Application of fluorescent label in polymer nanofibers

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ABSTRACT

The electrospinning of flurescent probe polyamide 6 doped by 7H-benzimidazo[2,1-a]benzo[de]isoquinolin-7-on is presented as a model processing photoluminescent nanofibers. The presence of the fluorescent probe in the fiber layers was confirmed by Attenuated total reflection - Fourier transform infrared spectroscopy (ATR - FTIR); the surface nanofiber structure was described by high-resulution fluorescence microscope and scanning electron microscope images. The prepared nanofibers with the fluorescent label was further characterized by fluorescence spectroscopy, both in the solid phase and in the solution.

KEY WORDS: fluorescent marker, nanofibers, electrospinning method, safety agent

INTRODUCTION

The electrospinning technique is, principally, very simple and efficient method for producing polymer nanofibers from solutions or melts [1]. This technique has been known since the early 20th century. However, a significant progress in this area has been achieved during the past few years due to the wide range of nanofibers applications as novel materials in various devices and the producing of the synthetic polymer nanofibers on an industrial scale [2-5]. Polymer fiber could be applied, for example, as tissue scaffolds [6-9], filtration devices [10-11] and fibrous reinforcements in synthetic and biological composite materials [12-14]. Nanomaterials might be used simultaneously in production of synthetic blood vessels, skin or healing agents. Such materials could also be used as suitable matrices for the growth of stem cells. Moreover, during recent decades, the fluorescent conjugated polymers and their use as active components in organic light-emitting diodes, photoconductors, and sensors give the electrospinning technique farther attractiveness to be leader technology [15-17]. The electrospinning is applicable practically to every soluble or fusible polymer. Nevertheless, beside the most common polymer nanofibers, this technique has found an application in the preparation of metal, glass, ceramic and carbon nanofibers [18-19].

Using the electrospinning method, a different type of polymer can be fabricated as nanofibers. Their diameters, shapes and configurations can be control using the adjustable parameters in the actual spinning process. At the same time, it is possible to apply a thin layer of selected polymers on virtually any surface.

Among large quantities of nanomaterials, polyamide-based nanocomposites are mostly applied in industry. This material has been used in connection with elecrospun in many applications [20-23], for example as part of the sensor [24]. PA6 has an ever wider application in nanomaterials [25-31]. The importance of such materials was capped by the first patent by Toyota on nylon 6 nanocomposite [38]. The main problem is the difficulty of transferring the results from the laboratory to industrial processing [32-33].

In this work, we have chosen 7H-benzimidazo[2,1-a]benzo[de]isoquinolin-7-one (NBI) for incorporation into PA 6 matrix by the electrospinning process. NBI is a very known fluorescent marker for labeling of various materials, and not least as a fluorescent color in reflective material [34]. The potential of labeled fibers is, for example, as a component of composite materials (epoxy resins) or as safety agent in the industry.

EXPERIMENT

Materials and instrumentation

NBI was prepared according to procedure described in the literature by the reaction of 1,2-benzenediamine and 1H,3H-naphtho[1,8-cd]pyran-1,3-dione [35] as shown in the reaction scheme 1. The host matrix PA6 were purchased from BASF company. The concentration of polymer (PA6) in a solvent was 12 wt.%, the concentration of NBI in dissolved polymer was 10 wt.%, the electrode distance 180 mm, the frequency of AC voltage applied is 50 Hz, and the current 0.004 mA.

ATR-FTIR spectra were measured using Brucker Vertex 80V spectrometer. The solid samples were measured in attenuation total reflection mode using a diamond crystal. The sample was evacuated to 2.51 hPa. Spectral range was calibrated from 4000 to 600 cm^{-1}. Each spectrum was scanned 100 x. Bruker OPUS software was used for spectra evaluation and the baseline correction was done by "rubber - band".

The images of the nanofibers were made by scanning electron microscope (SEM) Tescan MIRA3. Created nanolayer was fitted using sticky label on the pad and its surface was covered by 30 nm gold layer. The samples were measured using secondary emission mode, depth regime with 15 kV, working distance (WD) of 10 mm and 20.000x magnification. The contrast-enhancing coloring was used for contrast visualization. The fibre size was determined using software ImageJ software with manually located fibers [36-37].

Fluorescence microscop was used to image the features of prepared sample. The images are in 5 Mpx resolution, acquired and processed using Lvenhuk ToupWiev used UV source with a wavelength of 365 nm, corrected image on the light temperature of 5500 K (white light), the images in reflected light were also corrected to white light (light source was originally 4000 K). All image were taken under identical exposure settings.

The fluorescence emission spectra were measured on Perkin-Elmer LS55 Spectrofluorimeter equipped with special commercial cuvette for the solid state measurements. The fluorescent spectra in solid phase were recorded from the surface of the pressed powder/film. Fluorescent spectra were corrected for the characteristics of the emission mono-chromator and for the photomultiplier response and by excitation at the wavelengths of the NBI absorption maximum in 1,4-dioxane.

The fluorescence of Fibre UV-PA6 was investigated using Vickers instruments M41 Photoplan modified at Lancshire University. The images were processed by Lvenhuk ToupWiev programme using resolution 5 megapixels, the light source was 365 nm wavelengths. The image was corrected to a temperature of 5500K light (white light). The

reflected light images are also corrected to white light (a temperature of light source - 4000K). All photos are taken at identical exposure settings (58 ms).

The preparation of the solutions for the formation of NBI doped PA6 nanofibers

The process of nanofibres production using electrospinning technique is influenced by many parameters, such as viscosity, temperature, polymer type and solvent. The selection of the optimum solvent plays especially in the mixture of polymeric matrix (in our case PA6) and additive (used NBI) a big role in the success electrospinning process [38].

The polymeric matrix PA6 was dissolved in 10 ml trifluoroacetic acid (TFA) to create 12 wt. % concentration of viscous polymer solution. NBI was added to the PA6 solution in 10 wt. % of the total weight. The solvent as well as the concetration was chosen on the base of literature [38]. The whole mixture had been homogenized for 30 minutes in ultrasound apparatus before fiberizing.

Nanofibers were formed from the rod electrode using Elmarco Nanospider™ NSLAB 500 pilot plant unit (Fig. 1). Using electrospinning technology, the nanofibers were obtained from the free surface of the polymer solution. Polypropylene (PP) foil was used as the supporting material covering the grounded electrode. The drops on the rod were formed in approximately of the same size and each drop was fiberized for at least 8 minutes. Nanofibers were excised together with the supporting PP foil and allowed to dry for one hour at 25°C in order to evaporate the rest of the solvent. The values of fiberized polymer solutions and parameters of the electrostatic field are shown in Table 1.
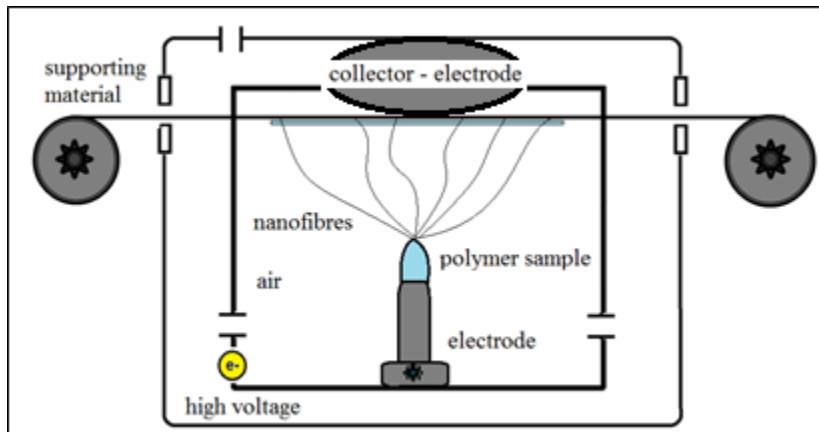

Fig. 1. Elmarco Nanospider™.

Table 1: Parameters of the electrostatic field for PA6 and NBIPA.

PA6		NBIPA	
Value	d [um]	Value	d [um]
Vol. count	10	Vol. count	10
Summation	2.51	Summation	20.22
Min. value	0.15	Min. value	0.70
Max. value	0.63	Max. value	3.12
Mean value	0.25	Mean value	2.02
Std. dev.	0.14	Std. dev.	0.81

RESULTS AND DISCUSSION

The fluorescent probe was synthesized according the following reaction scheme 1:
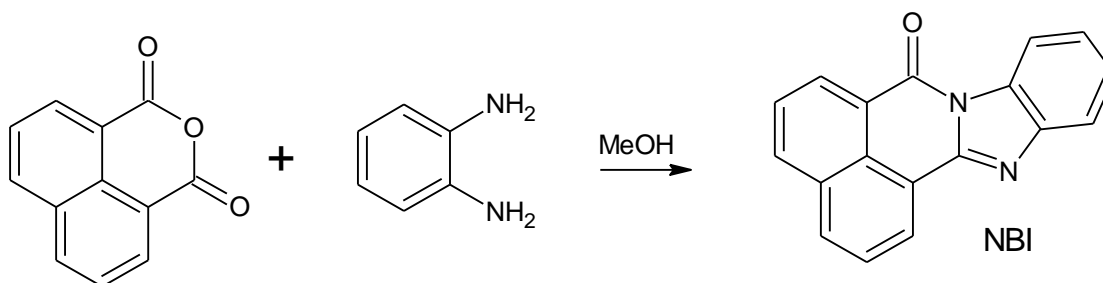
Scheme 1: Synthesis of the fluorescent probe NBI.

The synthesized NBI was used as the additive into the PA6 matrix (NBIPA) and the nanofibers were created. Fig. 2 show the electron microscopy images of nanofibers composed of PA6 without NBI (left) and PA6 with NBI (right) in different magnifications.

From the Fig.2, it is evident that the fibers created from the mixture of PA6 and NBI creat unsmooth fibers with randomly arranged needles, easily observed in the snaps of fibres (right) whereas the fibres created from pure PA6 seem to be relatively homogenous, smooth without structure irregularities (left).
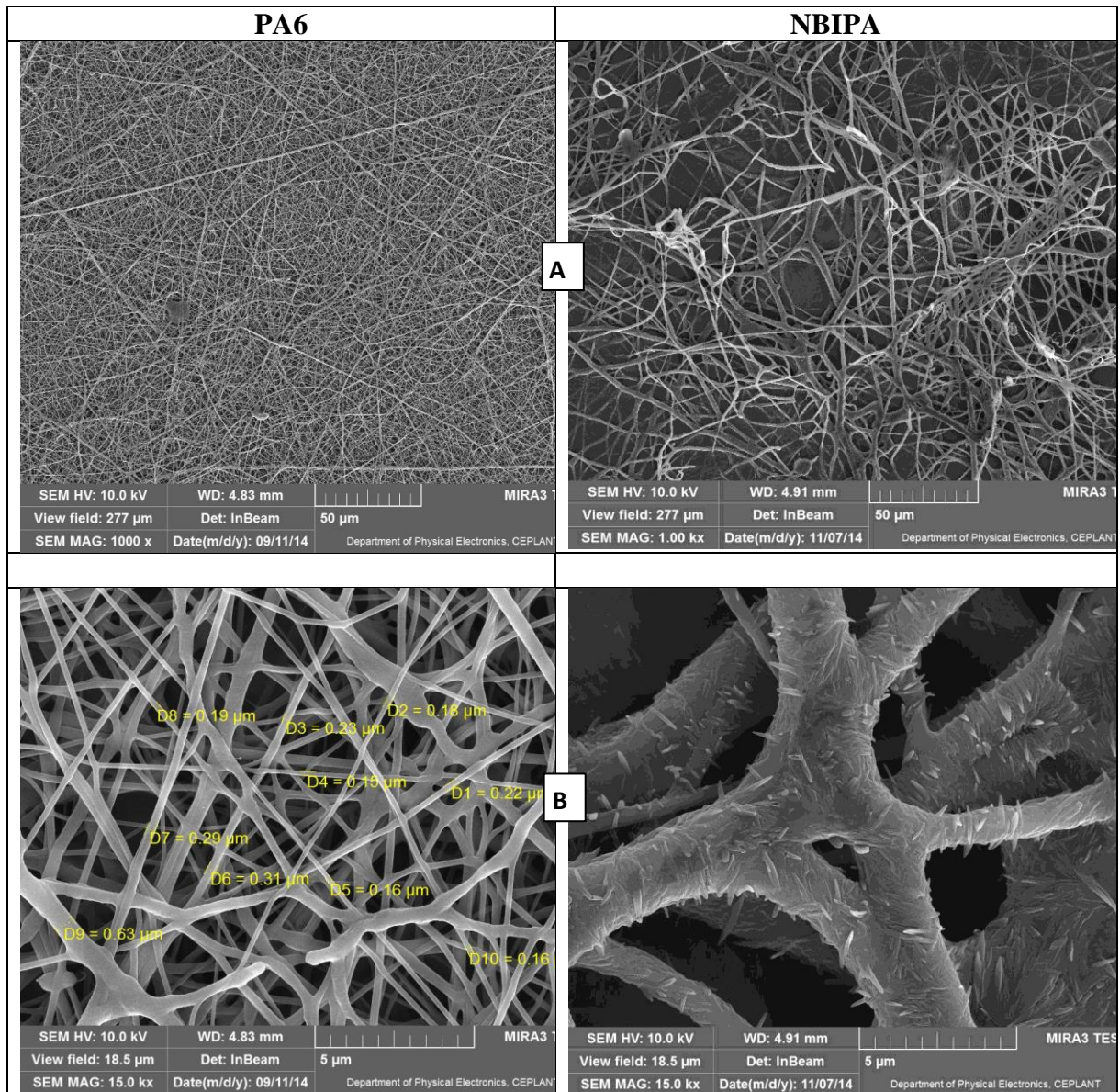
PA6	NBIPA

Fig. 2 SEM images of PA6 (left) and NBIPA (right) electrospun nanofibers in different magnifications.

The needle structures are probably created as a result of presence of NBI. Simultaneously, the average width was determined from the SEM snaps, where 10 representative fibers were manually chosen, their borders were marked, fibre width was determined (Tab. 2). Later, the presented average fiber width was calculated as the average value of the measurement of 10 fibres. The average fibre width for fibers from pure PA6 was 250 nm whereas the average fibre width for fibers containing PA6 and NBI was 2.02 um. It means that the presence of NBI additive increases the average width of the fibers up to eight times.

<p align="center">**Table 2**: Electrospinning characteristic of PA6 a NBIPA</p>

Fibre PA6 [um]		Fibre NBIPA [um]	
Min. value	0.15	Min. value	0.70
Max. value	0.63	Max. value	3.12
Mean value	**0.25**	**Mean value**	**2.02**

The electrospun nanofibers prepared from pure PA6 and that prepared from its mixture with NBI were characterized by ATR-FTIR (Fig. 3). The presence of NBI in created electrospun nanofibers is presented by the first two of four intense bands in the region between 1300-1700 cm^{-1} (the position of about 1350 and 1450 cm^{-1}). These bands are of low intensity for PA 6. Furthermore, the presence of NBI in the electrospun NBIPA nanofibers is evident from the small dual-band around the region 900-1000 cm^{-1}, which is absent for PA6.
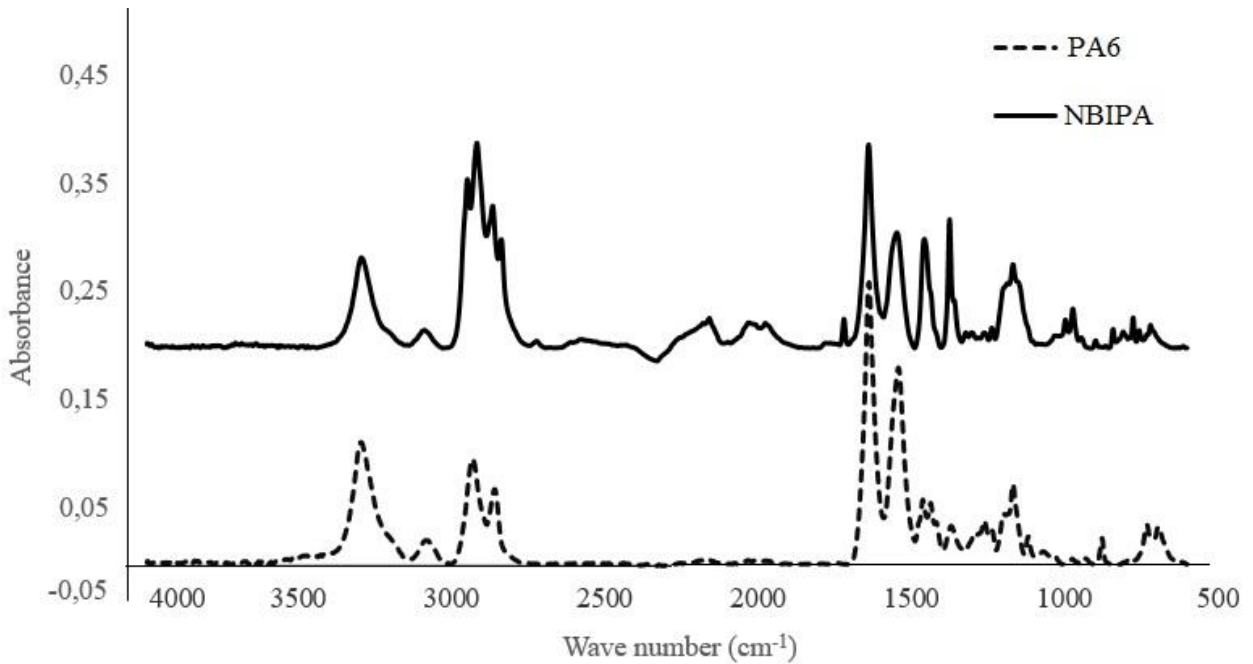
Fig. 3 ATR-FTIR analysis.

It was also investigated the fluorescence behaviour of NBI additive in the PA6 nanofibres. Fig. 4 shows the snaps of nanofibres textile, where the fibre structure significantly shines in a comparison with the nanofibres textile without NBI additive. For comparison, PA6 without NBI can be observed in Fig. 5 using a fluorescence microscope, so there is the evident difference between NBIPA and PA6.
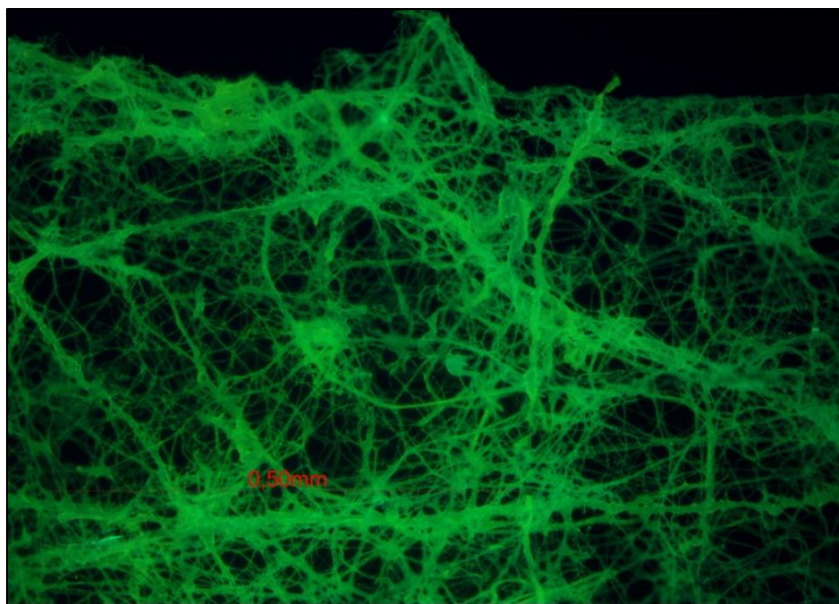
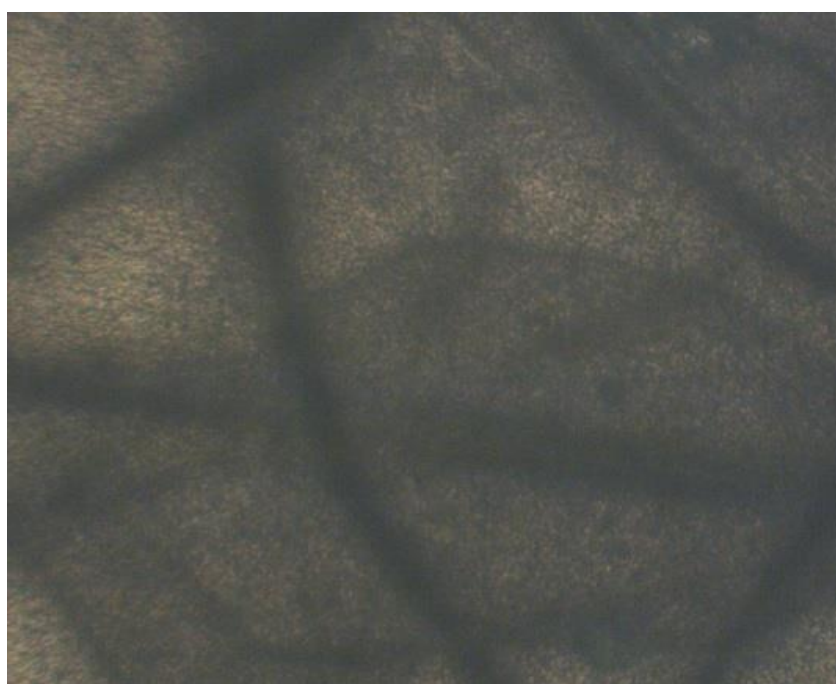**Fig. 4**. Fluorescence microscope image of NBIPA.

Fig. 5: Fluorescence microscope image of PA6.

The fluorescent spectra of both NBI in powder and NBIPA electrospun fibers are formed from a broad and structureless band. However, the fluorescence maxima of NBIPA is hypsochromicaly shifted by about 11 nm compared with the the fluorescence maximum of NBI in solid phase (Fig. 6). By comparison of the fluorescence intensity of NBI and NBIPA in solid phase, only insignificant difference was observed. Relative fluorescence intensities of NBI in solid phase are about ten times lower compared with the maximum intensity of NBIPA.
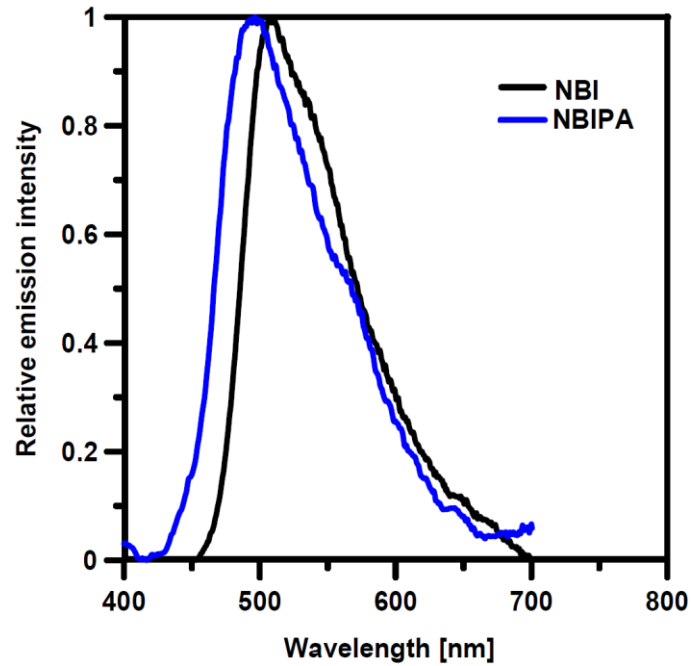
Fig. 6. Normalized fluorescence spectra of NBI (powder) and NBIPA electrospun nanofibers.

CONCLUSION

Electrospinning has been recognized as an efficient technique for the fabrication of polyamide 6 doping with NBI-fluorescent marker. Nanofibres with relatively regular structure, containing needle microstructures of NBI, was obtained. These modified fibers were studied using ATR-FTIR, SEM, fluorescence microscope and spectrofluorimeter. It has been confirmed that the fibers exhibit the same fluorescence spectrum in comparicon with NBI. The potential of the modified fibers is as a component of composite materials or as safety agent in the industry.

REFERENCE

[1] Greiner, A., Wendorff J.H.. "Electrospinning: a fascinating method for the preparation of ultrathin fibers." Angewandte Chemie International Edition 46.30 (2007): 5670-5703.

[2]   Carraher, Ch. E.: Seymour/Caraher's polymer chemistry. J. J. Lagowski. 6th edition, New York: Marcel Dekker, 2003. 913 s. ISBN 0-8247-0806-7

[3]   Cook, J: Handbook of textile fibres: Man-made fibres, 5th edition. Cambridge: Woodhead publishing, vol. II. p. 194-195. ISBN 978-1-85573-485-2

[4]   Mcintyre, J. E.: Synthetic fibres: nylon, polyester, acrylic, polyolefin. 1st edition. Cambridge: CRC Press, 2005. ISBN 0-8493-2592-7.

[5]   Vasanthan, N. Handbook of Textile Fibre Structure. 1st. edition. USA: Elsevier, 2009 Polyamide fibre formation: structure, properties and characterization. Page 232-239. ISBN 978-1-84569-380-0.

[6] Venugopal, J.; Vadgama, P.; Kumar, T. S. S.; Ramakrishna, S. Nanotechnology 2007, 18, 055101.

[7] Ito, Y., et al."A composite of hydroxyapatite with electrospun biodegradable nanofibers as a tissue engineering material." Journal of Bioscience and Bioengineering 100.1 (2005): 43-49.

[8] Zong, X., et al. "Electrospun fine-textured scaffolds for heart tissue constructs." Biomaterials 26.26 (2005): 5330-5338.

[9] Lee, Ch. H., et al. "Nanofiber alignment and direction of mechanical strain affect the ECM production of human ACL fibroblast." Biomaterials 26.11 (2005): 1261-1270.

[10] Yoon, K., et al. "High flux ultrafiltration membranes based on electrospun nanofibrous PAN scaffolds and chitosan coating." Polymer 47.7 (2006): 2434-2441.

[11] Tang, Z., et al. "Design and fabrication of electrospun polyethersulfone nanofibrous scaffold for high-flux nanofiltration membranes." Journal of Polymer Science Part B: Polymer Physics 47.22 (2009): 2288-2300.

[12] Barhate, R. S., et al. "Fine chemical processing: The potential of nanofibres in filtration." Filtration & Separation 45.4 (2008): 32-35.

[13] Stachewicz, U., Asa H. B.. "Enhanced wetting behavior at electrospun polyamide nanofiber surfaces." Langmuir 27.6 (2011): 3024-3029.

[14] Hang, F., Asa H. B.. "Nano-mechanical properties of individual mineralized collagen fibrils from bone tissue." Journal of the Royal Society Interface 8.57 (2011): 500-505.

[15] Jo, S., et al. "Conjugated polymer dots-on-electrospun fibers as a fluorescent nanofibrous sensor for nerve gas stimulant." ACS applied materials & interfaces 6.24 (2014): 22884-22893.

[16] Collister, J.. "Commercialization of polymer nanocomposites." 2002. 7-14.

[17] Fei, X., Yingchun G.. "Progress in modifications and applications of fluorescent dye probe." Progress in Natural Science 19.1 (2009): 1-7.

[18] Kampalanonwat, P., Supaphol P.. "Preparation and adsorption behavior of aminated electrospun polyacrylonitrile nanofiber mats for heavy metal ion removal." ACS applied materials & interfaces 2.12 (2010): 3619-3627.

[19] Wang, H., et al. "Polypyrrole-coated electrospun nanofibre membranes for recovery of Au (III) from aqueous solution." Journal of Membrane Science 303.1 (2007): 119-125.

[20] Matulevicius, J., et al. "Design and characterization of electrospun polyamide nanofiber media for air filtration applications." Journal of nanomaterials 2014 (2014): 14.

[21] Kim, G.-M., et al. "Temperature dependence of polymorphism in electrospun nanofibres of PA6 and PA6/clay nanocomposite." Polymer 48.16 (2007): 4814-4823.

[22] Dersch, R., et al. "Electrospun nanofibers: Internal structure and intrinsic orientation." Journal of Polymer Science Part A: Polymer Chemistry 41.4 (2003): 545-553.

[23] Dotti, F., et al. "Electrospun porous mats for high efficiency filtration." Journal of Industrial Textiles 37.2 (2007): 151-162.

[24] Mercante, L. A., et al. "Electrospun polyamide 6/poly (allylamine hydrochloride) nanofibers functionalized with carbon nanotubes for electrochemical detection of dopamine." ACS applied materials & interfaces 7.8 (2015): 4784-4790.

[25] Picouet, P. A., et al. "Influence of PA6 nanocomposite films on the stability of vacuum-aged beef loins during storage in modified atmospheres." Meat science 96.1 (2014): 574-580.

[26] Li, Cong-Ju, et al. "PA6@ FexOy nanofibrous membrane preparation and its strong Cr (VI)-removal performance." Chemical engineering journal 220 (2013): 294-301.

[27] Majka, T. M., et al. "Modification of organo-montmorillonite with disodium H-phosphonate to develop flame retarded polyamide 6 nanocomposites." Applied Clay Science 139 (2017): 28-39.

[28] Li, Y., Shimizu, H.. Novel morphologies of poly (phenylene oxide)(PPO)/polyamide 6 (PA6) blend nanocomposites. Polymer, 45(22) (2004), 7381-7388.

[29] Castro, L. D. C., et al. "Effect of organoclay incorporation and blending protocol on performance of PA6/ABS nanocomposites compatibilized with SANMA." Polymer Engineering & Science (2017).

[30] Tserpes, K. I., et al. "Experimental investigation of the effect of hygrothermal aging on the mechanical performance of carbon nanotube/PA6 nanocomposite." Plastics, Rubber and Composites (2017): 1-6.

[31] O'Neill, A., et al. "Polymer nanocomposites: In situ polymerization of polyamide 6 in the presence of graphene oxide." Polymer Composites 38.3 (2017): 528-537.

[32] Cook, J. Gordon. Handbook of textile fibres: man-made fibres. Elsevier, 1984.

[33] Mleziva, J., Snuparek, J. Polymery, Sobotales, 2000.

[34] Burger, M., Gebuhr, T., Stoll, I.. "Method of producing a cover element and an optoelectronic component, cover element and optoelectronic component." U.S. Patent Application No. 14/908,252.

[35] Arient, J.; Marhan, J. Imidazole dyes. VI. Synthesis and properties of 1,2-naphthoylenebenzimidazole, Collection of Czechoslovak Chemical Communications, 26, 2774-80; 1961

[36] Scheider, C. A, Rasband, W.S., Weliceiri, K.. NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 2012-6-28, 9 (7), 671-675.

[37] Advex Instruments - See system [online]. Advex Instruments, s. r. o., 2008 [cit. 2016-05-01]. http://www.advex-instruments.cz

[38] Bacovska, R., et al. "Phenyl-methyl phosphazene derivatives for preparation and modification of hydrophobic properties of polymeric nonwoven textiles." Reactive and Functional Polymers 100 (2016): 53-63.