



Research articles

Magnetically modified electrospun nanotextile exhibiting peroxidase-like activity



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ABSTRACT

The magnetic modification of four different electrospun nanofibrous textiles, based on polyamide, polyvinyl alcohol, polycaprolactone and polyurethane, is reported. The simple spray modification procedure employing two types of magnetic fluids was used. Magnetic modification led to the deposition of magnetic iron oxide nanoparticles on the surface of textile nanofibers. Magnetic nanotextile exhibited response to external magnetic field and was stable for a long time. Magnetic modification has not substantially changed the structure of the modified nanotextile. Magnetically modified nanotextile exhibited peroxidase-like activity and thus it represents a promising composite material applicable in various biochemical, biomedical and biotechnology applications.

1. Introduction

Magnetically responsive materials play an important role in various areas of biosciences, medicine, (bio)analytical chemistry, biotechnology and environmental technology. Such materials exhibit several types of responses to external magnetic field including selective separation, targeting and localization, heat generation, increase of a negative T_2 contrast during magnetic resonance imaging or increase of apparent viscosity of magnetorheological fluids [1]. In addition, magnetic nano- and microparticles can be used for magnetic modification of enormous amount of diamagnetic biomaterials, biopolymers, organic polymers and inorganic materials [2–4]. Recently a great attention has been also paid to electrospun nanofibers and nanotextile [5] and their magnetic derivatives [6].

Electrospinning is a broadly used technology for electrostatic fiber formation which utilizes electrical forces to produce polymer fibers with diameters ranging from 2 nm to several micrometers using polymer solutions of both natural and synthetic polymers. This process offers unique capabilities for producing novel natural nanofibers and fabrics with controllable pore structure. Electrospun fibers have been successfully applied in various fields, such as, nanocatalysis, tissue engineering scaffolds, protective clothing, filtration, biomedical, pharmaceutical, optical electronics, healthcare, biotechnology, defense and security, and environmental engineering. Stimuli-responsive nanofibers have received great attention during the last 10 years and have been exploited in a diverse range of biomedical applications [6,7].

Several strategies have already been employed to prepare magnetically responsive electrospun nanofibers and nanotextile. The most often used strategy is based on the direct mixing of magnetic nanoparticles with the polymer solution followed by electrospinning; in order to obtain homogeneous nanoparticle/polymer nanosuspension, mechanical stirring and ultrasonication are usually employed. Alternatively a polymer solution containing the magnetic nanoparticles salt precursors is electrospun first, followed by the immersion of the resulting electrospun nanotextile into a high pH solution and subsequent formation of iron oxide magnetic nanoparticles [6,8,9].

Magnetic fluids (MFs) can be successfully used for magnetic modification of various diamagnetic materials. Perchloric acid stabilized magnetic fluid in methanol was used for one-step magnetic modification of sawdust [10], peanut husks [11] or spent grain [12]. In this paper this simple approach was employed for magnetic modification of different types of nanotextile; in addition to formation of magnetically responsive nanomaterials, the bound iron oxide nanoparticles exhibited peroxidase-like activity.

2. Materials and methods

2.1. Materials

Water based magnetic fluid stabilized with perchloric acid was prepared using a standard procedure [13]; its concentration was 70.3 mg/mL and the diameter of majority of nanoparticles was between

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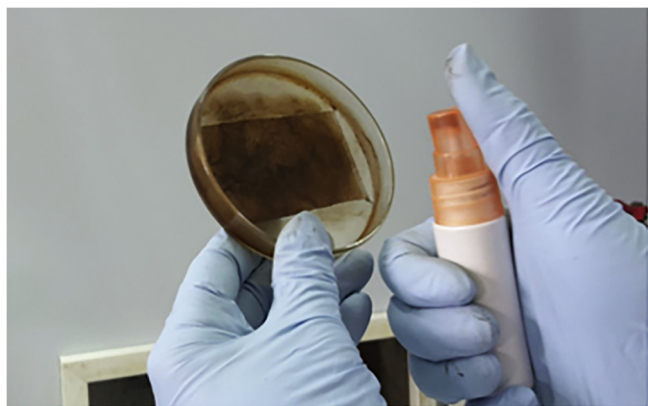


Fig. 1. Spray technique used for magnetic modification of nanotextile using chloroform-based magnetic fluid.

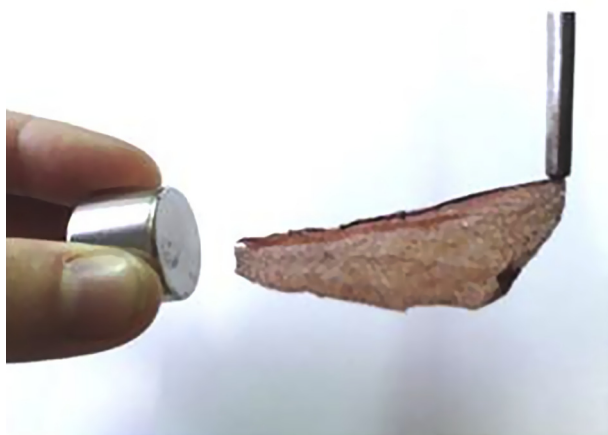


Fig. 2. Response of magnetic fluid modified nanotextile to external magnetic field.

10 and 18 nm [14]. *N,N*-diethyl-*p*-phenylenediamine sulfate salt (DPD) was purchased from Merck, Germany while nonanoic acid (96%) was from Sigma-Aldrich, Czech Republic. $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and hydrogen peroxide were obtained from Lach-ner, Czech Republic. Methanol, chloroform and ammonium hydroxide were from Penta (Czech Republic). Electrospun textile formed from polyamide 6, polyurethane, polyvinyl alcohol and polycaprolactone was obtained from Nanoprogres, Pardubice, Czech Republic; it was prepared using a DC Spinner at 38 kV, distance between needle and surface was 20 cm.

2.2. Preparation of chloroform-based magnetic fluid

Chloroform-based magnetic fluid was prepared as follows. 1.72 g $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and 4.7 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were dissolved in 80 mL of water with constant stirring (500 rpm) at 80 °C. After dissolution of iron salts 10 mL of acetone containing 200 μL of nonanoic acid was added dropwise, and then 10 mL of concentrated ammonium hydroxide solution was gradually added. After the suspension formation 200 μL portions of nonanoic acid (in total 10 doses) were added each 5 min. After the last addition of nonanoic acid the suspension was stirred (500 rpm) for the next 30 min at 80 °C. The produced black precipitate was cooled to room temperature, magnetically separated and washed with methanol (100 mL) five times. After that 40 mL of water was added under stirring (500 rpm) and heated at 70 °C. In the next step 12 mL of the mixture of nonanoic acid and ammonium hydroxide solution (1:5, v/v) was added dropwise (1 drop per 2 s). After water evaporation at 70 °C, ammonia was evaporated in the next day at room temperature in a fume hood. Subsequently the precipitate was suspended in 100 mL of chloroform.

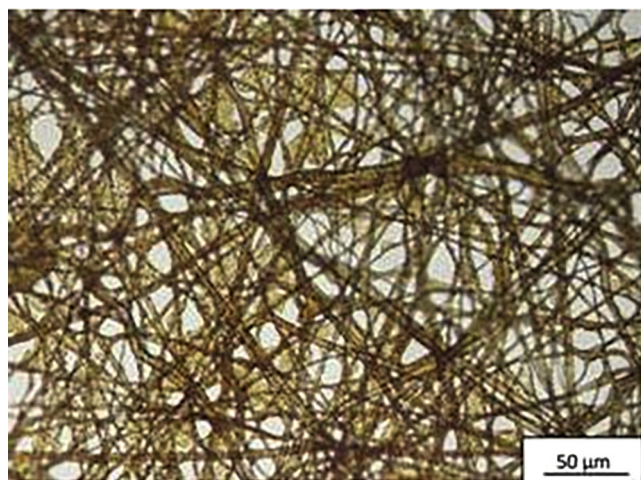
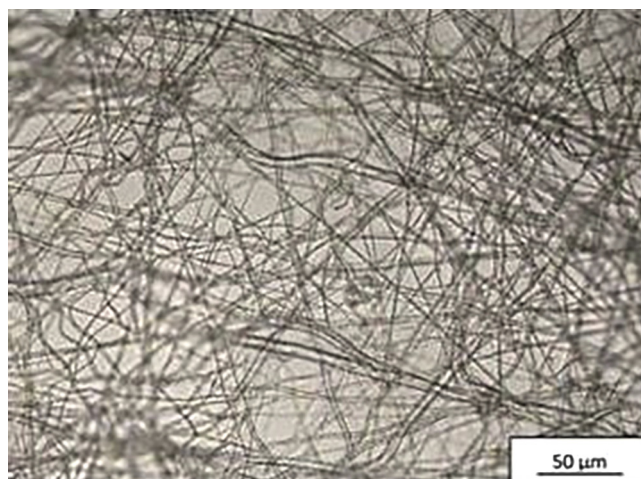


Fig. 3. Optical microscopy of native polyurethane nanotextile (top) and the same nanotextile after magnetic modification with perchloric acid stabilized magnetic fluid (bottom).

This suspension was centrifuged using Universal 320 (Hettich, Germany) centrifuge for 30 min at 4,000 rpm (1771g). The supernatant was used as the final magnetic fluid; its concentration was 7.5 mg/mL.

2.3. Magnetic modification with acid and chloroform-based magnetic fluids

Electrospun textile was modified with perchloric acid stabilized MF and chloroform-based MF using the spray procedure. Commercially available glass spray bottle was filled with the MF. Chloroform-based MF was kept in the freezer at −18 °C before use and then directly applied, while perchloric acid stabilized MF was mixed with methanol in the ratio 1:4 (v/v) before use at laboratory temperature. Approximately 1 mL of MF was applied on 1 cm² of the textile. Modified nanotextiles were dried on a laboratory hot plate at ca 45–50 °C in a fume hood to fix the magnetic nanoparticles on the fiber surface. Then the magnetized materials were washed several times with distilled water to release non-bound magnetic particles and air-dried. Dried material was cut into squares 2 × 2 cm.

2.4. Determination of peroxidase-like activity

Peroxidase-like activity of magnetically modified nanotextiles was determined by spectrophotometry using *N,N*-diethyl-*p*-phenylenediamine sulfate (DPD) as a chromogenic substrate. Washed, dried samples of magnetic nanotextile (squares 2 × 2 cm) were preincubated several minutes in test tubes containing 3.4 mL of water. Then 400 μL of

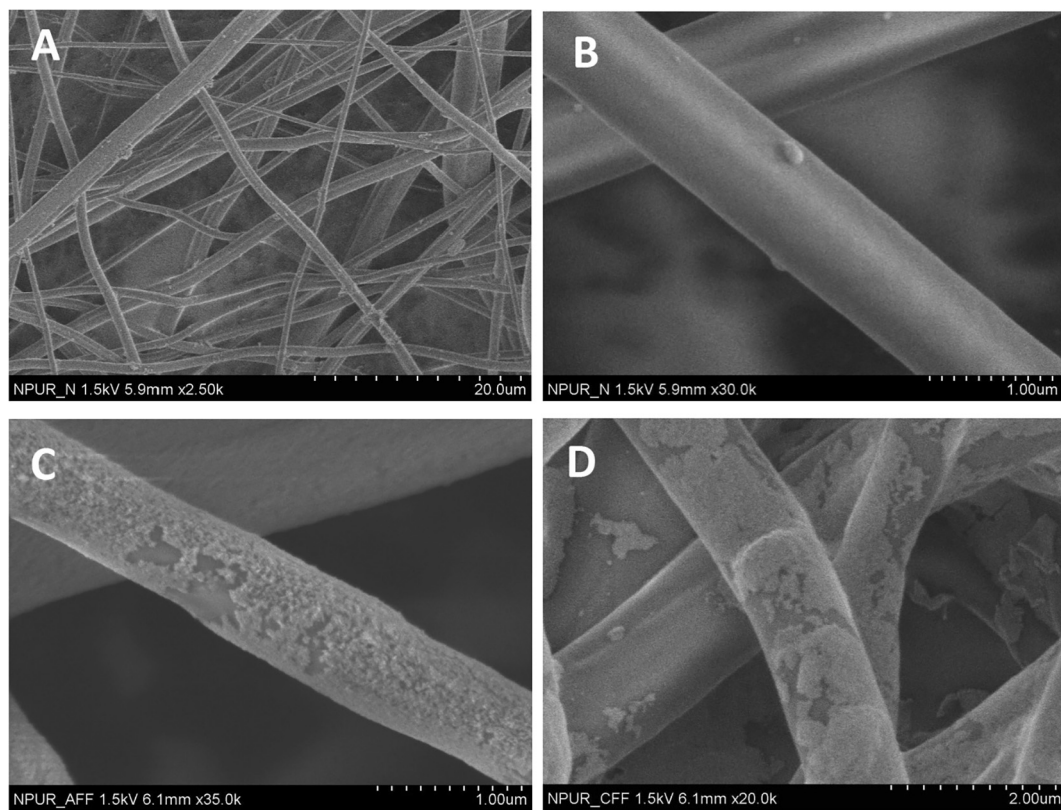


Fig. 4. Scanning electron microscopy of native polyurethane nanotextile (A, B), the same nanotextile modified with perchloric acid stabilized magnetic fluid (C) and nanotextile modified with chloroform-based magnetic fluid (D).

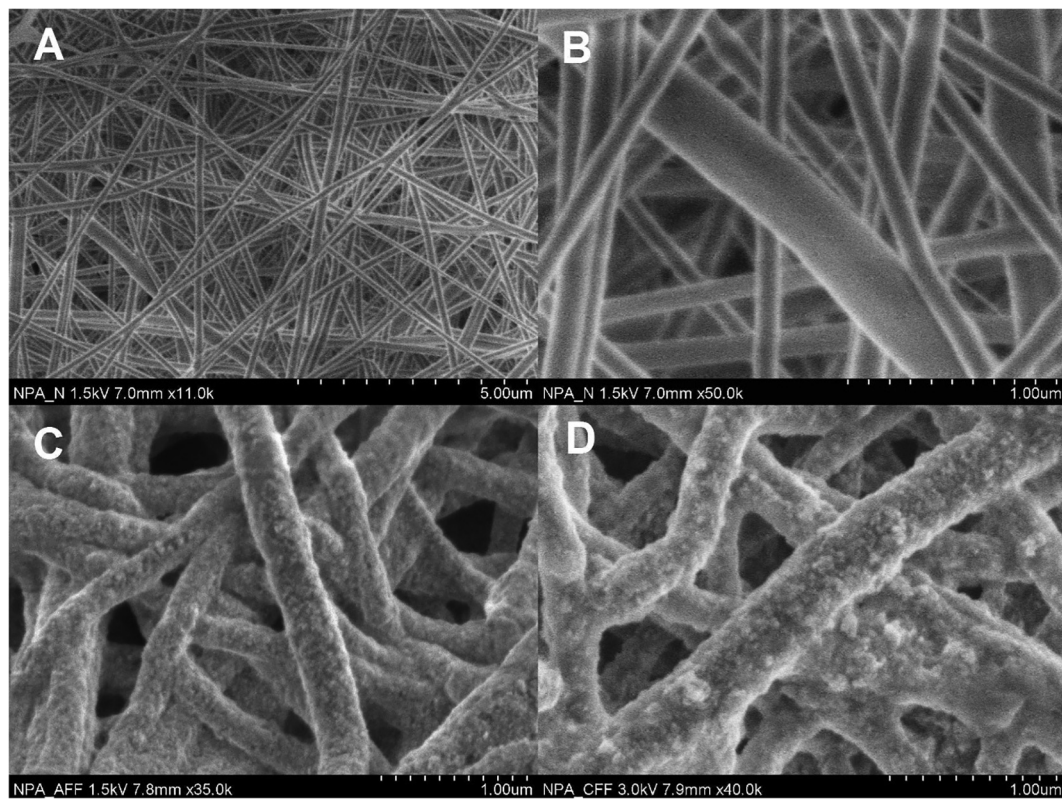


Fig. 5. Scanning electron microscopy of native polyamide nanotextile (A, B), the same nanotextile modified with perchloric acid stabilized magnetic fluid (C) and nanotextile modified with chloroform-based magnetic fluid (D).

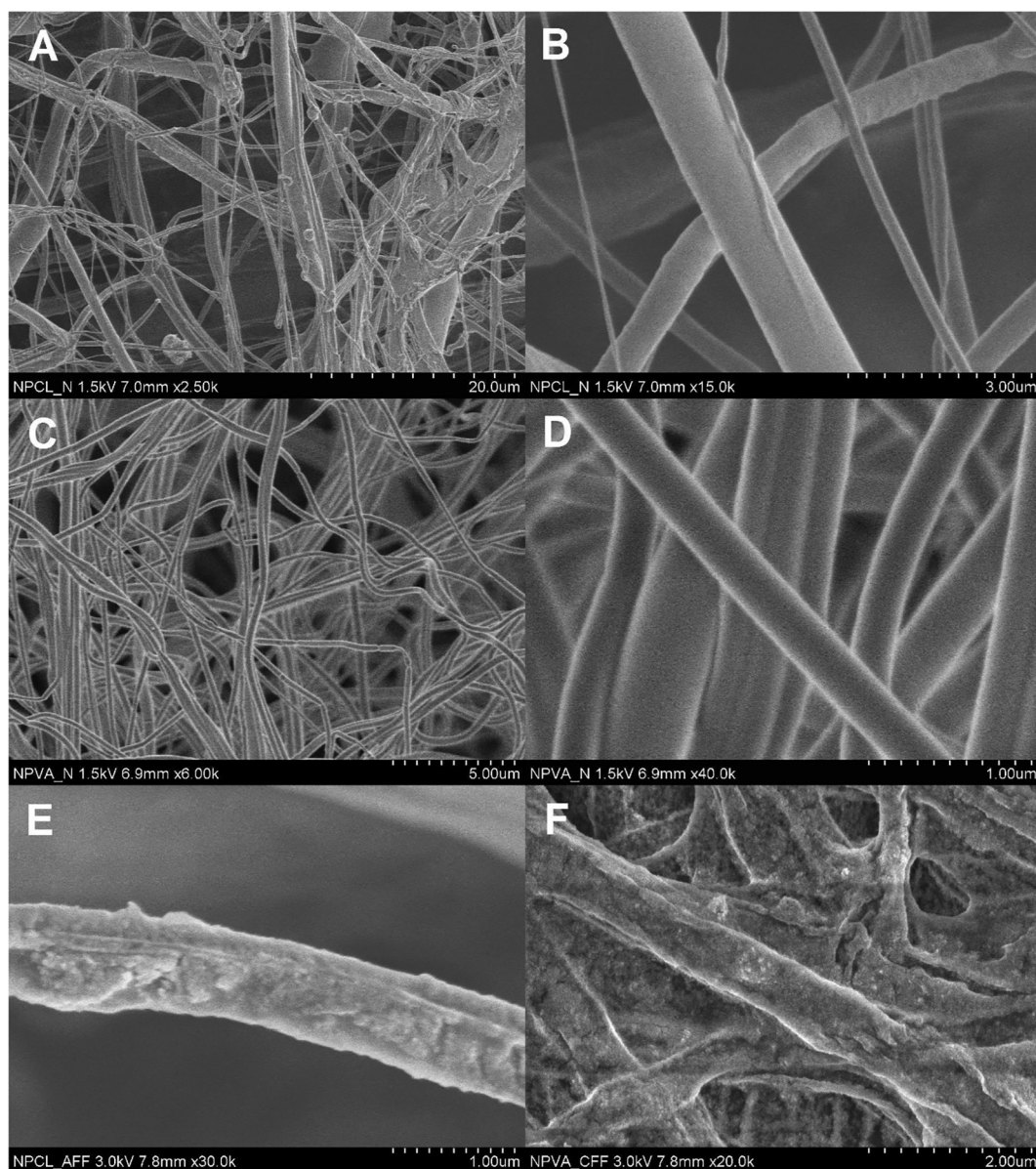


Fig. 6. Scanning electron microscopy of native polycaprolactone nanotextile (A, B), native polyvinyl alcohol nanotextile (C, D), polycaprolactone nanotextile modified with perchloric acid stabilized magnetic fluid (E) and polyvinyl alcohol nanotextile modified with chloroform-based magnetic fluid (F).

12.5 mmol DPD solution was added, followed by 200 μ L of 2% hydrogen peroxide. Samples were mixed at 16 rpm at the rotary mixer and after 5 min of incubation at room temperature magnetic nanotextile was captured by a permanent magnet and purple-colored supernatants were collected. Reaction mixture was immediately measured spectrophotometrically at 551 nm using Cintra 20 spectrophotometer (GBC Scientific Equipment, Braeside, Australia). Magnetically modified nanotextile materials in test tube were rinsed with water and repeatedly used for peroxidase-like activity measurement. Native nanotextile squares of the same size were used to obtain blank values.

2.5. Materials characterization

The morphological study was performed using optical microscopy (Arsenal) and scanning electron microscopy (SEM) measurements. The samples were analyzed by SEM 120 Hitachi SU6600 (Hitachi, Tokyo, Japan) with accelerating voltage 5 kV, equipped with energy-dispersive X-ray spectroscopy (EDS)—Thermo Noran System 7 (Thermo Scientific, Waltham, MA, USA) with Si (Li) detector (accelerating voltage of 10 kV

and acquisition time 300 s).

3. Results and discussion

Four types of electrospun nanofibers prepared from polyamide 6 and polyvinyl alcohol (typical examples of hydrophilic nanofibers) or polyurethane and polycaprolactone (typical examples of hydrophobic nanofibers) were used for subsequent magnetic modification using two types of magnetic fluids, namely water based, perchloric acid stabilized MF, and chloroform-based, nonanoic acid stabilized MF. A simple procedure was used for magnetic modification, namely spraying the nanotextile with either native chloroform-based MF or with acid MF/methanol mixture (Fig. 1). Approximately 17–18 mg of magnetic iron oxides from perchloric acid stabilized MF and 7–8 mg of magnetic iron oxides from chloroform-based, nonanoic acid stabilized MF were loaded on 1 cm² of the modified nanotextile. The solubility of nanofiber forming polymers in MF used for modification has to be taken into account, that's why polyvinyl alcohol nanofibers were modified only with chloroform-based magnetic fluid, and polycaprolactone nanofibers

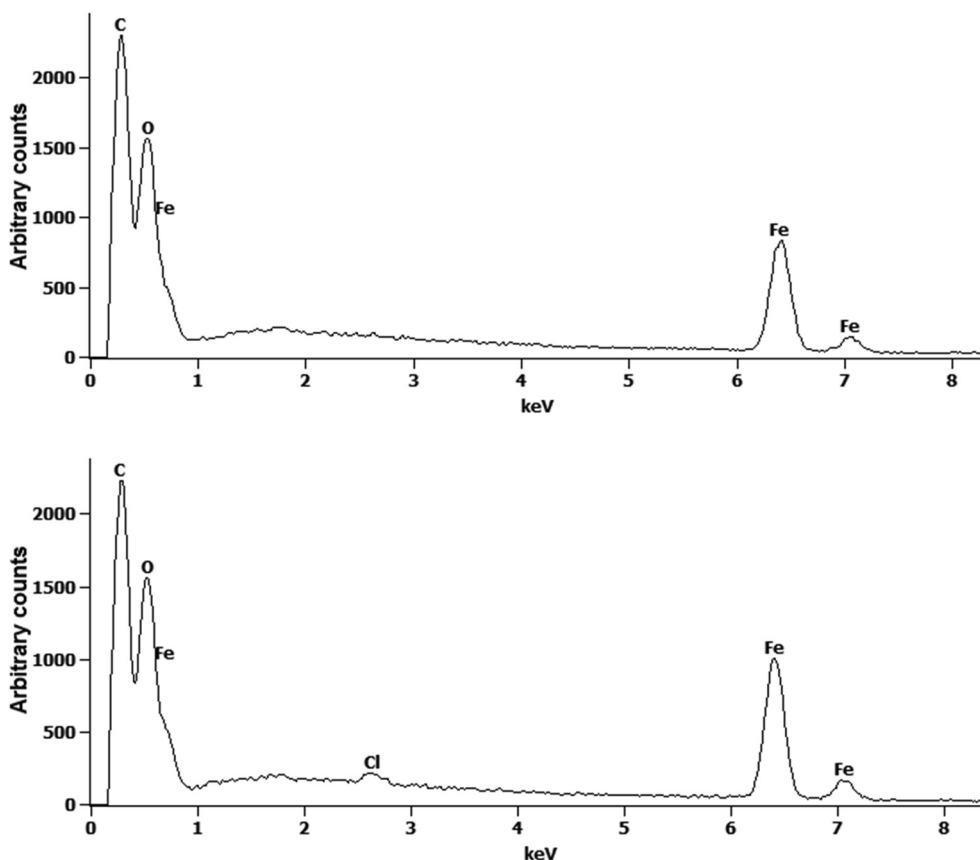


Fig. 7. Energy-dispersive X-ray spectroscopy of polyurethane nanotextile modified with perchloric acid stabilized MF (top) and chloroform-based MF (bottom).



Fig. 8. Typical example of peroxidase-like activity of polyurethane nanotextile modified with chloroform-based MF leading to the formation of pink reaction product (oxidized DPD). Left – before the assay; right – after the assay.

were modified only with water-based MF. After spraying the iron oxide nanoparticles were thermally fixed on the fiber surface; the magnetically modified nanotextile was very stable in water and no visible release of magnetic nanoparticles was observed after three months storage in water. Magnetic modification has not substantially changed the structure of the modified nanotextile.

In both cases ferrofluid modification of electrospun textile led to the formation of magnetic composite material (see Figs. 2 and 8 showing the response of modified nanotextile to external magnetic field and Fig. 3 for optical microscopy of native and magnetically modified

polyurethane nanotextile). The fibers surface was modified with magnetic iron oxide nanoparticles (see Figs. 4–6 presenting SEM images of native nanotextile materials and nanotextile magnetically modified with both acid and chloroform-based MFs). The presence of iron oxides on the surface of magnetically modified polyurethane nanofibers was confirmed using energy-dispersive X-ray spectroscopy (Fig. 7).

Magnetically responsive electrospun nanotextile was already used in several applications including magnetic drug targeting, magnetic scaffold formation, magnetic bioseparation, hyperthermia cancer treatment and contrast enhancement in magnetic resonance imaging [6]. In this paper we present another important application of magnetically modified nanotextile, namely as a potential catalyst exhibiting peroxidase-like activity.

Magnetically modified nanotextile exhibited peroxidase-like activity; this activity was determined by a photometric method in which a well-known chromogenic substrate *N,N*-diethyl-*p*-phenylenediamine (DPD) was oxidized by a peroxidase-like catalyzed reaction. After the assay purple-colored reaction product was formed (see Fig. 8).

Nanotextiles modified with both MFs exhibit high peroxidase activity, however, in the case of modification with acid MF progressive decrease of peroxidase-like activity was observed during repeating measurements. Substantially better results were observed when nanotextiles were modified with chloroform-based MF.

Fig. 9 presents the course of peroxidase-like activity of polyamide 6 and polyurethane nanotextiles modified with chloroform-based MF; the same amount of magnetic iron oxide nanoparticles was used for modification of both nanotextiles. The magnetic polyamide 6 nanotextile was always kept in water solutions; on the contrary, one of the magnetic polyurethane nanotextile squares was dried before subsequent enzyme-like assay (before the cycle 2). As can be seen, high and relatively quite stable peroxidase-like activity was observed during the series of catalytic assays for individual nanotextiles (similar peroxidase-

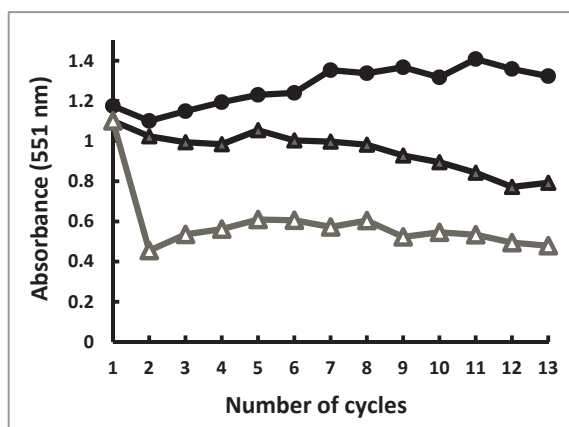


Fig. 9. Peroxidase-like activity of nanotextiles modified with chloroform-based MF (area $2 \times 2 \text{ cm}^2$) expressed as absorption at 551 nm. Modified nanotextile was repeatedly used for activity measurements. ● – polyamide 6; ▲ – polyurethane (wet material); △ – polyurethane (dried material).

like activity was observed after 40 cycles using magnetic polyamide 6 (data not shown)). However, the magnetic nanotextile drying can partially decrease the peroxidase-like activity (the absorbance change at 551 nm was ca 0.7 between the first and second cycle). The native nanotextile exhibited negligible peroxidase-like activity.

Taking into account the extinction coefficient of the oxidized form of DPD ($21000 \text{ M}^{-1} \text{ cm}^{-1}$) [15] it is possible to calculate the amount of formed colored product per one square centimeter of the magnetically modified nanotextile during the reaction time (5 min). These values were $52.4 - 67.0 \text{ nmol/cm}^2$ for polyamide 6, $36.8 - 52.4 \text{ nmol/cm}^2$ for wet polyurethane and $20.2 - 29.0 \text{ nmol/cm}^2$ for dried polyurethane.

4. Conclusions

Iron oxide nano- and microparticles have been widely used in many important fields due to their excellent physical properties, including magnetism and enzyme-like activity. In this paper a simple spray procedure for magnetic modification of electrospun textile using both perchloric acid stabilized magnetic fluid and chloroform-based magnetic fluid is described. Long-term stability of magnetic composites was observed. The prepared magnetic nanotextiles exhibited high peroxidase-like activity and thus they can find interesting applications in biosciences, biomedicine and biotechnology as a low-cost substitute of standard peroxidases. The potential applications include e.g., substitution of horseradish peroxidase (HRP) in the enzyme-linked immunosorbent assay (ELISA) and other HRP-related molecular detection methods, for the detection of glucose, galactose, cholesterol or alcohol (in combination with glucose oxidase, galactose oxidase, cholesterol oxidase or alcohol oxidase), or as a potential bioremediation agent for

the treatment of a broad range of toxic, carcinogenic and hazardous environmental pollutants at low cost [16,17].

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