

Antibacterial property on Gram-positive bacteria of polypyrrole-coated fabrics

Alessio Varesano,¹ Claudia Vineis,¹ Cinzia Tonetti,¹ Giorgio Mazzuchetti,¹ Vittorio Bobba²

¹CNR-ISMAR, Institute for Macromolecular Studies – National Research Council of Italy, C.so Giuseppe Pella 16 I-13900 Biella, Italy

²Yanga srl, via Monte Orfano 21, I-13864 Crevacuore (Biella), Italy

Correspondence to: C. Vineis (E-mail: c.vineis@bi.ismar.cnr.it)

ABSTRACT: Antibacterial activity against Gram-negative bacteria of polypyrrole-coated fabrics has been demonstrated in the past. In this work, biocidal efficacy of polypyrrole has been evaluated against Gram-positive bacteria on textiles with different polypyrrole loading. Excellent bacterial reduction ($\geq 99\%$) was found on cotton fabrics containing more than ~ 9 wt % of polypyrrole. Polypyrrole loading can be greatly reduced in presence of silver. Silver-containing fabrics used in this work alone does not guarantee a complete biocidal effect, but the addition of just 2 wt % of polypyrrole showed a bacteria reduction of 99%. Moreover, stability to different washing procedures of the antibacterial activity was evaluated. Fabrics were characterized by scanning electron microscopy, energy dispersive X-ray analysis, and infrared spectroscopy. Stability of the coating was assessed by abrasion tests. PPy showed excellent fastness to abrasion. © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2015**, *132*, 41670.

KEYWORDS: conducting polymers; fibers; textiles

Received 21 May 2014; accepted 21 October 2014

DOI: 10.1002/app.41670

INTRODUCTION

Conjugated polymers are materials useful for several applications (i.e. electrostatic dissipation, heating devices, sensors, energy-storage devices and capacitors, microwave attenuation, and EMI shielding) related to their capability of electrical charge transfer. For this reason, the conjugated polymers are also named as “conducting polymers” or “electroactive polymers”.

Polypyrrole (PPy) is one of the most studied conjugated polymers. It can be easily synthesized by electrochemical or chemical oxidative polymerization from water solution of the monomer. Moreover, PPy resulted a biocompatible polymer,¹ and it was proposed in biomedical field for nerve growth^{2,3} and electrically stimulated cell growth⁴⁻⁶.

Besides, PPy showed excellent antibacterial properties against *Escherichia coli* (Gram negative).^{7,8} Bioactivity of PPy is likely due to the presence of positive charges in the backbone chains and no leaching of biocidal substances has been already demonstrated using PPy-coated fabrics.⁷ The “non-leaching” approach would avoid or limit the release of the biocidal agents to the environment or to the skin of the wearers, in the case of garments. Recently, PPy has been also used for antimicrobial applications in combination with silver on textiles.^{9,10}

Emerging applications for biocidal polymers such as PPy are envisaged in textile products for many different fields. In sportswear (e.g., garments, socks, underwear) biocidal activities are mainly required for odor control. Anti-microbial textile-based medical devices like bandages, disposable, wipes, white coats, and bedding are proposed for disease and infection control, in particular to fight multiple-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), as well as in textile products for home (e.g., sheets, towels, tablecloths, curtains). Another wide field of applications is in filtration (white rooms, hospital and operating theatre, food and pharma industries, water depuration and drinkable water, air conditioning, public transport, for instance).

In this work, PPy-coated fabrics were tested against Gram-positive bacteria (namely *S. aureus*). Fastness to laundering was evaluated using four different detergents. Moreover, a comparison between silver-containing fabric and PPy coating was carried out, a synergic effect of silver and PPy in increasing biocidal efficacy at low PPy content has been found. Fabrics were characterized by scanning electron microscopy, energy dispersive X-ray analysis, and infrared spectroscopy. Stability of the coating was assessed by abrasion tests.

Table I. Chemical Concentrations and Weight Uptake at Different Coating Conditions

Compound	Concentration (g/L)			
Pyrrrole	0.5	1.0	2.0	4.0
Ferric sulphate	5.6	11.2	22.3	44.7
DSS	1.1	2.3	4.6	9.2
Weight increase (%)	2.0	3.4	9.1	25

EXPERIMENTAL

Materials

Raw cotton knit-fabric and silver-containing cotton fabric were produced by Yanga srl (Italy). Silver-containing cotton fabric (Redisilver®) contains 10 wt % of silver-coated polyester (polyethylene terephthalate) filaments. Chemicals used for the synthesis of PPy were: pyrrole (97%) and dicyclohexyl sulfosuccinate sodium salt (DSS) by Fluka (Germany), and ferric sulphate hydrate (21–23 wt % Fe) by Sigma-Aldrich (Germany).

The bacterium used for antibacterial test was *S. aureus* (Gram positive) ATCC 6538 by Microbiologics. Yeast extract agar and peptone were supplied by Liofilchem (Italy). Other chemicals were petroleum ether and tetrachloroethylene by Riedel-de

Haën (Germany), ECE detergent (according to ISO 105-C06) by EMPA Testmaterials (Switzerland) and Tween® 20 by Sigma-Aldrich (Germany), commercial Marseille soap by Industria Saponi (Italy)-containing olive oil-derived anionic surfactants, in particular sodium palmitate and sodium dodecanoate.

PPy Coating Procedure

Fabrics were cleaned by Soxhlet extraction using petroleum ether for 2 h, and dried in air. PPy depositions were carried out at room temperature by plunging the fabrics (about 5 g) in stirred solution of ferric sulphate and DSS with a liquor ratio of 50 : 1 mL/g. After an impregnation of 15 minutes, pyrrole was drop-wise added to the solution, up to the final concentrations. Concentrations of the chemical used are reported in Table I for different monomer concentrations. The solution became dark because of PPy production. The samples were pulled out from the polymerization bath after 4 h, rinsed in cold water, squeezed, and dried overnight at room temperature. The samples were weighted before and after PPy deposition. The percentage of weight increase ($w\%$) was measured using the following eq. (1):

$$w\% = \frac{b-a}{a} \times 100 \quad (1)$$

where a is the weight of the fabric before the deposition and b is the weight after the deposition. The results are reported in Table I.

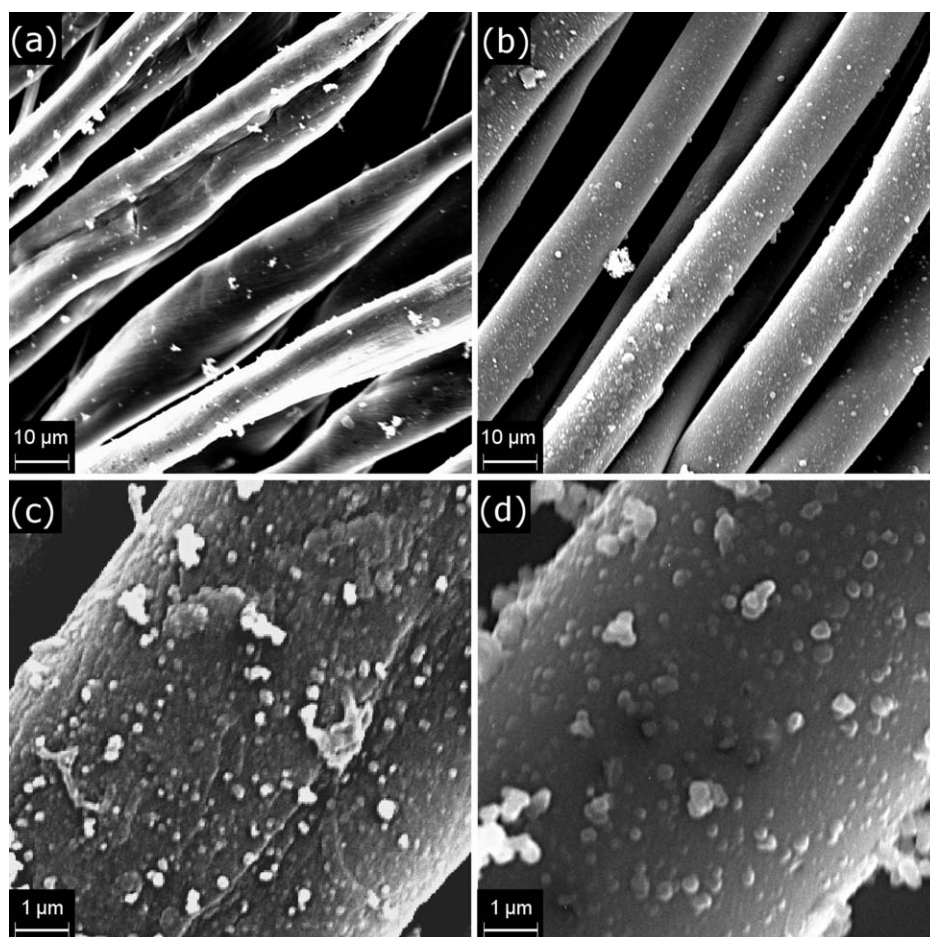


Figure 1. SEM pictures of (a) and (c) PPy-coated cotton fabric, (b) and (d) PPy-coated silver-containing fabric (pyrrole concentration of 2 g/L).

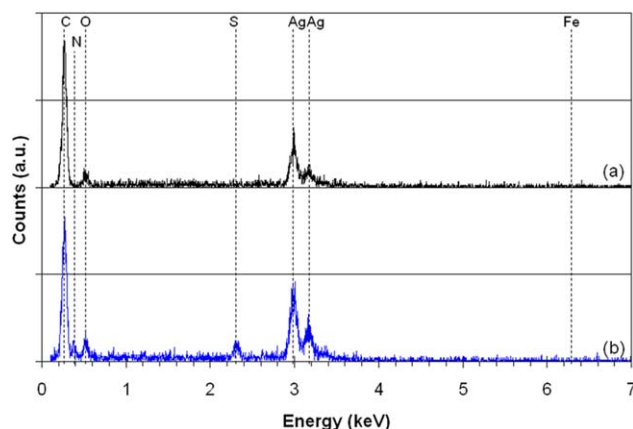


Figure 2. EDX spectra of silver-containing cotton fabrics (a) before PPy coating and (b) after PPy coating (pyrrole concentration of 2 g/L). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Washing Conditions

Samples of the resulting PPy-coated fabrics were washed five times in a wash-wheel in four different ways:

1. 5 g/L standard soap (ECE) in water at 40°C with a liquor ratio of 50 : 1 mL/g in order to simulate a domestic laundering, according to ISO 105-C01 (domestic laundering);
2. 1 g/L non-ionic detergent (Tween® 20) in water at 40°C with a liquor ratio of 50 : 1 mL/g (non-ionic laundering);
3. 4 g/L Marseille soap in water at 40°C with a liquor ratio of 50 : 1 mL/g (Marseille soap).
4. pure tetrachloroethylene at room temperature with a liquor ratio of 40 : 1 mL/g, according to ISO 105-X05 (dry-cleaning);

Each washing cycle lasted 30 minutes. For the procedures (a), (b), and (c) after each cycle the samples were rinsed in cold water for 10 minutes, as requested by ISO 105-C01. Final drying was carried out at room temperature. For the procedure (d), the samples were squeezed and residual solvent evaporated in air in <1 h.

Characterizations

Scanning electron microscopy (SEM) investigations were performed with a Leica Electron Optics (LEO) 435 VP SEM, at an acceleration voltage of 15 kV and a 20 mm working distance. No metallization was carried out before SEM analysis because the samples already had good conductivities. Energy dispersive X-ray (EDX) analysis was performed by an Oxford Instruments Model 7060 Link ISIS interfaced to a PC using 4096 channels in the range of 10 keV.

Fourier transform infrared (FT-IR) spectra were acquired with Attenuated Total Reflection (ATR) technique in the range from 4000 to 550 cm^{-1} with 100 scans and 4 cm^{-1} of band resolution by means of a Thermo Nicolet Nexus spectrometer equipped with a Smart Endurance™ (ZnSe crystal).

Round specimens with a diameter of 3.9 cm were cut and subjected to abrasion. Abrasion tests were performed using a Nu-Martindale abrasion and pilling tester from James Heal & Co.

(UK), with a load of 12 kN, according to ISO 12947 test method.

Antibacterial Test

Antibacterial activity was evaluated following the antibacterial standard AATCC Test Method 100–2012,¹¹ which is a quantitative method for the assessment of antibacterial finished textile materials. The procedure is briefly described in here. Test bacterium culture grown for 24 h in a suitable nutrient broth was diluted to give a concentration of $1.5\text{--}3.0 \times 10^5$ CFU/mL (inoculum). A specimen of each fabric (about 1 g) was transferred to a flask. 1.0 ± 0.1 mL of the inoculum were added on each specimen. All flasks were incubated at 37°C for 1 h (contact time). After incubation, 100 ± 1 mL of a buffer solution at pH 7 were added in each flask and shaken vigorously for 1 minute. Total of 1.0 ± 0.1 mL of the solution was diluted 1 : 10 in the same buffer solution. 1.0 ± 0.1 mL of diluted solution was plated in nutrient agar. In this way, a maximum of 150–300 CFU were transferred to each plate. The inoculated plates were incubated at 37°C for at least 24 h and surviving colonies were counted. The antibacterial activity is expressed as percentage reduction of the micro-organisms after contact with the test specimen compared to the number of bacterial cells originally present in the dilute inoculum solution contacted with each specimen (so-called “0” contact time). The tests were performed in duplicate. The results are the average of two separate counting.

RESULTS AND DISCUSSION

SEM Observations and EDX Analysis

Freshly PPy-coated specimens of raw cotton knit-fabric and silver-containing cotton fabric were observed by SEM. Pictures are reported in Figure 1. Each fibre shows a very uniform film-like dense layer coating the surface. The layer appears quite rough due to the presence of PPy particles and agglomerates of particles deposited on the fibre surface. In particular, Figure 1(a) shows PPy-coated cotton fibres recognizable by the typical

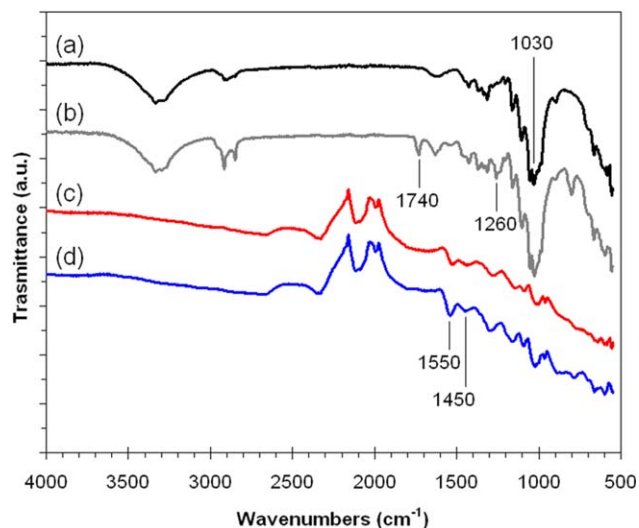


Figure 3. FT-IR spectra of pure cotton fabrics (a), silver-containing cotton fabrics (b), PPy-coated cotton fabric (c), and PPy-coated silver-containing cotton fabric (d). Pyrrole concentration of 2 g/L. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



Figure 4. Picture of the specimens after abrasion tests (200, 1000, 2000, and 10,000 abrasion cycles). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

bean-shaped cross-section. While the Figure 1(b) shows a bundle of polyester filaments (round cross-section and regular diameter) within the fabric structure of the silver-containing fabric sample. Pictures (c) and (d) of Figure 1 show PPy-coated fibre surfaces at high magnification of a cotton fibre and a polyester filament, respectively. The morphology of PPy layer is similar in both cases. The layer on the fibre surface is composed of particles of around 300 nm.

Polyester filaments are coated with a silver layer on which PPy had been deposited. The presence of both PPy and silver was confirmed by elemental analysis carried out with EDX spectroscopy and reported in Figure 2. The spectrum (a) refers to silver-containing fabric without PPy coating. It is characterized by peaks of carbon, oxygen, and silver. After the coating with PPy, other peaks (in addition to C, O, and Ag) were observed related to nitrogen (contained in the pyrrole ring) and sulphur (contained in the dopant DSS used), as spectrum (b) shows. The peak related to iron (FeK 6.3 keV) was not found in the spectra (b). Therefore, it is possible to suppose that there are not iron ions as residues of oxidation agent embedded in the PPy layer. This is particularly important because iron ions can interfere in antibacterial test results.^{12–14}

FT-IR Analysis

Figure 3 shows FT-IR spectra of uncoated and PPy-coated cotton fabrics with and without silver. The spectrum of cotton (a) is characterized by broad bands at about 3300 and 2900 cm^{-1} assigned to O—H and C—H stretching vibrations, and a strong absorption band at 1030 cm^{-1} attributed to overlapping bands of chemical groups of cellulose, such as C—C, C—O, and C—O—C. The spectrum of silver-containing cotton fabric (b) shows weak adsorption bands of polyester due to the silver-coated polyester filaments at 1740 and 1260 cm^{-1} (O—C=O ester vibrations).¹⁵ The spectral feature of the cellulose appears

Table II. Bacterial Reduction Against *S. aureus* of PPy-Coated (2 g/L of pyrrole) Cotton Fabric Compared to Pristine Cotton Fabric

Sample	Bacterial reduction
Uncoated	16%
Coated	100%

greatly attenuated in the spectra of PPy-coated fabrics. Since the infrared beam of the ATR technique analyses only the fibre surface with a penetration $<1 \mu\text{m}$, the evenness of the PPy layer on the fibres was excellent (fibre surfaces are fully covered) and PPy thickness was comparable with the infrared-ray penetration. The spectra (c) and (d) in Figure 2 show the characteristic tail of the electronic absorption of PPy in the range between 4000 and 1700 cm^{-1} .¹⁶ The bands at 1550 and 1450 cm^{-1} are assigned to C—C and C—N stretching vibrations in the pyrrole ring.¹⁷

Abrasion Tests

PPy-coated cotton fabrics showed excellent fastness to abrasion. Figure 4 shows the specimens of fabrics subjected to 200, 1000, 2000, and 10000 abrasion cycles. PPy layer deposited on the fabric is black and no color changes can be observed on the samples, even at 10,000 abrasion cycles.

Antibacterial Tests

Antibacterial performances against *S. aureus* of PPy-coated fabrics were quantified following the AATCC 100 test method. Table II reports the results of bacterial reduction of uncoated and PPy-coated raw cotton fabrics for comparison. PPy-coated cotton fabrics showed excellent biocidal properties (i.e. 100% of bacterial reduction) when 2 g/L of monomer concentration was used in the polymerization bath in the deposition phase. At this condition a PPy content of $\sim 9\%$ was measured on the cotton fabrics as weight increase after deposition. On the contrary, uncoated raw cotton fabrics showed poor activity against bacteria.

Antibacterial performances of PPy-coated raw cotton fabrics were evaluated also after washing. Four different washing

Table III. Bacterial Reduction Against *S. aureus* of PPy-Coated (2 g/L of pyrrole) Cotton Fabric After Five Washing Cycles

Laundering	Bacterial reduction
Domestic (ECE detergent)	9%
Marseille soap	52%
Non-ionic (Tween® 20)	78%
Dry-cleaning	98%

Table IV. Bacterial Reduction on *S. aureus* of PPy-Coated Cotton Fabrics with and Without Silver at Different Monomer Concentration

Pyrrrole concentration	Without silver ^a	With silver ^b
Without PPy	16%	91%
0.5 g/L	18%	99%
1.0 g/L	95%	100%
2.0 g/L	100%	100%
4.0 g/L	100%	100%

^aPure cotton fabric.

^bSilver-containing cotton fabric (10 wt % of silver-coated polyester).

procedures and surfactants were used. Each sample was washed five times before antibacterial testing. The results of bacterial reduction percentage are reported in Table III. PPy-coating resulted sensitive to water, in particular when an ionic surfactant is used in the washing solution (e.g., ECE detergent, Marseille soap). Fastness to washing of PPy biocidal activity was found to be good using non-ionic surfactants (i.e. Tween® 20). Five times dry-cleaned PPy-coated fabrics attained an excellent bacterial reduction of 98%. The results can be easily explained considering the ionic chemical structure of PPy backbone chain, widely reported in literature^{17–21}: the ionic surfactant in water seems to destabilize the polar structure resulting in loss of positive charges borne by PPy chain.

Finally, a synergic biocidal effect between silver ions and PPy was observed by treating silver-containing cotton fabrics with the proposed PPy-coating procedure. As Table IV shows, antibacterial efficacy increases with the increase of pyrrole concentration in the polymerization bath of the deposition phase. Obviously, increasing pyrrole concentration resulted in an increase of PPy content on the treated fabrics, as reported in Table I. High-bacteria reductions (>95%) were obtained by treating cotton fabrics (without silver) with pyrrole concentration of 2 g/L or above. While, using cotton fabrics with 10 wt % of silver-coated fibres resulted in excellent bacterial reductions (99% or higher) already when 0.5 g/L of pyrrole was used. It is worth to note that the uncoated fabric with silver-coated fibres showed a high-bacterial reduction, but excellent results were attained only after PPy coating. It is known that only silver ions (Ag⁺) actually act against bacteria; whereas, silver in the fabric is mainly metallic (Ag⁰), nevertheless, Ag⁺ is produced by natural oxidation due to air oxygen and humidity.^{22–24} Even at low content, PPy seems to promote silver ions formation and, in turn, PPy and Ag⁺ contribute in increasing biocidal efficacy of the fabric.

CONCLUSION

In this work, antibacterial activity against Gram-positive bacteria of PPy-coated fabrics has been evaluated. In particular, PPy-coated cotton fabrics showed 100% bacterial reduction using 2 g/L of monomer concentration in the polymerization bath. On the contrary, uncoated raw cotton fabrics showed poor activity against bacteria. Moreover, antibacterial properties of PPy-coated raw cotton fabrics were evaluated also after

washing using four different washing procedures and surfactants. The results have been very good using non-ionic surfactants and dry-cleaning. A synergic antibacterial effect between silver ions and PPy was demonstrated by treating silver-containing cotton fabrics with the proposed PPy coating procedure. Finally, the PPy layer showed an excellent adhesion to cotton fibres.

ACKNOWLEDGMENTS

This work was funded by the EU FP7 - ManuNET project (call 2010) within the ERA-Net network through the ANFIBIO project “Antibacterial and electromagnetic interference shielding finishing for BIOMedical and technical textiles”.

REFERENCES

- Sajesh, K. M.; Jayakumar, R.; Nair, S. V.; Chennazhi, K. P. *Int. J. Biol. Macromol.* **2013**, *62*, 465.
- Thompson, B. C.; Moulton, S. E.; Richardson, R. T.; Wallace, G. G. *Biomaterials* **2011**, *32*, 3822.
- Llinas, R. R.; Walton, K. D.; Nakao, M.; Hunter, I.; Anquetil, P. A. *J. Nanopart. Res.* **2005**, *7*, 111.
- Ateh, D. D.; Vadgama, P.; Navsaria, H. A. *Tiss. Eng.* **2006**, *12*, 645.
- Rowlands, A. S.; Cooper-White, J. J. *Biomaterials* **2008**, *29*, 4510.
- Aoki, T.; Tanino, M.; Sanui, K.; Ogata, N.; Kumakura, K.; Okano, T.; Sakurai, Y.; Watanabe, M. *Synth. Met.* **1995**, *71*, 2229.
- Varesano, A.; Aluigi, A.; Florio, L.; Fabris, R. *Synth. Met.* **2009**, *159*, 1082.
- Varesano, A.; Vineis, C.; Aluigi, A.; Rombaldoni, F.; Tonetti, C.; Mazzuchetti, G. *Fibers Polym.* **2013**, *14*, 36.
- Shi, Z.; Zhou, H.; Qing, X.; Dai, T.; Lu, Y. *Appl. Surf. Sci.* **2012**, *258*, 6359.
- Firoz Babu, K.; Dhandapani, P.; Maruthamuthu, S.; Anbu Kulandainathan, M. *Carbohydr. Polym.* **2012**, *90*, 1557.
- American Association of Textile Colorists and Chemists. AATCC Test Method 100–2012, Antimicrobial Finishes on Textile Materials. Available at: <http://www.aatcc.org/> (accessed May 13, 2014).
- Arumugam, M.; Jaisankar, P.; Mukherjee, J. *Nat. Prod. Res.* **2012**, *26*, 1942.
- Wang, W.; Zhang, Sun, L. *Fish Shellfish Immunol.* **2011**, *31*, 269.
- Zafari, M.; Jafarpour, M.; Biazar, E.; Heidari, K. S. *J. Pure Appl. Microbiol.* **2013**, *7*, 143.
- Fonseca Caetano, V.; Vinhas, G. M.; Pimentel, M. F.; da Silva Simões, S.; Ugulino de Araújo, M. C. *J. Appl. Polym. Sci.* **2013**, *127*, 3441.
- Ghosh, S.; Bowmaker, G. A.; Cooney, R. P.; Seakins, J. M. *Synth. Metal.* **1998**, *95*, 63.
- Omastová, M.; Trchová, M.; Kovářová, J.; Stejskal, J. *Synth. Met.* **2003**, *138*, 447.
- Dhibar, S.; Sahoo, S.; Das, C. K. *J. Appl. Polym. Sci.* **2013**, *130*, 554.

19. Otero, T. F.; Martinez, J. G. *Adv. Funct. Mater.* **2014**, *24*, 1259.
20. Wernet, W.; Monkenbusch, M.; Wegner, G. *Die Makromolekulare Chemie, Rapid Commun.* **1984**, *5*, 157.
21. McNeill, R.; Siudak, R.; Wardlaw, J. H.; Weiss, D. E. *Aust. J. Chem.* **1963**, *16*, 1056.
22. Kumar, R.; Howdle, S.; Münstedt, H. J. *Biomed. Mater. Res. Part B: Appl. Biomater.* **2005**, *75B*, 311.
23. Pal, S.; Tak, Y. K.; Song, J. M. *Appl. Environ. Microbiol.* **2007**, *73*, 1712.
24. Lalueza, P.; Monzón, M.; Arruebo, M.; Santamaría, J. *Mater. Res. Bull.* **2011**, *46*, 2070.