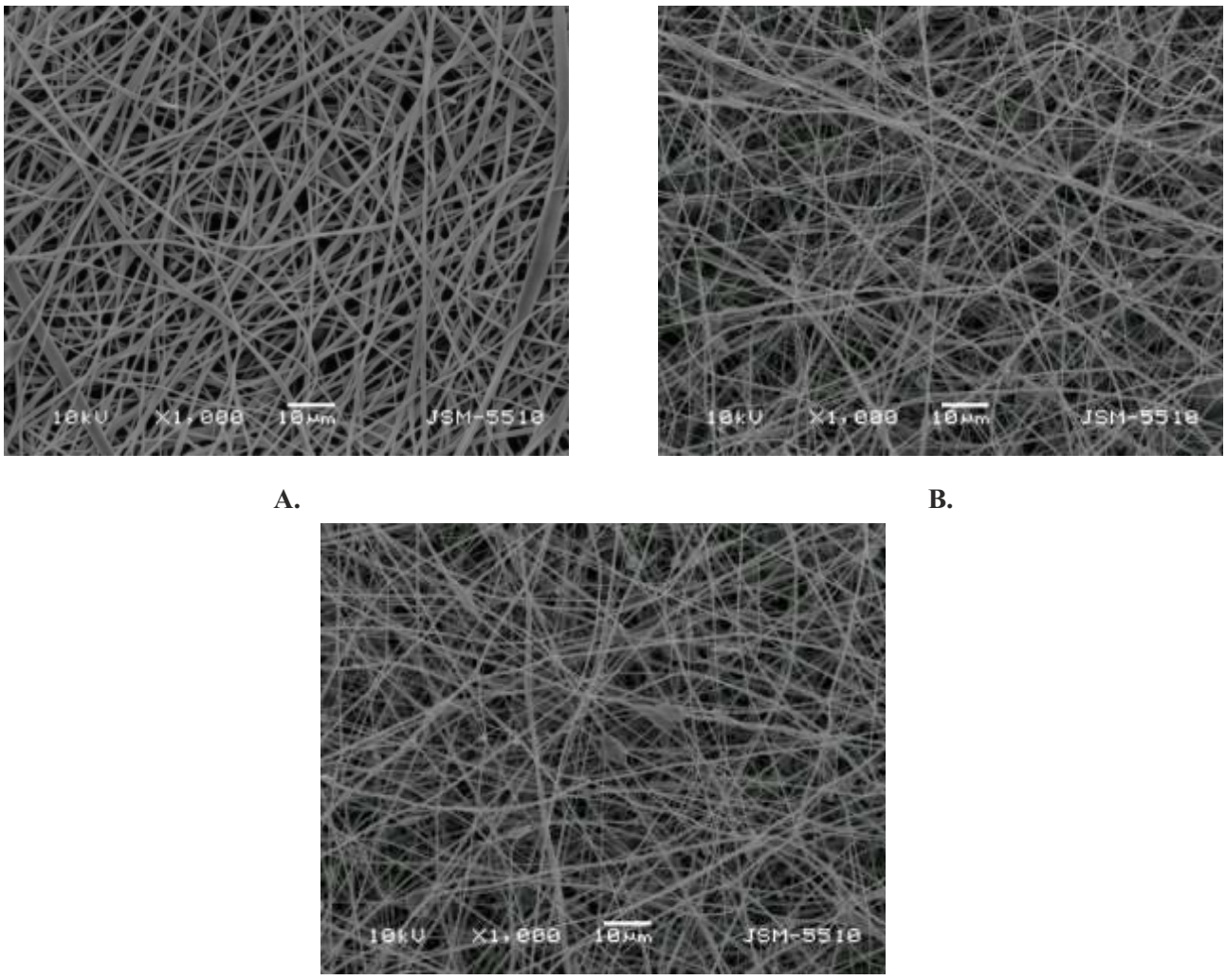


Figure 1. Schematic representation of fibers:
A. CA, B. CA/PEG and C. CA/PEG/QUE.

SEM micrographs of the obtained fibrous materials are shown in ***Figure 2***. Electrospinning of CA solution under the selected conditions resulted in obtaining defect-free fibers with mean fiber diameter of 780 ± 80 nm (***Figure 2A***). It was found that the incorporation of PEG resulted in decrease of the mean fiber diameter. The mean fiber diameter of CA/PEG fibrous material was 530 ± 150 nm (***Figure 2B***). This is probably due to presence of PEG, which lowers the solution viscosity thus leading to the fabrication of fibers with smaller diameters. Further decrease in the mean fiber diameters to 390 ± 150 nm was observed in the case of CA/PEG/QUE fibrous materials (***Figure 2C***).



A. **B.**

C.

Figure 2 SEM micrographs of fibrous materials:
A. CA, B. CA/PEG and C. CA/PEG/QUE.

The FTIR spectra of quercetin (powder), CA/PEG mat and CA/PEG/QUE mat are presented in Figure 3. Characteristic bands for the C=O functional groups at 1740 cm^{-1}, for the CH_3 groups at 1369 and 1226 cm^{-1}, as well as the ether C-O-C functional groups at 1037 cm^{-1} characteristic for the CA were observed (***Figure 3***) [11]. The presence of PEG in CA/PEG and CA/PEG/QUE mats resulted in bands at 1100 cm^{-1} characteristic of the PEG ether groups and at 2875 cm^{-1} due to vC–H. In the IR spectrum of CA/PEG/QUE fibers a shift of the characteristic band for C=O stretching vibrations up to 1747 cm^{-1} compared to the IR spectra of the CA/PEG

fibers without QUE (1739 cm^{-1}) was detected. Furthermore, in the IR spectrum of the CA/PEG/QUE mat, there is another shift of the characteristic bands for C=C down to 1600 cm^{-1} and 1508 cm^{-1} compared to the spectrum of the CA/PEG mat (1604 cm^{-1} and 1512 cm^{-1}, respectively). A similar shift for the band characteristic of the C=O of the aryl ketone groups of QUE (from 1666 cm^{-1} to 1651 cm^{-1} for the QUE-containing CA/PEG fibers) was also observed. These shifts suggest that hydrogen bonding between CA or PEG and QUE molecules occurs.

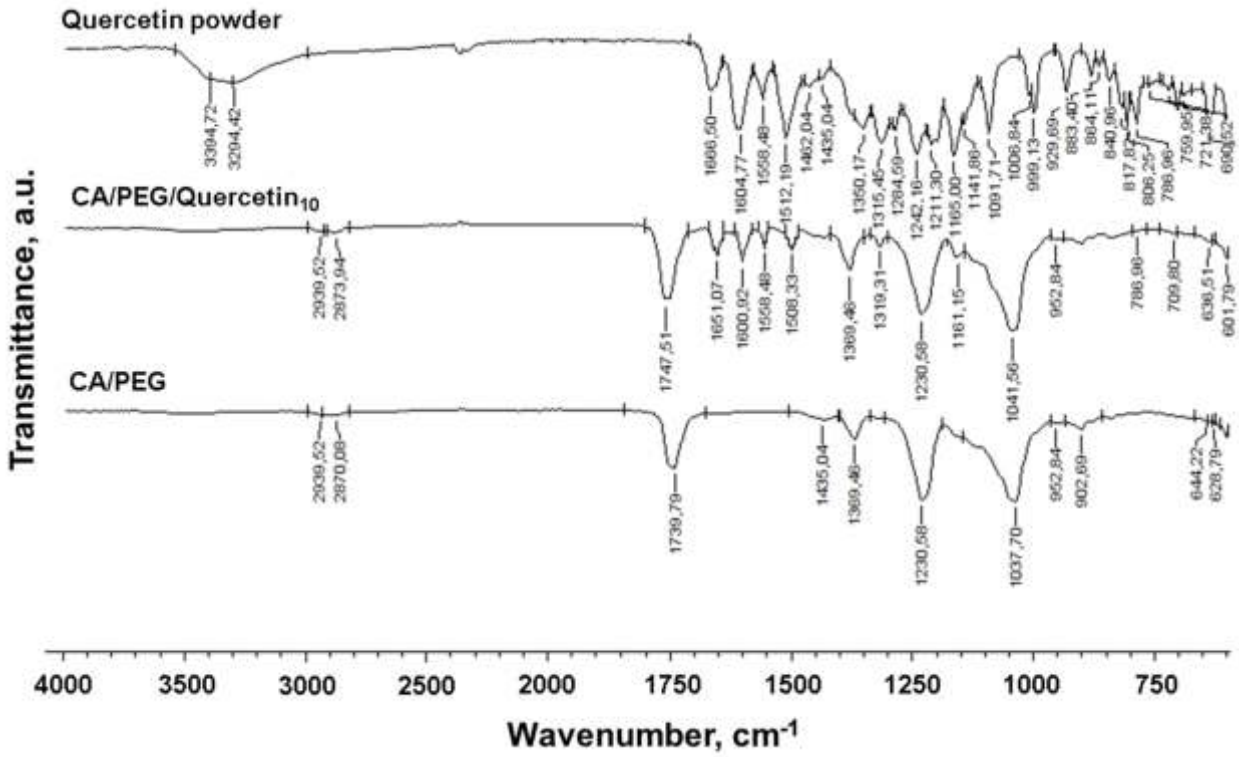


Figure 3. IR spectra of: quercetin (powder), CA/PEG and CA/ PEG/QUE fibrous mats.

The water contact angle of the obtained fibrous materials was measured as well. The digital photographs of distilled water droplets (10 μl) deposited on the surface of mats are shown in ***Figure 4***. It was found out that the CA mat is hydrophobic with water contact angle value ca. 120°, while the water contact angle of CA/PEG and CA/PEG/QUE was 0°.

A. **B.**

Figure 4. Digital photographs of distilled water droplets deposited on the surface of mats: CA and CA/PEG/QUE.

XRD patterns of QUE powder, CA/PEG mat and CA/PEG/QUE mat recorded in the range 2θ from 10 to 60° are presented in Figure 5. X-ray diffraction analysis revealed that the CA/PEG mat was amorphous. This result was in accordance with results obtained by Zhou et al. [12]. From the X-ray pattern of the QUE is it clearly seen that the main diffraction peaks for quercetin are detected at 2θ = 12.5°, 15.7°, 17.3° and 27.3°. The presence of these sharp diffraction peaks indicated that the QUE (powder) is highly crystalline. In contrast, amorphous halo was recorded in the XRD patterns of CA/PEG/QUE mat. No diffraction peaks for the crystalline phase of QUE were observed, revealing that the biologically active substance incorporated into the fibers was in amorphous state.

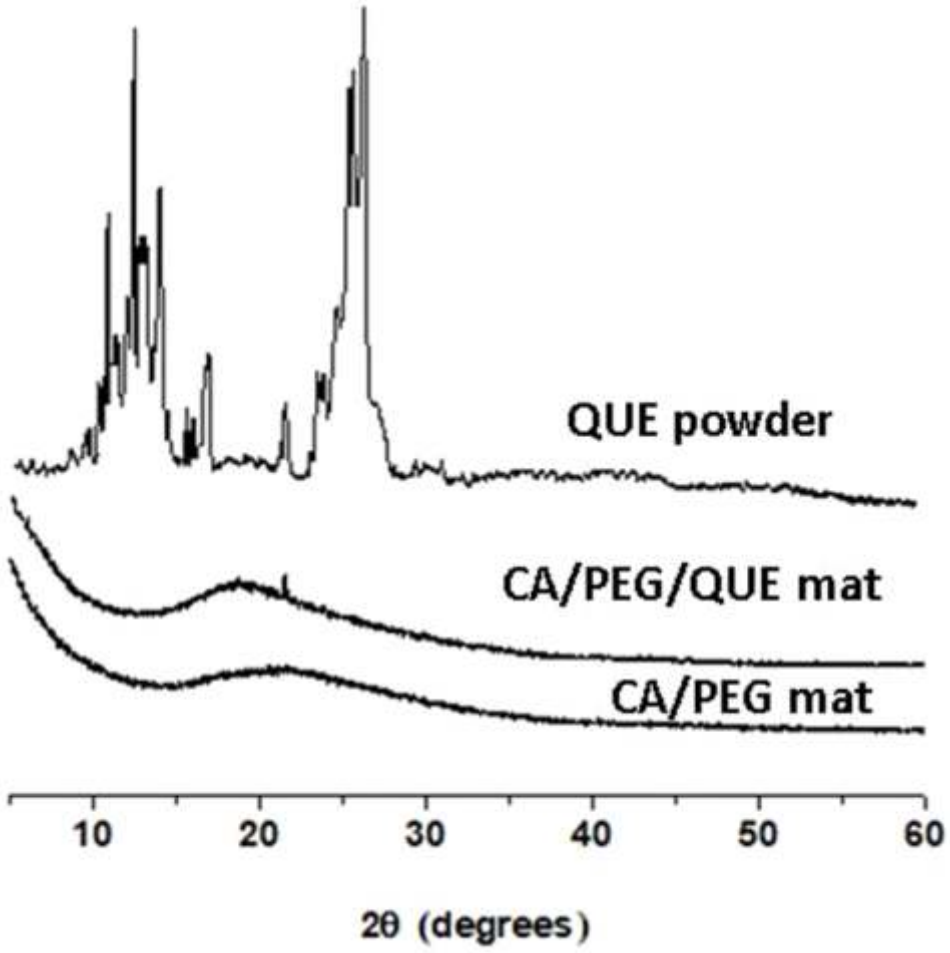


Figure 5. XRD patterns of: QUE powder, CA/PEG/QUE and CA/PEG mat.

In the present study, the release of QUE from the CA/PEG/QUE fibrous mat was performed under model conditions using a procedure for quercetin release by the addition of Tween 80 [4]. The QUE release was carried out in acetate buffer/Tween 80 = 99.2/0.8 v/v. It was found that quercetin was rapidly released when the water-soluble polymer PEG was in the fibers. The amount of biologically active compound released from the CA/PEG/QUE fibers was about ca. 85.3% for 360 minutes. PEG has been reported to improve the solubility of poorly water-soluble biologically active compounds and drugs [13]. Several factors affect the release of quercetin from fibrous materials. In general, the diffusion of the low-molecular-weight biologically active compound

from micro- and nanofibrous materials is a result of the wetting of the mats, which depends on their hydrophilic/hydrophobic characteristics and of the polymer crystallinity. The release is also affected by the crystalline/amorphous state of the biologically active compound. The diffusion of the biologically active compound also depends on the fiber morphology (mean fiber diameter and presence of defects or pores along the fiber). In the present study it was found that the hydrophilicity of PEG-containing mats assisted the penetration of the buffer medium as well as the release of the biologically active compound - quercetin.

We evaluated the antioxidant capacity of CA/PEG/QUE mats using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. DPPH is a stable free radical and its ethanol solution gives a strong absorption band at 517 nm and a deep violet colour. The reaction was studied using UV-visible spectrophotometry by monitoring the decrease in the absorbance of DPPH in the presence of CA/PEG/QUE mat. For comparison, the antioxidant activity of CA/PEG mats was studied as well. The antioxidant activity and digital photos of the corresponding solutions are shown in Figure 6. It was found that the CA/PEG mats exhibited very low antioxidant activity (absorbance of DPPH decreased by approximately 6.3%. Moreover, the colour of the solution of DPPH in contact with CA/PEG mat was not substantially altered, as can be seen from Figure 6. In contrast, after 30 minutes of contact with DPPH solution, QUE-containing mats exhibited high antioxidant activity (DPPH absorbance decreased by approximately 94.4%). The colour of the DPPH solution changed to pale yellow upon contact with CA/PEG/QUE mat. Moreover, the change in absorbance of DPPH solution upon contact with ethanol solution of QUE was similar to that obtained by contact with the fibrous mat containing QUE at the same QUE content.

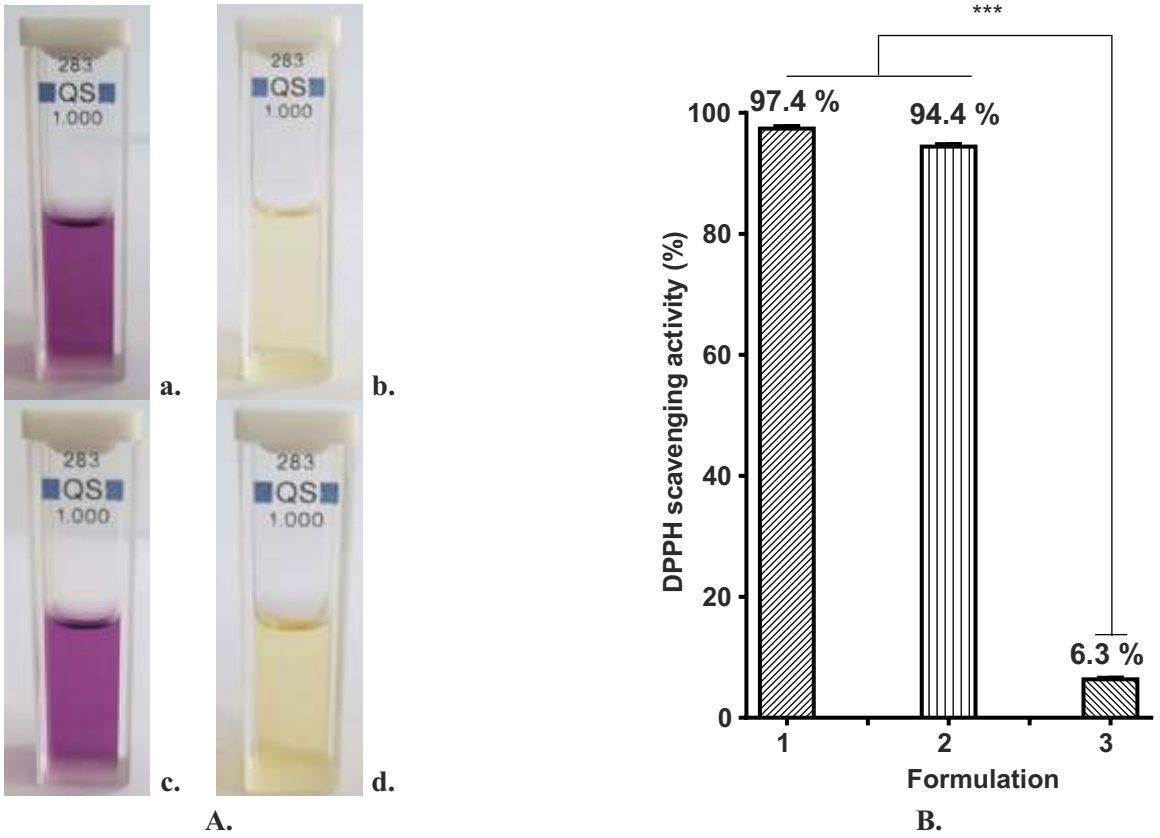


Figure 6. **A.** Digital photos of the tested solutions:
a) DPPH, b) DPPH+QUE, c) DPPH+ CA/PEG mat and d) DPPH+ CA/PEG/QUE mat.
B. Graph of the antioxidant activity of:
1- ethanol solution of quercetin, 2- CA/PEG/QUE mat, 3 -CA/PEG mat. *** p <0.001.

3. Conclusions

Fibrous materials based on cellulose acetate, polyethylene glycol and quercetin were successfully obtained by electrospinning It was found that the incorporation of PEG in the polymer matrix led to hydrophilization of the material and facilitated the release of the biologically active compound - quercetin. In addition, it was shown that the quercetin-containing fibrous materials exhibited high antioxidant activity.

Acknowledgments: Financial support from the Bulgarian Ministry of Education and Science (Grant D01-217/30.11.2018) under the National Research Programme "Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)" approved by DCM # 658/14.09.2018 is gratefully acknowledged.

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