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Electrospun Polyacrylonitrile Nanofibers Modified by Quaternary Ammonium Salts

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ABSTRACT: Electrospinning from a capillary is one of the methods for the production of nanofibers. The specific properties of such fibers result first of all from their large specific surface and the high porosity of the fiber mat. This article presents a process for producing functional nanofibers with antimicrobiological properties by electrospinning from polyacrylonitrile/dimethyl sulphoxide solution containing a bioactive agent based on quaternary ammonium salts (*N*, *N*, *n*, *n*,-didecyl-*N*,*N*-dimethylammonium chloride, Bis-(3-aminopropyl)-dodecylamine) and 2-propanol. The structure of the nanofibers obtained and their antimicrobial activity are investigated. A 5 wt % addition of bioactive preparation to the polymer solution (concentration of active substance in solution about 1.5 wt %) makes it possible to obtain fibers showing good bactericidal properties. After 6 h in contact with these fibers, *Escherichia coli* are eliminated to a level of 99.84% and *Staphylococcus aureus* to 99.99%. The IR spectrophotometric measurements do not indicate a residue of solvent in the bioactive nanofibers and show an increase in content of CH and CH_2 groups in relation to the pure nanofibers, which is connected with the presence of the biocide. Their degree of crystallinity determined by the X-ray scattering method is 44.4%. The nanofibers obtained can be designed for medical and filtration applications. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: QAS; bioactive polymer; antimicrobiological properties; nanofiber; electrospinning

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INTRODUCTION

The applications of synthetic fibers produced by the technique of electrospinning result mainly from their exceptionally small diameters (from several micrometers to several nanometres), high ratio of surface area to mass or volume, and the high porosity of the fiber mat. To obtain fibers with the required properties and structural parameters from a spinning liquid it is necessary to select the proper method and conditions for the electrospinning process. Generally, electrospinning is a technique in which a polymer solution or molten polymer is pumped through metal needles with diameters of the order of approximately 1 mm. On applying the power supply voltage, electrical induction occurs and the spinning liquid becomes charged. Consequently, the polymer stream is stretched, thinned, and collected on a grounded collecting element.[1,2] Other methods of electrospinning from a polymer solution are also known, e.g., with the use of a rotating roller[3] or a porous tube as feed electrode[4] or from the open surface of the polymer solution.[5] The choice of method is limited by the properties of the polymer solution used and especially its concentration and solvent volatility.[6] The possibility of producing fibers with specified features by a given method is influenced, besides the polymer solution properties, by the climatic conditions, under which electrospinning is performed and by parameters such as voltage applied to the system, distance between the feeding and collecting electrodes, collector material, capillary material, and diameter.[2,6,7–11]

Another very important factor that enables specific applications of such fibers is the possibility of their functionalization by modification with an appropriate process or additive fulfilling specified functions, e.g., a biocide capable of inhibiting the growth of undesirable microorganisms such as bacteria, fungi,

or yeasts, and even destroying them. Such fibers can be used for the production of fabrics resistant to the action of microorganisms, e.g., medical materials, implants, protective, health-promoting or sports clothing, bed-clothes, materials for filtration, ventilation or air conditioning, as well as materials used in the pharmaceutical, food, footwear, cosmetic and automotive industries, interior decorative fabrics, and many other fabrics for special applications. Bioactive polymers which are biocompatible or bioresorbable include chitin, chitosan, dibutyrylchitin, alginates, and other polymers.[12–20]

The antimicrobiological modification of fibers may involve special processing or incorporating a biologically active additive into the fiber, onto its surface or into the fiber spinning liquid. Nanofibers can be enriched with ions or nanoparticles of silver and additives as in the case of filtration materials. Their surface can be subjected to ionic plasma treatment. The necessity of such modification results from the particular use of the material, which depending on its application must interact with various microorganisms such as *Staphylococcus, Serratia, Klebsiella, Cladosporium, and Aspergillus.*[21]

When we deal with very thin and long fibers, such as those obtained by electrospinning that are mutually tangled in the form of a layer, modification is difficult to perform, especially the treatment of the whole surface of particular fibers. Some of the fibers in a layer are inaccessible and unexposed to the direct action of the modifier. It would be most easy to modify such fibers by performing a process that would partly affect their internal structure or only their surface to change their properties, e.g., by plasma or chemical treatment. From the literature[22–24] a photodynamic treatment of fibers is also known. TiO_2 nanofibers, as a photosensitizer, show a unique photocatalytic activity, which can lead to good results in antimicrobial photodynamic therapy. Upon UV illumination, TiO_2 nanofibers generate reactive oxygen species, which are toxic to microorganisms and can kill cancer cells.

The situation is different when the modification takes place during the stage of polymer solution preparation and an antimicrobiological agent can be added to this solution. Alternatively, one can incorporate the modifier into the polymer melt. Such an approach involves the selection of an appropriate modifier and the process of its combination with the polymer solution or polymer melt. It is also important to select an optimal concentration of modifier in the spinning liquids. On the one hand, it must not unduly deteriorate the liquid's spinnability, and on the other it should ensure a satisfactory level of biological activity of the fibers being formed. A problem that might appear during the preparation of polymer solution is the sedimentation of the added substance, aggregation of its particles and also, at a subsequent stage, a temporary or externally conditioned instability of the polymer solution. A separate issue is the selection of electrospinning conditions depending on the method selected, polymer solution parameters, and expected parameters of the fiber to be formed.

To impart antimicrobiological properties to nanofibers, reports in the literature most often propose the use of nanoparticles of metals such as copper, zinc, titanium, magnesium, gold, and especially nontoxic nanoparticles or ions of silver. The most popular source of silver nanoparticles is silver nitrate. The effectiveness of the antimicrobiological action of silver nanoparticles depends on their size and shape; the smaller the nanoparticles and the more complex their shape, the higher is their activity.[25] Moreover, silver nanoparticles can impart electrochemical activity to the material being modified. As shown by Kang et al.,[26] using the sol-gel method and electrospinning from a capillary, one can obtain silica Ag-doped nanostructured ribbons suitable for use in biosensing systems.

Silver nitrate is used as a antimicrobial modifier of nanofibers electrospun from different polymers, such as poly(vinyl alcohol),[27] poly(L-lactide),[28] acetylcellulose,[29] poly(vinyl chloride),[30] polyacrylonitrile (PAN),[30–32] polyurethane,[33] or from gelatine solution.[34]

The examples of antimicrobial modification presented above are connected with additional processes responsible for the activity of nanofibers and/or with silver. Unfortunately, silver is cytotoxic, and may cause undesired effects on contact with the skin.[35]

Besides silver nanoparticles, a few other bioactive agents with various biological activities against bacteria are known, for example moxifloxacin antibiotic used in modification of dextran nanofibers,[36] calcium peroxide used in polycaprolactone nanofibers,[37] and various *N*-halamines as modifiers of polyamide-6 nanofibers.[38]

To avoid an increase in resistance of microorganisms, microbiocides should continue to be modified and changed.

The aim of this study was to develop a process for the electrospinning of nanofibers with antibacterial properties imparted by the addition of a bioactive agent to the polymer solution. From the technological point of view, this process is relatively simple without additional operations involving antimicrobial modification of the fibers.

PAN was selected to produce nanofibers for medical and filtration applications because of its special properties such as thermal stability and tolerance to most solvents, atmosphere, and photo irradiation.[39]

As a antimicrobial modifier, the organic formulation Microbiocide N750 based on quaternary ammonium salts (QASs), with a wide spectrum of biocidal activity and high efficiency, characterized by very low minimal biocidal concentration (MBC) values, was used.

Quaternary ammonium microbiocides are known for their high antimicrobial activity.[40–44] The mechanism of their biocidal activity against microorganisms lies in destroying the cytoplasmic membrane, loss of K+ cations from the cytoplasm and changes in the structure of DNA and RNA.[44,45] Quaternary ammonium microbiocides are recommended for use as agents inhibiting the growth of microorganisms in textile materials and paints.[44,46–48]

The QAS nanofibers presented are an alternative to silver nanofibers, and do not require much procedure to obtain high antimicrobiological effectiveness (in the case of silver it must be in

the form of nanoparticles and must be in ionic form, and technologies ensuring uniform distribution of nanoparticles must be used; these are not necessary in the case of QAS). Moreover, silver is not easy to remove from the environment, it is not biodegradable, and its use is increasingly coming to be considered harmful to the natural environment, while QAS are easily degraded after use.

The bioactive nanofibers obtained can be used as one of the filtering layers in filters and respirators for individual protection of the human respiratory system.[49] This use of nanofibers requires them to behave, on account of their small diameters, as a specific mechanical sieve for microorganisms, and also, thanks to their modification with biocide, to show bacteriostatic or bactericidal effects. The antibacterial properties of nanofibers were investigated by means of appropriate tests.

EXPERIMENTAL

Material

Polymer and Solvent. In selecting the polymer and solvent, we took into account the necessity of preparing from them solutions that would not pose a danger to human health or an environmental hazard, and would also not cause undue deterioration of the electrospinning process. Based on literature data,[39] PAN was selected, as fulfilling these criteria and able to be used as a raw material in the production of bioactive nanofibers for medical and filtration applications.

PAN powder including 5% of comonomers of acrylic acid and methyl methacrylate (MMA) was produced by Zoltek Rt, Hungary. The intrinsic viscosity of PAN was equal to 1.3 $\pm$ 0.02 dL/g. Dimethyl sulphoxide was used as a solvent of PAN, as it is a substance used in medicine and does not pose any hazard to humans.[50] Pure dimethyl sulphoxide (DMSO) with molecular weight $M_r = 78.13$ g/mol was produced by POCh S.A., Poland.

Bioactive Agent. The following bioactive agent, suitable for use in materials to be in contact with human skin, was used in the study:

MICROBIOCIDE-N750 – a homogeneous liquid from INTERIODEX Sp. z o.o., Poland. The preparation contained: *N*, *N*, *n*, *n*,-didecyl-*N*, *N*-dimethylammonium chloride (> 25%), Bis-(3-aminopropyl)-dodecylamine (< 5%), 2-propanol (< 20%).[40] The Microbiocide-N750 preparation is designed for bioactive products, including textile goods.

Spinning Solutions

The polymer to be used was flooded with solvent and left to swell followed by stirring in an aqueous bath at a temperature of 40°C for about 5 h by means of a magnetic stirrer. The base solutions were of different concentrations: 13 wt %, 15 wt %, and 17 wt %.

The conductivity and surface tension of the base solutions were determined to select the most appropriate concentration of polymer solution from the point of view of electrospinning.

Conductivity measurements were carried out by means of a conductometer, type KO-102 (Elmetron, Czech Republic), using the differential measuring method with use of a three-electrode probe OK-902. The conductivity of the solutions was defined as electrolytic conductivity [S/cm]. The ambient temperature during the measurements was 25°C.

Table I. Properties of Solutions

Solution	Electrolytic conductivity, S/cm	Surface tension, 10^{-3}N/m
13 wt %PAN/DMSO	23×10^{-6}	45.40
15 wt % PAN/DMSO	24×10^{-6}	45.84
17 wt % PAN/DMSO	25×10^{-6}	46.86

The surface tension of solutions was determined by the method of detaching Du Nouy's ring.[51] The tensiometer developed by du Nouy is recommended by the American Society for Testing Materials to measure surface and interfacial tension of liquids, aqueous, and nonaqueous solutions.[52]

The electrolytic conductivity and surface tension of solutions increase with an increase in their concentrations. The higher the solution's concentration, the thicker will be the fibers electrospun from it.[53] During electrospinning from solutions with higher surface tensions, one should use a higher feed electrode voltage.[54]

Based on literature data and previous studies[6,55,56] as well as the results of conductivity and surface tension measurements (Table I), for the preparation of spinning solutions with bioactive properties 15 wt % PAN/DMSO solution was selected. This base solution made it possible to obtain very thin fibers with diameter of the order of several nanometres in a disturbance-free electrospinning process.

The base 15 wt % PAN/DMSO solution was used to prepare the spinning solutions containing MICROBIOCIDE-N750 in a quantity of 0.1 wt %, 0. 5 wt %, or 5 wt %.

To prepare a spinning solution modified with a biocide, the bioactive agent was added to the base polymer solution and the whole was stirred with a magnetic stirrer until a completely homogeneous solution was obtained. The stirring took place under ambient conditions at a temperature of about 20°C, which caused no changes in the biocide's properties.

To the polymer solution with a chosen concentration was added an appropriate quantity of biocide to obtain a homogeneous consistency, invariable with time and at a small difference in ambient temperature.

Electrospinning of Nanofibers

Nanofibers were spun by the standard method of electrospinning from a capillary. All experiments were carried out at room temperature (21–22°C) and air relative humidity of 38%.

The major parts of the spinning stand included a high-voltage direct current generator, a spinneret in the form of a syringe with capillary and a grounded collector in the form of a metallic plate. The spinning solution was loaded into the syringe and a high-voltage current was supplied to the metal capillary to create an electrostatic field between the spinning solution droplet at the tip of the capillary and the collector.[6,57]

Table II. Technological Parameters of the Electrospinning Process

Solution	Supply voltage, kV	Distance between capillary tip and collector, cm	Capillary diameter, mm
15 wt % PAN/DMSO (X)	15	15	0.9
15 wt % PAN/DMSO + 0.1 wt % of MICROBIOCIDE-N750	15	15	1.1
15 wt % PAN/DMSO + 0.5 wt % of MICROBIOCIDE-N750	15	15	1.1
15 wt % PAN/DMSO + 5 wt % of MICROBIOCIDE-N750	15	15	1.1

From the literature,[2,6–11] it is known that the quality of both the process and fibers is affected by atmospheric conditions, and by technological parameters such as voltage supplied to the system, distance between the capillary and collection screen, capillary diameter, solution concentration, type of collector (plate or rotating cylinder), and the material that covers the collector which directly collects the nanofibers. These conditions and parameters were appropriately selected for particular spinning solutions to provide a disturbance-free course of the electrospinning process (Table II).

Generally the nanofibers were collected on an alumina foil, while the fibers designed for biological tests were collected directly on a PAN spunlace nonwoven, which significantly facilitated the carrying out of tests.

Characteristics of Nanofibers

The nanofibers obtained were tested to assess their structure and properties, indicating possibilities for their potential applications.

Biological Properties. The antibacterial properties of PAN nanofibers modified with biocide were tested using unmodified nanofibers as reference samples. The tests were performed on the bacterial strains *Escherichia coli* (ATCC 10,536) and *Staphylococcus aureus* (ATCC 6538).

Preparation of samples. To prevent the delamination of the PAN spunlace/nanofibers layer system, samples were pressed in a press at elevated temperature under the following conditions: temperature 90°C, time 30 s, pressure 5065.8 kPa.

During pressing, samples were placed between Teflon foils previously washed with ethyl alcohol. Samples were not sterilized, to avoid changes in their physical and chemical properties. Prior to the experiments, the microbiological purity of the material was checked. Four samples with a surface area of about 25 cm^2 (5 cm × 5 cm) were put into a sterile solution of physiological saline, and the suspension was flooded with TSA medium (tryptic soy agar, Merck, Germany) and incubated at a temperature of 30°C for 72 h. The number of microorganisms was given in colony forming units (CFU) per 100 cm^2. The isolated microorganisms were subjected to microbiological diagnosis according to the relevant procedure (macroscopic and microscopic analyses, biochemical tests). The number of microorganisms in the materials tested was low and statistically made no contribution to the final result. The microorganisms belonged taxonomically to molds: *Aspergillus, Cladosporium* and bacteria: *Bacillus*, commonly present in air.

Testing the bactericidal effect during incubation with microorganisms. Samples of materials with a surface area of 4 cm^2 (2 cm × 2 cm) were inoculated with 0.1 mL of inoculation suspension of microorganisms. The inoculation suspension was prepared after the activation of bacteria in a TSB medium (malt extract broth, Merck, Germany) (37°C, 48 h) and then after centrifuging (5000 rpm, 10 min, Jouan B41 centrifuge) the separated bacterial biomass was suspended in 50 mL of physiological saline and mixed (10 min, 1000 rpm, Heidolph MultiReax mixer). The number of microorganisms in the inoculating suspension (inoculum) was first determined by a microscopic method using Thom's chamber and then by a culture method on TSA medium (incubation: 37°C, 48 h). The density of suspensions determined by the culture method was 3×10^9 for *Escherichia coli* and 1.5×10^9 for *Staphylococcus aureus.*

Each sample of the test material was inoculated with microorganisms. Samples were taken before and after incubation at 37°C for 6 h. Next, the microorganisms were washed out from the materials into physiological saline and shaken for 15 min in a water bath at a temperature of 37°C using a Water Bath Shaker 357 with a revolution frequency of 150 c.p.a. Dilutions and TSA medium were prepared from the solution. The culture was incubated for 48 h at a temperature of 37°C.

The antibacterial activity of the material tested, expressed as a biocide effect, was calculated from the results obtained as average numbers of microorganisms per sample. The criteria of material activity were established using standard calculations for the determination of biostatic and biocide effects of disinfectants against bacteria according to the PN-EN 1276 : 2000 standard.[58]

A value below 0.5 was accepted as low (this means a threefold drop in the number of microorganisms), while activity at a level of 3 was considered high (this means a 1000-fold drop in the number of microorganisms).

The biocidal activity was calculated from the following formula:

$$A = \log B/C$$

where A, biocidal activity; B, number of microorganisms per sample after time t = 0 with reference nanofibers (X); C, number of microorganisms per sample after time t of exposure with bioactive nanofibers.

Structure

Fineness. Fineness is one of the most important fiber parameters, especially in the case of nanofibers for use in filtration,

Table III. Number of *E.coli* and *S.aureus* After Incubation with Nanofibers, and Biocidal Activity of Nanofibers Against Bacteria. [Color table can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

	Number of bacteria *E.coli* [CFU/sample]*		Biocidal activity against *E.coli* after 6 h	Number of bacteria *S.aureus* [CFU/sample]*		Biocidal activity against *S.aureus* after 6 h
Nanofibers	time 0 h	time 6 h		time 0 h	time 6 h	
PAN -control	$2.61 \times 10^8 \pm 9.49 \times 10^7$	$1.68 \times 10^8 \pm 3.29 \times 10^7$	–	$2.35 \times 10^8 \pm 6.36 \times 10^7$	$1.65 \times 10^8 \pm 6.86 \times 10^7$	–
15 wt % PAN/DMSO						
PAN + 0.1 wt % of MICROBIOCIDE-N750	$1.23 \times 10^8 \pm 1.50 \times 10^8$	$1.16 \times 10^7 \pm 1.65 \times 10^7$	1.264	$2.16 \times 10^7 \pm 1.69 \times 10^7$	$2.28 \times 10^6 \pm 1.88 \times 10^6$	2.014
PAN + 0.5 wt % of MICROBIOCIDE-N750	$1.43 \times 10^8 \pm 1.85 \times 10^8$	$7.95 \times 10^6 \pm 1.05 \times 10^7$	1.428	$2.23 \times 10^7 \pm 9.90 \times 10^5$	$2.18 \times 10^5 \pm 3.04 \times 10^5$	3.033
PAN + 5 wt % of MICROBIOCIDE-N750	$2.32 \times 10^7 \pm 1.07 \times 10^7$	$3.69 \times 10^4 \pm 3.15 \times 10^4$	3.762	$1.14 \times 10^7 \pm 1.45 \times 10^7$	1.00 ± 0.57	8.371

Note: * mean (N = 3) ± SD (standard deviation)
Red shade — low activity, Yellow shade — average activity, Green shade — high activity

where they can be used as a so-called mechanical sieve. Nanofiber diameters were measured by means of SEM microphotography (scanning microscope JEOL JSM 5200 LV, Jeol LTD., Japan) and the Lucia G image analysis software (Laboratory Imaging s.r.o., Czech Republic). The average nanofiber diameter was calculated from 40 measurements carried out for individual nanofibers.

Molecular structure. The molecular structure of the nanofibers was examined by IR spectrophotometry using a monobeam spectrophotometer fourier transform infrared spectroscopy (FTIR)–8101 M (SHIMADZU, Japan) equipped with a control unit DR–8001. The transmission technique was used. In this technique, the investigated powdered material and crystalline potassium bromide (1 : 100) were prepared in the form of KBr pellet. By this method the existence of any trace impurities in the nanofibers can be detected.

Supramolecular structure. The wide angle X-ray scattering method was used to study comparatively the supramolecular structure of PAN nanofibers with and without biocide. Generally, the crystalline morphology of electrospun nanofibers influences their mechanical properties. If the degree of crystallinity is higher, the tensile strength of the nanofibers is higher, which means more possibilities for their application.[59,60]

The investigations were carried out with the use of a URD 6 Seifert diffractometer (Panalytical B. V., The Netherlands). The X-ray diffraction patterns were recorded over an angle range of 4–50° with a step of 0.1° using CuKα radiation. The impulse counting time was 20 s, the current intensity was 30 mA and the accelerating voltage was 40 kV. The diffraction maxima partition was carried out by the Hindeleh & Johnson method[61] with the use of the Rabiej program.[62] The degree of crystallinity was determined by comparing the domains which originated from the crystalline part with the total domains (from the crystalline and amorphous regions).

RESULTS AND DISCUSSION

Antibacterial Properties of the Bioactive Nanofibers

Biocidal activity of nanofibers with respect to *Escherichia coli* and *Staphylococcus aureus* was found after just 6 hours' contact at a temperature of 37°C, which is a very short period of time in comparison with those from similar tests reported in the literature, for example, 72 h at 25°C with respect to yeast,[27] 24 h at 37°C with respect to *Escherichia coli*,[63] 18 h at 37°C with respect to *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*,[29] 12 h at 37°C with respect to *Escherichia coli* and *Staphylococcus aureus*.[28] Despite the short time of incubation, some nanofibers showed biocidal activity (Table III), which indicates their fast and effective action against microorganisms.

High biocidal activity was shown by the nanofibers containing MICROBIOCIDE-N750, whose active substances acted very effectively on *Staphylococcus aureus*, but somewhat less well on *Escherichia coli*. A high biocidal effectiveness of PAN nanofibers was recorded even when the concentration of this preparation in solution was just 5 wt % (the content of active substances, i.e., ammonium compounds, was about 1.5 wt %); in this case after 6 h of incubation *Escherichia coli* bacteria were eliminated to a degree of 99.84% and *Staphylococcus aureus* bacteria were totally eliminated, i.e., to 99.99%. The reported differences in the antimicrobial activity of the tested nonwovens depend on the morphological properties of microorganisms, i.e., the structure of membranes and cell walls, and in the case of molds or other bacteria it also depends on the cells' physiological activity; e.g., production of surviving forms is a factor responsible for the high resistance of Gram-positive endospore-forming rods or mold spores. The membrane of Gram-negative rods (*Escherichia coli*) contains lipopolysaccharide (LPS) – the major component of the outer membrane, protecting the cell from chemical attack; in Gram-positive cocci (*Staphylococcus aureus*) LPS is absent and the peptidoglycan layer forming the cell wall is

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Table IV. Fineness of PAN Nanofibers (15 wt % PAN/DMSO) with MICROBIOCIDE-N750

Content of MICROBIOCIDE-N750, wt. %	Average nanofiber diameter, nm	Standard deviation, nm
0.1	514	60.9
0.5	474	67.7
5.0	628	86.4

substantially thicker than in Gram-negative bacteria. This is the reason for differences in susceptibility to disinfectants, in this case QAS, which are probably more effective for Gram-positive cocci. However, Kenawy et al.[44,45] concluded that polymers with the addition of triethyl ammonium salt react more effectively against Gram-negative bacteria (*Escherichia coli, P.aeruginosa, Salmonella typhae, Shigella* sp.) than Gram-positive bacteria – although the authors studied the highly resistant bacteria from the genus *Bacillus* (endospore-forming).

An increase in the content of quaternary ammonium salts in the spinning solution considerably intensified the biological activity of PAN nanofibers against the bacteria being tested.

Structure of the Bioactive Nanofibers

Fineness. The form of the nanofibers and their diameter are affected and controlled by the spinning solution characteristics, the type and quantity of added bioactive substances, as well as the technological parameters of electrospinning. The nanofibers obtained under the conditions given in Table II were characterized by different diameters.

The example of PAN nanofibers containing MICROBIOCIDE-N750 shows that a change in just one of the above factors influences the diameter of the nanofibers formed (Table IV). The thinnest nanofibers had a diameter of the order of 474 nm (Figure 1).

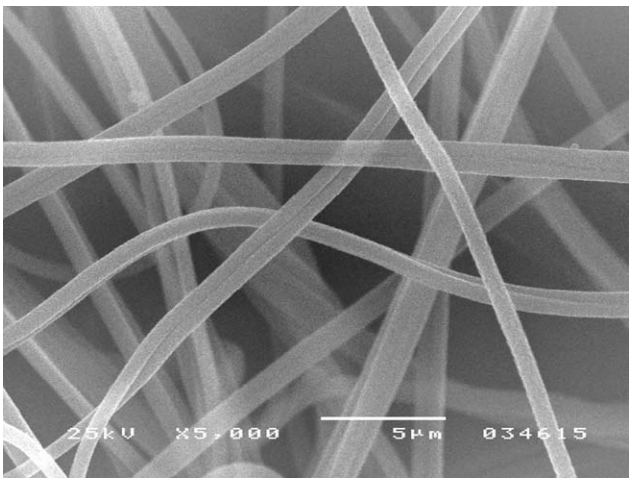

Figure 1. SEM image of nanofibers obtained from 15 wt % PAN/DMSO solution with 5 wt % of MICROBIOCIDE-N750; the average fiber diameter is ~ 474 nm.

Molecular Structure. The IR spectrophotometric measurements of nanofibers showed that: based on comparison of the spectrum of PAN nanofibers with that of pure PAN from the database and the spectrum of powdered PAN as used for the preparation of spinning solution, the occurrence of absorption bands indicates that the fiber material contains the co-monomers of methacrylic acid and MMA in a quantity of about 5%, but no new absorption bands are observed that would indicate a residue of solvent, i.e., DMSO (Figure 2); from comparison of the spectrum of PAN nanofibers containing 5 wt % of MICROBIOCIDE-N750 with that of pure PAN nanofibers, the increased

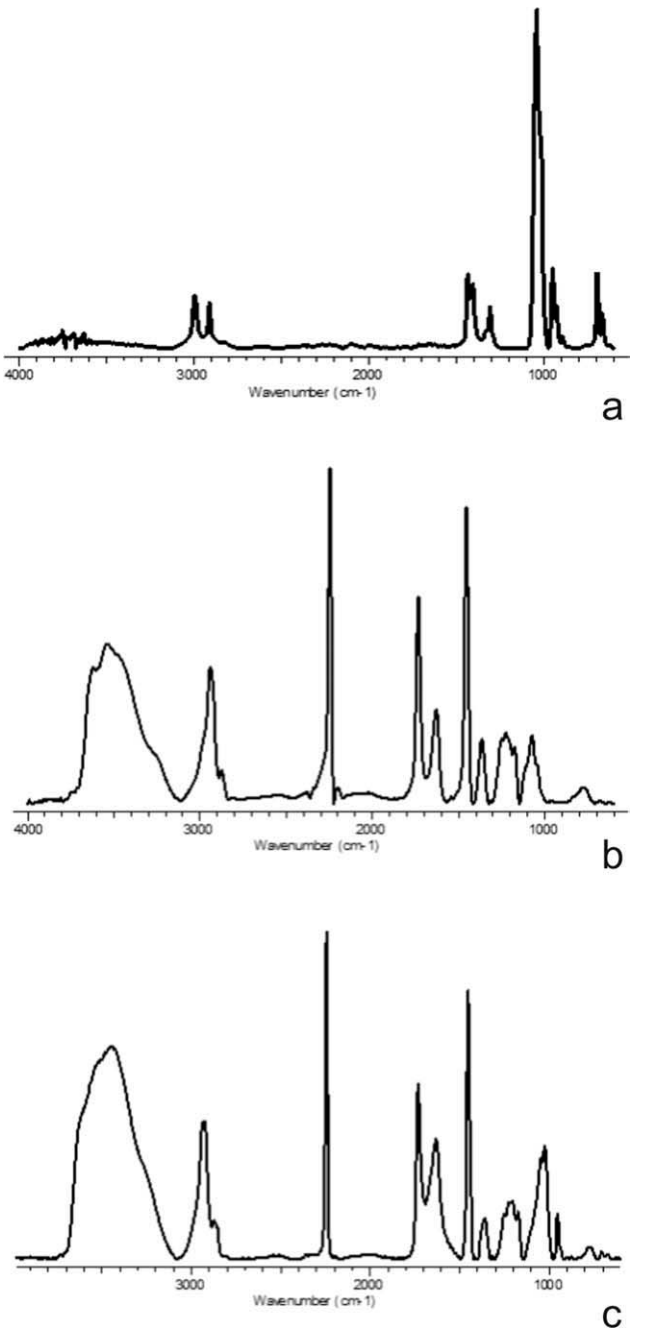

Figure 2. FTIR spectra of (a) DMSO, (b) PAN powder, (c) pure PAN nanofibers.

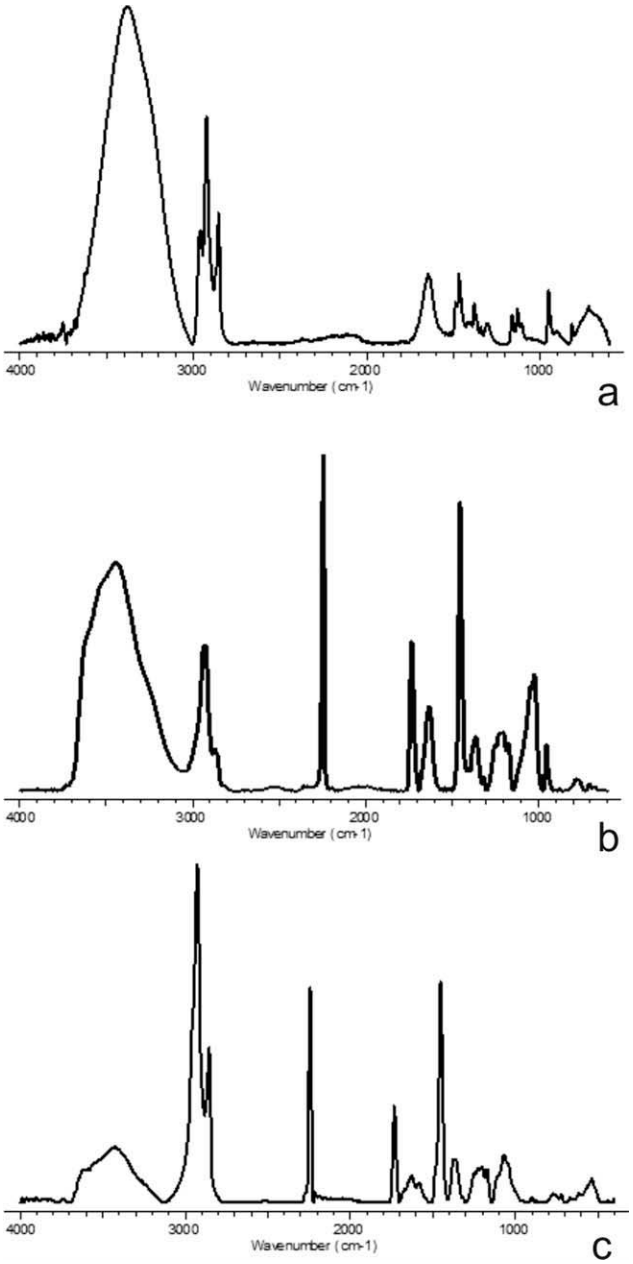

Figure 3. FTIR spectra of (a) MICROBIOCIDE-N750, (b) pure PAN nanofibers, (c) bioactive PAN nanofibers.

intensity of absorption bands ~ 2900 cm^{-1} indicates an increased content of CH and CH_2 groups, which is connected with the presence of the biocide (Figure 3).

Supramolecular Structure. Comparative structural examinations were performed for pure unmodified PAN nanofibers and bioactive PAN nanofibers. Based on literature data,[64–66] the diffraction curves of the samples under investigation were separated into crystalline peaks with appropriate Miller's indicators and halo of diffusion dissipation from the amorphous phase (Figure 4). Moreover, the diffraction patterns show two broadened maxima, whose angular positions 2θ are about 38° and 46°. These maxima are connected with different conformation of PAN macromolecular chains.[65,66]

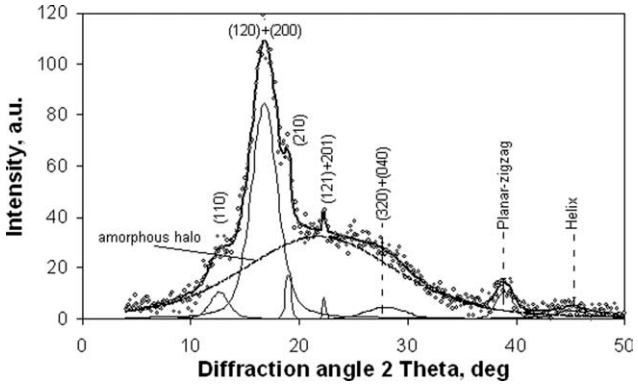

Figure 4. Diffraction pattern of PAN nanofibers obtained from 15 wt % PAN/DMSO solution with 5 wt % of MICROBIOCIDE-N750.

As follows from Table V, the tested PAN nanofibers with and without biocide show a crystalline structure. The crystallinity degree values of these nanofibers are similar, while the nanofibers containing 5 wt % of MICROBIOCIDE-N750 show a minimally higher degree of crystallinity, i.e., 44.4%, compared with pure nanofibers with a degree of crystallinity of 43.5%. It can be concluded also that the PAN nanofibers obtained in the electrospinning process have a different supramolecular structure than standard PAN fibers. The latter fibers show a specific supramolecular structure discriminating them from other synthetic and man-made fibers, referred to in the literature as a paracrystalline structure.[67–69] Standard PAN fibers are characterized by, for example, nonoccurrence of crystalline structure, despite the fact that PAN is capable of developing crystalline structures. A fundamental element of the supramolecular structure of standard PAN fibers is the fibril. This is a bundle of more or less straightened macromolecules, in which areas with an ordered mesomorphic state of aggregation of macromolecules occur in sections. These areas are not strictly crystalline, however, but are merely similar to crystalline areas, as they do not contain a regular spatial grid. They are called paracrystalline areas, and they contain only lateral ordering, without lengthwise ordering (in the direction of the axis). Confirmation of the paracrystalline structure of PAN fibers is provided by their x-ray diffraction image, which lacks reflexes on the meridian and distinctive layer reflexes.

Table V. Degree of Crystallinity and Mean Dimensions of the PAN Crystallites Perpendicular to the Planes with (110) and (120) + (200) Miller Indices in the Nanofibers

Nanofibers	Crystallinity %	Dimensions of the PAN crystallites $D_{(110)}$ nm	$D_{(120)+(200)}$ nm
15 wt % PAN/DMSO	43.5	4.3	2.7
15 wt % PAN/DMSO + 5 wt % of MICROBIOCIDE-N750	44.4	4.9	2.6

Nonetheless, PAN is capable of forming crystalline structures (it is possible to obtain monocrystals and spherolitic aggregates). Crystallized PAN displays a spatial grid with a rhombic crystallographic arrangement.

In other basic man-made fibers such as polyester, polypropylene, or polyamide, the fibril contains alternating crystalline and noncrystalline areas. Macromolecules in a noncrystalline area occur in amorphous and mesomorphic states.

CONCLUSIONS

Using MICROBIOCIDE-N750 preparation containing quaternary ammonium salts (*N*, *N*, *n*, *n*,-didecyl-*N*, *N*-dimethylammonium chloride, Bis-(3-aminopropyl)-dodecylamine) as an additive to polymer solution, the possibility of producing antibacterial nanofibers by electrospinning has been assessed.

Even at a concentration of just 5 wt % (the content of active substances, i.e., ammonium compounds, being about 1.5 wt %) in the spinning solution it makes it possible to obtain nanofibers with excellent antibacterial properties with respect to *Escherichia coli* and *Staphylococcus aureus* bacteria, the latter being completely eliminated after 6 hours' incubation.

PAN nanofibers display a crystalline structure, as distinct from standard PAN fibers. Nanofibers containing 5 wt % of MICROBIOCIDE-N750 show a minimally higher degree of crystallinity, i.e., 44.4%, and an increased content of CH and CH_2 groups compared with the pure nanofibers.

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