Solvent-Crazed PET Fibers Imparting Antibacterial Activity by Release of Zn^{2+}

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ABSTRACT: Poly(ethylene terephthalate) fibers containing zinc chloride in the fiber bulk were prepared by solvent crazing. Fibers containing 6 g/Kg and 13 g/Kg Zn^{2+} were investigated. SEM-EDX analyses and the formation of the pink bis(1,5-dithiocarbazonato-*N*,*S*) complex inside the fibers confirm the presence of zinc. UV-Vis spectroscopy indicates a slow release of zinc ions into the aqueous media and, thus, the fibers serve as a release system to inhibit the growth of *Escherichia coli* during the first exposure. Thermal annealing of the freshly prepared fibers above T_g was shown to modify the release profile so that bacterial growth was also inhibited during repetitive and prolonged exposures. The washing fastness is fair and after 10 washing cycles, $\sim$ 30% of the original zinc content still remains in the fiber.
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Key words: solvent crazing; poly(ethylene terephthalate); fibers; antibacterial activity; controlled release; zinc ions

INTRODUCTION

Functional fibers are gaining increased importance in advanced applications such as scaffolds for tissue engineering or as drug-delivery systems,[1,2] sensor, laser and switchable textiles,[3–6] wearable electronics,[7,8] and wound healing.[9,10] There are three principle ways to introduce functionality into synthetic fibers, namely by using functional monomers for the polymer chain or side chain, by incorporating functional additives into the melt or gel before spinning, or by applying a functional coating after production. Mixing additives to the melt or gel leads to a modification of the fiber bulk and has been realized e.g., for conductive,[11–14] flame retardant,[15–17] and antibacterial[18–21] fibers. However, this requires a high extent of miscibility or dispersability of the additive in the melt or gel. Coatings on the other hand rely less on a chemical compatibility of the actual functional compound with the fiber material. They are usually very thin compared to the fiber diameter and, thus, the active compounds are readily accessible from the surroundings. However, the volume of the fiber can hold more material than thin coatings and, therefore, fiber-bulk modification is more suitable for achieving sustained effects. Combining the advantages of fiber-bulk modification and coating would be most beneficial.

One approach to achieving this is the process of solvent crazing,[22] which makes use of the Rebinder effect in polymers.[23,24] Plastic deformation of polymer fibers in a medium that wets the fiber surface leads to the formation of dispersed polymer fibrils separated by microvoids.[22,25,26] The microvoids are 1–100 nm in size and are filled with the surrounding medium. If the surrounding medium contains nonvolatile additives, these remain inside the fiber after evaporation of the solvent. In total, solvent crazing leads to a modification of the fiber bulk, but the porosity generated by fibrillation of the polymer during the deformation provides easy access to the incorporated compounds. Additionally, since the dissolved compounds are swept into the fiber by active wetting of the microvoids and retained inside the fiber for steric reasons, chemical compatibility of the additive and the polymeric material is not necessary. A number of unusual material combinations such as AgCl in poly(propylene),[27] nickel in poly (propylene), and metallic copper in poly(ethylene terephthalate) (PET)[28,29] have been prepared by solvent crazing, but potential industrial or medical applications have not yet been shown.

Herein, we present an application of the solvent crazing process for the preparation of PET, fibers exhibiting antibacterial activity. Although solvent

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crazing is not limited to a particular polymer, PET was chosen as fiber material since it is frequently used alone or in combination with natural fibers for various medical and apparel applications.[30,31] The antibacterial effects are due to incorporated Zn^{2+} ions, which in contrast to silver nanoparticles do not have a color of their own.

EXPERIMENTAL

Continuous, low-oriented PET multifilament material (T_g = 74.3°C) with a filament diameter of 23.1 μm was obtained from Märkische Faser GmbH (Premnitz, Germany). Commercial PET contains metastable crystallites melting at 250–265°C.[32] The material used in this study shows an endothermic signal at 250°C in the DSC, from which a degree of crystallinity = 31% ± 5% was calculated using the ratio of melting enthalpies. The original fiber bundle was divided into finer batches of ~ 3430 dtex for better handling. Zinc chloride, *n*-propanol, and 1,5-diphenylthiocarbazone (DPTC, dithizone) were obtained from Merck. Sodium hydroxide and 0.1*M* Na_2-EDTA were from KMF, xylenol orange from Fluka AG, and sulfuric acid from Aldrich. All chemicals were of analytical grade and used as received without further purification.

A Zeiss Axioplan 2 microscope was used for the optical analyses. SEM-EDX images were recorded on a Hitachi *S*-3000*N* with an Amedek EDAX detector. The zinc content in the fibers was quantified by either ICP-AES on a PerkinElmer Plasma-400 after dissolving the fibers in a mixture of nitric and sulfuric acid or by titration with Na_2-EDTA against xylenol orange. In the latter case, the fiber sample was dissolved in conc. sulfuric acid at 40°C for 10 min, after which the resulting clear solution was diluted and the pH adjusted to ~ 5.5 before titration. The color reaction with 1,5-diphenylthiocarbazone was used as qualitative test for Zn^{2+}.[33,34] UV-Vis spectra were recorded on a Varian Carry Win Bio spectrometer. The growth of *Escherichia coli* (DSMZ 498) was monitored according to ATCC 23716 in a Tecan GENIOS Pro microwell-plate reader.

The solvent crazing was performed as described earlier.[7,22,27] 0.55 g of fibers were stretched in 1 L of 10 and 20 wt % solutions of zinc chloride at room temperature, giving samples designated as PET-10 and PET-20, respectively. The volume of the bath depends on the actual set-up, but at the given bath ratio, the Zn^{2+} concentration in solution does not change significantly from batch to batch so that the same solutions could be used for the entire study. The final elongation of the samples was 200%. Immediately after stretching, the fibers were removed from the bath, washed thoroughly with *n*-propanol, and dried at room temperature in air over night. Thermal annealing of the stretched fibers was achieved by keeping the samples at 80°C for 30 min.

For the antibacterial test, the fiber samples were sterilized by UV irradiation for 45 min. A nutrient solution containing 5 g/L peptone and 3 g/L of meat extract in bi-distilled water at pH 7 was used as a growth medium. The solution was autoclaved for 15 min at 120°C before use. 0.3–0.4 g of the fiber sample was placed in a 50 mL Erlenmeyer flask containing a suspension of 3 mL *E. coli* with 2.8×10^7 colony-forming units (CFU) per millilitre in 3 mL nutrient solution. The initial 1.5 h exposure was run at 37°C and 240 rpm in a thermal shaker. The subsequent 20 h exposures were run at 25°C. The optical density (OD) of the growth medium was recorded in the tecan reader at 612 nm and 37°C using a 50 μL aliquot to which 150 μL of fresh nutrient solution were added. The value given is an average of three individual measurements. Time-growth profiles were measured overnight in the Tecan microwell-plate reader at 612 nm and 37°C on a 100 μL aliquot of the growth medium to which 10 μL of fresh *E. coli* (2.8×10^8 CFU/mL) was added. The microwell plates were prepared in a laminar airflow to maintain strict sterile and aseptic conditions. In all cases, the aliquots were inoculated with the same amount of bacteria (i.e., the starting concentration of fresh *E. coli* was 2.8×10^7 CFU/mL).

The washing fastness was determined according to ISO-C01. 10 g of fiber material were washed in 500 mL soap solution for 30 min on a shaker at 42°C ± 2°C with 150 rpm. The fibers were then dried between filter paper and washed three times with distilled water for 5 min at room temperature and on a shaker at 150 rpm to complete one 45 min cycle. The fibers were then dried at 40°C.

RESULTS AND DISCUSSION

Stretching of low-oriented PET fibers in alcoholic solutions of $ZnCl_2$ to an elongation of 200% leads to the characteristic necking. The necks are generated at the transition from elastic to plastic deformation and propagate along the filaments upon further stretching,[35,36] which create the repetitive pattern of stretched (thin) and unstretched (thick) sections (Fig. 1). The number and distribution of the crazes and with it the distribution of stretched and unstretched parts along the filament is random, but the final elongation is similar for all filaments in the bundle stretched under identical conditions.

Upon stretching, the degree of crystallinity increases from 31% ± 5% for the as received material to 41% ± 5% and 47% ± 5% for the PET-10 and PET-20 samples, respectively. The increase indicates the formation of oriented parts upon stretching in accordance with the general mechanism of solvent crazing.[22]

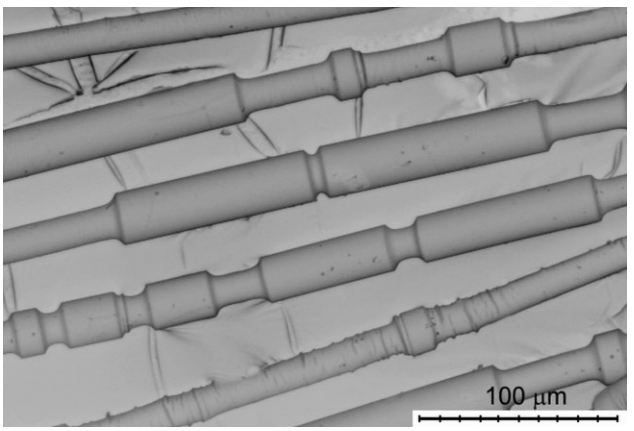

Figure 1 SEM image of PET fibers after mechanical stretching in $ZnCl^2$ solution.

Solutions of 10 and 20 wt % of $ZnCl_2$ were used as stretching medium, since Zn^{2+} was found to inhibit the growth and bacterial conjugation in *E. coli*.[37,38] The corresponding samples were designated PET-10 and PET-20. ICP-AES analyses confirm the presence of zinc and indicate that doubling the concentration in solution—under otherwise identical conditions—doubles the initial concentration of zinc inside the fibers (Table 1). Although the distribution of stretched and unstretched parts along the filament is random, the zinc content of individual fiber bundles stretched under the given conditions is within the given limits.

SEM-EDX analysis showed that the surface of the stretched fibers is free of zinc, while the cross section contains zinc. Additionally, a small batch of the stretched fibers was subjected to a second stretching in a solution of dithizone, which turns the previously colorless, stretched parts pink (Fig. 2). This indicates the formation of the Zn(DPTC) complex. The zinc complex is exclusively formed in the stretched (thin) and crazed parts, i.e., the parts which are about to form a neck [Fig. 2(A)]. In the cross-sectional view, the colored complex is only found in the fibers with smaller diameter, i.e., the stretched parts, and fully penetrates the cross section of the filament [Fig. 2(B)]. However, some of the stretched parts are colorless, which are assumed to be stretched parts in which the crazes have collapsed and are no longer accessible from the outside.

To test for a possible release of Zn^{2+} from the fibers, time-dependent UV-Vis measurements (350–700 nm) were performed in the presence of dithizone. For this, ~ 15 individual filaments of the PET-10 sample of 1 cm length were placed in a cuvette containing a 5 μ*M* solution of dithizone.

TABLE I
Zinc Content of the Treated Fibres

Sample	Zinc concentration mg/Kg[a]
Untreated fibres	1.62 ± 1.62
PET-10	6229 ± 426
PET-20	13517 ± 426

[a] Determined by ICP-AES spectrometry.

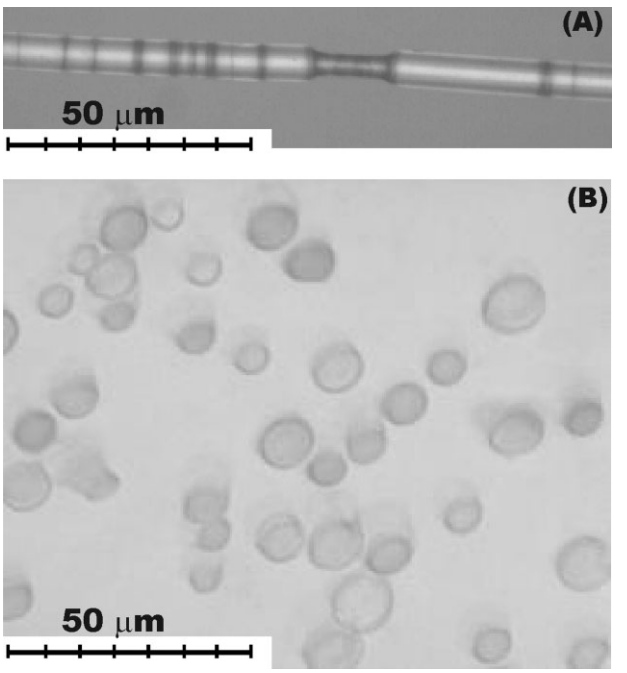

Figure 2 Optical appearance of zinc-containing PET filaments additionally stretched in dithizone solution. Dark areas indicate the formation of the zinc dithizonate complex. (A) side-view and (B) crosssectional view.

The free ligand exhibited two characteristic absorption maxima at $\lambda_1 = 471$ nm and $\lambda_2 = 595$ nm, as indicated by the dashed line (Fig. 3), while the pure complex has a single λ_{max} at 515 nm. In the

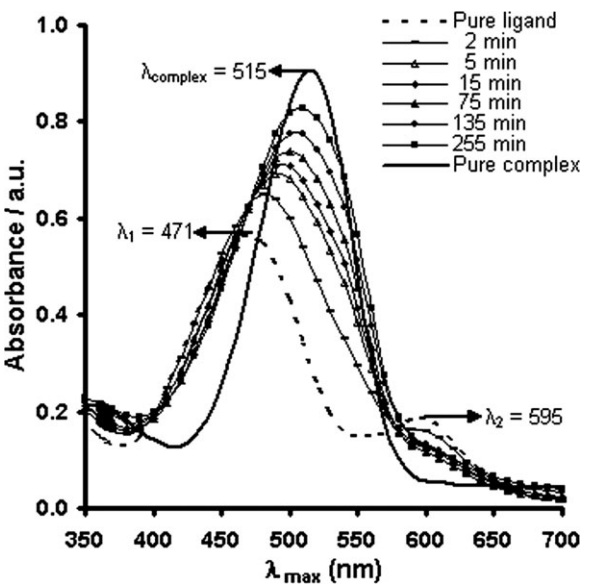

Figure 3 Time-dependent release of Zn^{2+} from PET-10 fibers in a solution of dithizone monitored by UV-Vis spectrometry.

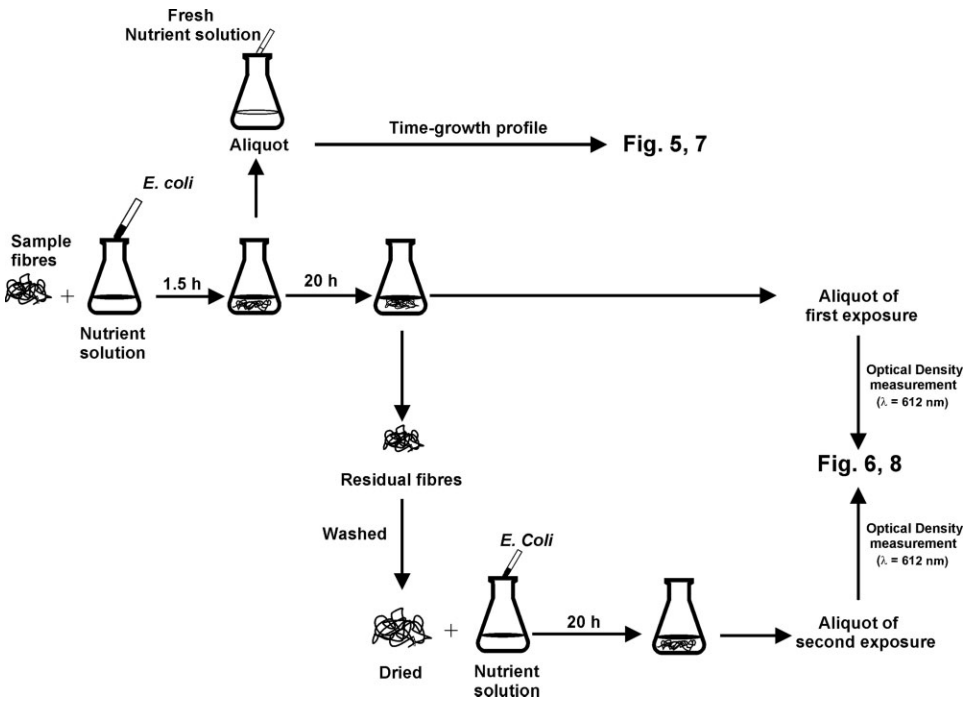

Figure 4 Analytical protocol of the antibacterial tests.

presence of the zinc-containing filaments, the maximum at $\lambda = 595$ nm disappears over time, while the one at 471 nm shifts to larger wavelengths. This clearly indicates the formation of Zn(DPTC).

The results above indicate that low-oriented PET fibers stretched in alcoholic $ZnCl_2$ solution contain zinc ions and that such fibers can serve as release systems. Consequently, the fibers were subjected to antibacterial tests using *E. coli*.

The analytical protocol for the antibacterial tests is outlined in Figure 4. Fibers stretched in pure *n*-propanol were used as reference. During the test, aliquots of the supernatant nutrient-medium were taken and analyzed. The extent of bacterial growth in the medium during exposure to the fibers was tested using OD measurements, while the potential of the medium to promote bacterial growth after removal of the fibers was checked by time-growth profiles.

The stretched fibers were exposed to a solution of *E. coli* under growth conditions. After 1.5 h of exposure, a first aliquot was taken, of which a time-growth profile was recorded to test for the initial release of zinc ions from the fibers (Fig. 5). Compared to the reference, the treated fibers show delayed growth and, especially the PET-20 sample, some retardation in bacterial growth. After an additional 20 h, the fibers were separated from the solution, washed, dried, and saved for a second exposure. Optical-density measurements of the media indicated almost complete inhibition of bacterial growth during exposure (Fig. 6, dark bars). Time-growth profiles after the exposure using fresh *E. coli* revealed that enough zinc ions had been released to prevent further bacterial growth even in the absence of the fibers.

To test for residual zinc ions in the fibers, the separated fibers were subjected to a second exposure using fresh nutrient and *E. coli* solution. Optical-density measurements after an additional 20 h exposure showed no inhibition for the PET-10 sample, while the PET-20 sample decreased the bacterial growth

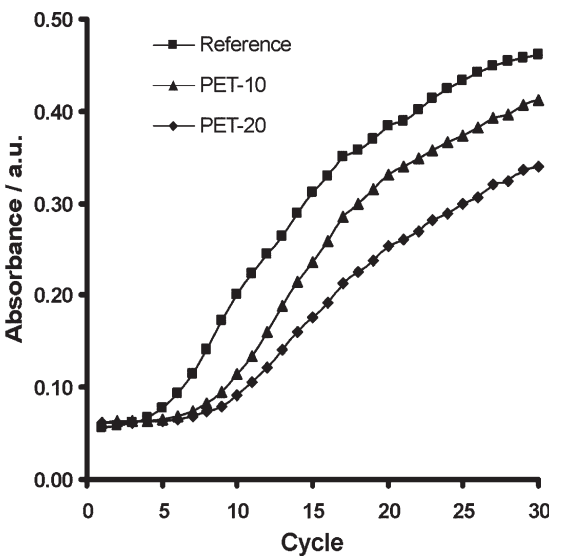

Figure 5 Time-growth profile of the aliquot taken after 1.5 h during the first exposure of the nontempered fibers (1 cycle = 30 min).

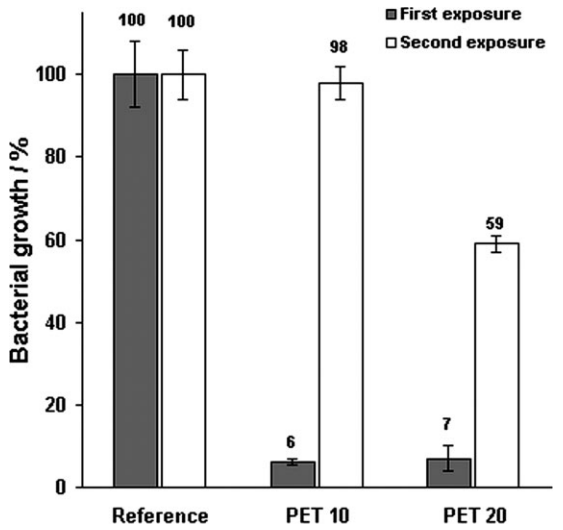

Figure 6 Extent of bacterial growth in the medium exposed to the nontempered fibers calculated from optical-density measurements.

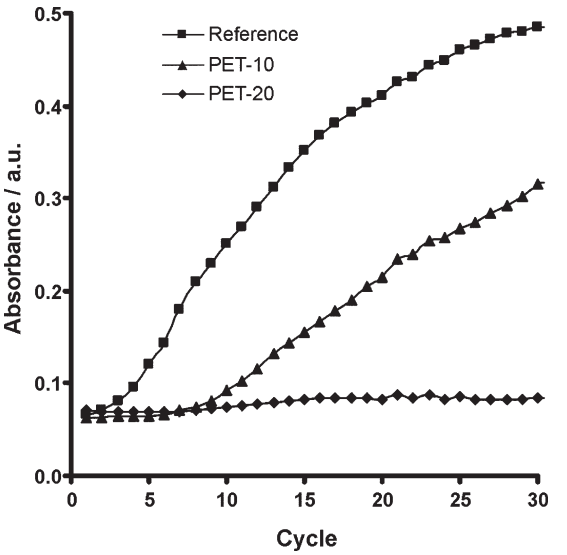

Figure 7 Time-growth profile of the aliquot taken after 1.5 h during the first exposure of the thermally annealed fibers (1 cycle = 30 min).

by ~ 60% (Fig. 6, light bars). Apparently, the fibers had been largely depleted during the first exposure, but enough zinc was left in the PET-20 sample to show an effect during the second exposure.

It would be highly beneficial for potential applications, if the release profile could be tuned to a more sustained antibacterial activity. To achieve this, a more detailed look at the crazing mechanism is necessary.[22] On a molecular level, crazes form when the polymer chains uncoil under external load in the region of plastic deformation.[39] This creates voids between the chains or bundles of chains, which usually collapse in air due to the intermolecular cohesion, but can be stabilized with the help of appropriate solvents.[40] As outlined before, if the solvent contains nonvolatile compounds, these remain inside the fiber after evaporation of the solvent. Thermal treatment above T_g causes morphological changes in the oriented parts of the necks and at the transition from oriented (fibrills) to unoriented (bulk) material.[22,41] This alters the local environment of material trapped between the fibrils, along with its accessibility from outside and, consequently, should influence the release profile.

To test this, PET-10 and PET-20 samples were annealed at 80°C for 30 min before being subjected to the analytical protocol outlined in Figure 4. The first time-growth profile recorded after 1.5 h of exposure shows significant differences in the release behavior. The PET-10 sample shows a marked retardation of the bacterial growth, while no growth was observed in the aliquot taken from the tempered PET-20 sample (Fig. 7).

After an additional 20 h of exposure, the optical-density measurements of both media again showed almost complete inhibition of bacterial growth (Fig. 8, dark bars) as was also observed for the nontempered fibers (compare Fig. 6). Likewise, the time-growth profile indicated the presence of enough zinc ions to prevent further growth of fresh *E. coli* after removal of the fibers. During the second exposure, the tempered PET-10 fibers exhibit only weak inhibition of bacterial growth, indicating a pronounced depletion of the fibers during the first exposure. In contrast, the tempered PET-20 show sustained antibacterial activity during the second exposure, since after the additional 20 h optical-density measurements of the medium confirms no bacterial growth (Fig. 8, light bars).

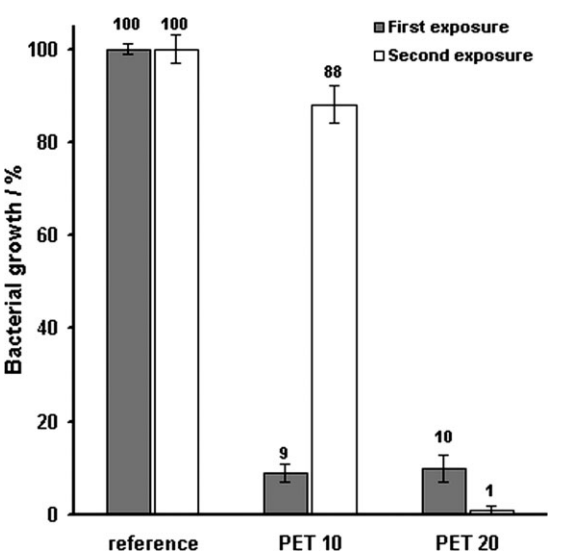

Figure 8 Extent of bacterial growth in the medium exposed to the tempered fibers calculated from optical density measurements.

There are at least two possible explanations as to why the tempered fibers exhibit a stronger antibacterial activity after 1.5 h of exposure compared to the nontempered ones: (i) the morphological changes induced by annealing above T_g cause a microphase separation of polymer and inorganic material, by which the zinc is transported from the core of the filaments towards near-surface regions, thus, increasing the local concentration of Zn^{2+} in these areas; (ii) the loss of orientation in the stretched parts of the filaments promotes diffusion of Zn^{2+} through the polymer material and facilitates the penetration of water. Both processes alone or a combination of both will lead to more Zn^{2+} being released during the initial time of exposure as well as more Zn^{2+} being easily available for a sustained release.

The PET-20 fibers were tested for washing fastness according to ISO-C01 (Fig. 9). For both nontempered and tempered fibers, the zinc content decreased rapidly during the first two washing cycles, but even after 10 washing cycles $\sim$ 30% of the original zinc content still remained in the fiber. The decrease is less pronounced for the tempered fibers. The absolute amount of zinc in the fibers during the test could be fitted by a simple exponential decay of the type $y = a + b \times e^{-kx}$. The results are shown in Table 2. The parameters a, b, and k are associated with the chemical and physical properties of the release system, but their wider significance is still under investigation.

CONCLUSIONS

Solvent crazing is a straight-forward method to incorporate zinc ions into PET fibers. The amount incorporated can be controlled by the solution concentration and the prepared fibers can serve as release systems. Complete inhibition of bacterial growth in the surrounding medium was observed even after repetitive and prolonged exposure. Given the fact that the fibers are designed as a release system, they exhibit a fair washing fastness. The described material could, therefore, be used in medical textiles applications such as dressings, band-aids, masks and other single-use items, in technical textiles such as filters and insulation material, in home furniture as padding and mattresses as well as in clothing, which are subject to dry cleaning.

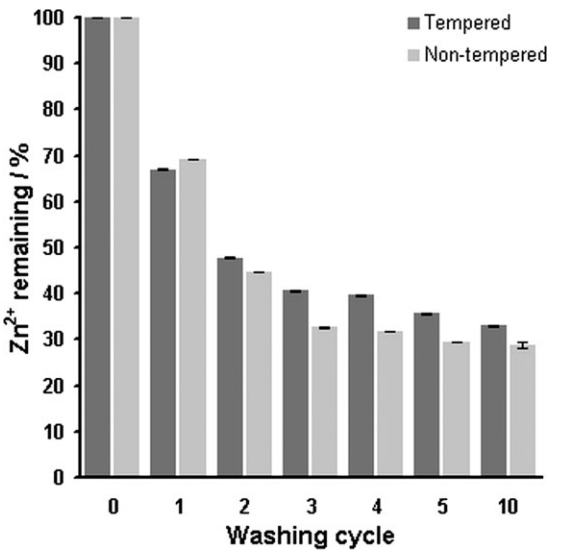

Figure 9 Washing fastness of the treated PET-20 fibers.

TABLE II
Fitting Parameters of the Washing Fastness Using the Exponential Decay $y = a + b \times e^{-kx}$

	Nontempered	Tempered
a	4.00 ± 0.37	5.22 ± 0.21
b	11.2 ± 0.57	9.82 ± 0.34
k	0.672 ± 0.84	0.746 ± 0.064
r^2	0.990	0.995

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