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# Antibacterial properties of polypyrrole-treated fabrics by ultrasound deposition

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ABSTRACT

Antimicrobial textiles can contribute to the fighting against antibiotic resistance pathogenic microorganisms. Polypyrrole is a conjugated polymer that exerts a biocidal action thanks to positive charges on its backbone chain produced during it synthesis. In this work, dispersions of stable polypyrrole nanoparticles were produced by chemical oxidative polymerization at room temperature in water. An ultrasound-assisted coating process was then used to effectively treat a polyester fabric with the nanoparticles to obtain an optimal antibacterial coating which efficiently eradicates the bacteria. The results showed that the treated fabric with about 4 g/m$^2$ of polypyrrole had log bacteria reductions of 6.0 against *Staphylococcus aureus* and 7.5 against *Escherichia coli*. The combination of a polypyrrole synthesis in the form of water nanoparticles dispersions and a continuous coating of fabrics supported by ultrasound overcomes some issues of upscaling of the traditional *in-situ* chemical deposition used until now for the production of polypyrrole-coated textiles.

## 1. Introduction

Antibiotic resistance is one of the most urgent threats to global health. It occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process. A large number of infections (such as pneumonia, salmonellosis, gonorrhoea and tuberculosis) are becoming harder to treat as the antibiotics used become ineffective. In turn, hospital stays are longer, resulting in increased mortality and medical costs [1]. The emergence of resistant bacteria has stimulated intensive research in different fields, including the development of antimicrobial fabrics and novel broad-range biocides for textiles that can potentially better control and reduce the spread of antibiotic resistant bacterial strains. [2–5].

Since laundry does not effectively protects the textile surface from microbial contamination [6], antimicrobial treatments have been proposed for textile products that can be subjected to contaminations, such as hospital textiles and home-textiles (such as bedding, sheets, bed underlays and pyjamas) [7–10], as well as for garments (such as clothing, sportswear, underwear, socks and shoes) [11–13], and technical applications (such as protective clothing, air filtration and water depuration) [14,15]. Moreover, biocidal products in textiles can avoid unpleasant odour in clothing [16,17] and can help to control biofouling in filtration and water depuration [18,19].

Emerging antimicrobial treatments for textile materials include chitosan-based finishing [20,21], sol-gel processes [22–25], immobilized/controlled release of biocidal agents [26–29], and silver nanoparticles deposition [30,31].

Conjugated polymers [32,33], and polypyrrole (PPy) [11,34–37] in particular, was also reported to have excellent antibacterial properties on textiles. Bioactivity of PPy is likely due to the presence of positive charges produced during its polymerization along the backbone chain. The positive charges are stabilized by the introduction of anions in the polymer matrix as counter-ions [38].

PPy can be easily synthesized by electrochemical or chemical oxidative polymerization from solutions of the monomer. PPy is a biocompatible polymer, [39], it was suggested as platforms for nerve growth [40] and as substrates for electrically stimulated cell growth [41] because of its electrical properties.

In the past, electrochemical synthesis of PPy has been studied also in presence of polyelectrolytes. A broad spectrum of sulfonated polymers were used as polyelectrolytes [42–45], including poly(styrene sulphate) (PSS), in order to produce polymer composite films and to enhance

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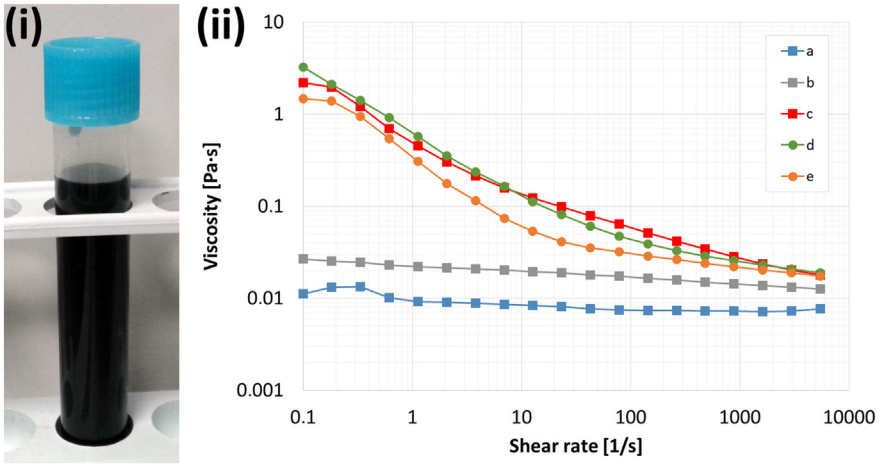


**Fig. 1.** (i) Picture of the PPy nanoparticles dispersion after 3 months. (ii) flow curves of (a) APS 3.9% w/w and PSS 14% w/w solution in water at pH 1.0; PPy nanoparticles dispersions in water (b) after 2 h at pH 1.0, (c) after 7 days at pH 1.0, (d) after 7 day at pH 5.0 (2 h after the addition of NaOH), and (e) after 3 months at pH 5.0.

electrical properties of PPy. PSS has been employed also in chemical oxidative synthesis to produce water and solvent dispersible PPy nanoparticles [46,47]. Moreover, a previous study, which examined the effect of doping agents on the deposition of PPy on fabrics by *in situ* chemical oxidative polymerization [38], showed that PSS hinders the direct deposition of PPy on fabrics. In fact, chemical synthesis of PPy in presence of PSS produces stable dispersions of PPy nanoparticles. The resulting PPy dispersions can be potentially spread on fabrics as a coating by continuous textile finishing processes. At industrial level, the separation between polymer synthesis and fabric coating could lead to benefits in terms of productivity, evenness, cost, wastewater, and chemicals usage. At laboratory scale, fabric coating of poly(3,4-ethylenedioxythiophene)/PSS dispersions has been recently proposed by spraying [48] and by screen printing [49].

Studies on cytotoxicity of PPy have been carried out recently. It seems that the size of PPy nanoparticles synthetized with ferric chloride and poly(vinyl alcohol) can influence cells viability. Moreover, the results of apoptosis/necrosis and oxidative stress were consistent with the viability results [50]. On the other hand, another study [51] demonstrated that PPy particles produced with hydrogen peroxide had good biocompatibility in animals (*i.e.* no cytotoxic effect on mouse peritoneum cells, no allergic response, no effects on spleen, kidney or liver, no effect on immune-related haematological parameters, no inflammation in the peritoneum of mice after 6 weeks). In addition, an *in vivo* study has confirmed that PPy produced by electrodeposition in solution with polystyrene sulfonate (PSS) or sodium dodecylbenzene sulfonate has good biocompatibility [52]. Finally, PPy-coated polyester fabrics showed no cytotoxicity and then they could improve cells adhesion promoting cell growth [53].

The ultrasound-assisted coating process was developed by Gedanken's group and has been applied on various surfaces such as polymers [54], metals [55], and textiles [56]. Different properties were imparted by coating active nanoparticles on the substrates using the sonochemical method. The coating technique is considered as very effective since the coating was tested for the durability and was found stable even after 65 washing cycles at 75 °C [57], the textiles remain antibacterial with very limited loss of the coated nanoparticles. A major advantage of the sonochemical technique includes not only the coating stage, but also the synthesis of the active nanomaterials. The process is one-step process in which the particles are created and coated on the surface [58,59]. In the current work, ultrasound (US) was used to coat the fabrics from a water PPy dispersions. The mode of coating is what has been previously termed the "throwing stones" method, namely, the particles are prepared in advance and the ultrasound waves are only used for the coating. Such an approach was previously applied for coating $Al_2O_3$ and MgO on textiles [60]. An optimization of the coating was performed by changing US parameters. The target in the current work was to obtain an optimal PPy coating which efficiently eradicates the bacteria overcoming the upscaling problem related to the traditional *in-situ* chemical deposition currently proposed for the production of polypyrrole-coated textiles that generally is operated as a batch process. The combination of a polypyrrole synthesis in the form of water nanoparticles dispersions and a continuous coating of fabrics supported by ultrasound can improve productivity, evenness and speed of deposition.

## 2. Experimental

### 2.1. Materials

The chemicals used for PPy synthesis were pyrrole 97% (Fluka) as monomer, ammonium persulfate 98 + % (APS, Sigma–Aldrich), polystyrene sulphate Mw ~70,000 (PSS, Sigma–Aldrich), and sodium hydroxide (NaOH, Sigma–Aldrich). All chemicals were used as received.

The fabric used was a 100% polyester (PET) plain weave fabric with a weight of 0.136 kg $m^{-2}$, supplied by Klopman, Italy.

### 2.2. Methods

For the synthesis of PPy nanoparticles, a solution of 3.9% w/w of APS and 14% w/w of PSS was prepared in distilled water. The solution had a pH of 1.0. As the chemicals were completely dissolved, pyrrole was dropwise added under stirring reaching a final concentration of 4.2% v/w. A light exothermic reaction started immediately and a uniform, black dispersion was produced after a few seconds. The pH of the solution did not change during synthesis. After 7 days, 5 N NaOH solution was added to the dispersion under stirring until the pH reached 5.0 in order to stop the polymerization and to allow the ultrasound process. The dispersions were stored under ambient laboratory conditions at about 20 °C before the viscosity was measured. A picture of the PPy dispersion after 3 months is reported in Fig. 1(i).

Viscosity of the dispersions was measured by means of an Anton Paar Physica MCR 301 rheometer, equipped with a PTD 200 Peltier temperature control device at 25.0 ± 0.1 °C, using a cone-plate geometry (75 mm diameter, 1° angle and 45 μm truncation) in controlled shear rate mode. The shear rate was logarithmically increased from 0.1 to 10,000 $s^{-1}$. Data were acquired and elaborated with the Rheoplus v2.66 (Anton Paar) software. Viscosity of the dispersions was measured at different times and pHs, before and after the addition of NaOH in order to evaluate both the shear rate dependency and stability of the dispersions over the time. In particular, the viscosities were measured at pH 1.0 after 2 h and 7 days, and at pH 5.0 after 7 days (2 h after the addition of NaOH) and after 3 months. Moreover, the flow curves of the PPy dispersions were compared with a solution of the reagents (APS

and PSS) without PPy in order to assess the effect of PPy nanoparticles.

Various concentrations of PPy dispersion was used during the coating optimization. The dispersion was diluted with double distilled water for reaching the desired concentration. The solution was irradiated for 30 min with an ultrasonic corn (Ti horn, with booster 30% efficiency 750 W) in the presence of a piece of fabrics (7 × 7 cm). After 10 min, the sonication beaker was placed in a cooling bath, maintaining a constant temperature of 40 °C during the sonication. At the end of the sonication, the textile was removed from the solution, washed with water and dried at room temperature.

The particle morphology and size were studied using high-resolution scanning electron microscopy (HR-SEM) with a Magellan, FEI microscope, at an accelerating voltage, over the range of 5–15 kV.

Colour evaluation was performed by Datacolor Spectraflash SF600 (Dietlikon) spectrophotometer, under CIE standard illuminant D65 and a 10° observer. A calibration curve has been produced on specimens with a known amount of PPy dispersion confirmed by weight increase measurements. The colour evaluation was then used to quantify the amount of PPy on the fabrics produced by US.

The thermogravimetric analysis was carried out with a Mettler Toledo TGA-DSC 1. About 2.5 mg of fabric was put in a 70 mL aluminium oxide crucible for the analysis. The calorimeter cell was flushed with nitrogen at 70 mL $min^{-1}$. The TGA data were recorded with a Mettler Toledo STARe system. The run was performed from 30 to 600 °C with a heating rate of 10 °C $min^{-1}$.

Fourier transform infrared (FT-IR) spectra were acquired using the Attenuated Total Reflection (ATR) technique in the range from 4000 to 650 $cm^{-1}$ with 50 scansions and 4 $cm^{-1}$ of band resolution by means of a Thermo Nicolet Nexus spectrometer equipped with a Smart Ark™ (ZnSe 45° crystal).

Antibacterial activity of PPy-coated fabrics was evaluated against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213. In all experiments, *E. coli* and *S. aureus*, were grown overnight at 37 °C under agitation (250 rpm) in Muller Hinton (MH, Difco) growth medium. The ability of coated surfaces to kill free-living bacteria (*i.e.* planktonic bacteria) in solution was evaluated as previously described [61].

Bacteria grown overnight were diluted in the following day in MH 1% to obtain a working population corresponding to $10^5$ colony forming units (CFU)/mL. 1 mL from each bacterial stock suspension was taken into each well in a 24-well plate (DE-GROOT). Each of the different fabrics was added to the well (1 cm diameter). The plates were then incubated for 20 h at 37 °C. Following the incubation step, 200 µl were transferred into the first line of a 96-well plate (Greiner Bio-One), while the rest of the lines were filled with 180 µl of MH. Serial dilutions were carried out and the bacterial cells were spotted onto LB agar plates, which were then incubated at 37 °C for 20 h. Cell growth was monitored and determined by viable cell counts.

## 3. Results and discussion

### 3.1. Viscosity measurement

The viscosity flow curves of a solution with APS 3.9% w/w and PSS 14% w/w in water (at pH 1.0) and PPy nanoparticles dispersions at pH 1.0 and 5.0 after 2 h, 7 days and 3 months were measured in order to observe the effect of PPy nanoparticles and the stability of the dispersion. The shear rate-dependence of viscosities is reported in Fig. 1(ii). The solution of APS and PSS showed a Newtonian behaviour, *i.e.* the viscosity was independent of the linear shear rate, with a viscosity around 0.01 Pa·s (curve a). The same behaviour was observed on a fresh PPy dispersion (curve b), but the viscosity value was almost doubled. This result confirms the presence of nanoparticles and the dependence of viscosity on the concentration of PPy nanoparticles. It is important to highlight that after 2 h the nanoparticles formation and the reaction of PPy have not been completely finished. As a result, the number of nanoparticles in solution was increasing as a function of time until a change of the pH occurred.

After 7 days, the flowing behaviour was completely changed (curves c, d and e). The viscosity at low shear rate considerably increased by two orders of magnitude (> 1 Pa·s) and the characteristic flowing behaviour of a nanoparticles dispersion in the polymer solution was observed [62–64]. This variation of viscosity demonstrated that the concentration of nanoparticles after 7 days increased considerably in comparison with the dispersion after 2 h. At low shear rate, the high content of PPy nanoparticles with PSS macromolecules hinder the flow of solution on account of the presence of neighbouring nanoparticles, and make the resulting viscosity high. As the shear rate increases, PSS macromolecules orientated along the flow direction meet less resistance to move, thereby the shear thinning is evident and the viscosity decreases by about 100 times. At high shear rate, the viscosity became minimal (close to the viscosity value of curve a, related to 2 h of synthesis) because all PPy nanoparticles in solution moved in the flow direction. Moreover, this behaviour seems to be positive for the high shear rates locally generated during an ultrasonic process.

It is worth noting that the modification of pH from 1.0 to 5.0 after seven days does not produce significant variations on the flowing behaviour and the concentration of PPy nanoparticles is stable for at least 3 months after synthesis. The slight differences that can be observed between the viscosities of dispersion after 3 months and 7 days are a consequence of a small modification in the particle size distribution, probably because of aggregation of nanoparticles [65,66].

### 3.2. US coating process

One of the main goals of this research is to obtain a homogeneous, uniform coating of PPy nanoparticles on the fibre surface. The initial amount of the PPy was found to be the critical parameter in obtaining a sufficient amount of coating which possesses good antibacterial property. PPy dispersion was diluted by addition of double distilled water. The different compositions used for the coating are presented in Table 1.

After US depositions, the fabrics became grey to black depending on the PPy concentration. Fig. 2 depicts the difference in the colour as the function of different concentrations of PPy.

### 3.3. Fabrics characterization

HR-SEM observations of the PPy-coated fabrics at high magnification revealed a layer composed of nanoparticles on the fibre surface and some large aggregates between the fibres. Fig. 3 presents SEM pictures of the fabrics after PPy deposition by US at increasing PPy amount. The quality of the coating conducted by US depends on the initial concentration of PPy. Sample D showed the most homogeneous coating with no aggregates observed. Generally, polyester fibre surfaces after US deposition appeared smoother as compared to fibres coated with PPy produced by direct *in-situ* chemical polymerization [67–70].

Thermogravimetric analysis is reported in Fig. 4. The thermal degradation of PET fabric started at about 370 °C, with the maximum mass rate loss at 435 °C. All the PPy-coated samples showed the same thermal behaviour, therefore the coating amount and the US process had no effect on the thermal properties of the fibres. Interestingly, the

**Table 1**
Concentrations of the PPy dispersions used for coating.

| Sample | Dilution | Resulting PPy concentration (w/w %) |
|---|---|---|
| A | 1:25 | 0.16 |
| B | 1:12.5 | 0.32 |
| C | 1:5 | 0.80 |
| D | 1:2.5 | 1.60 |

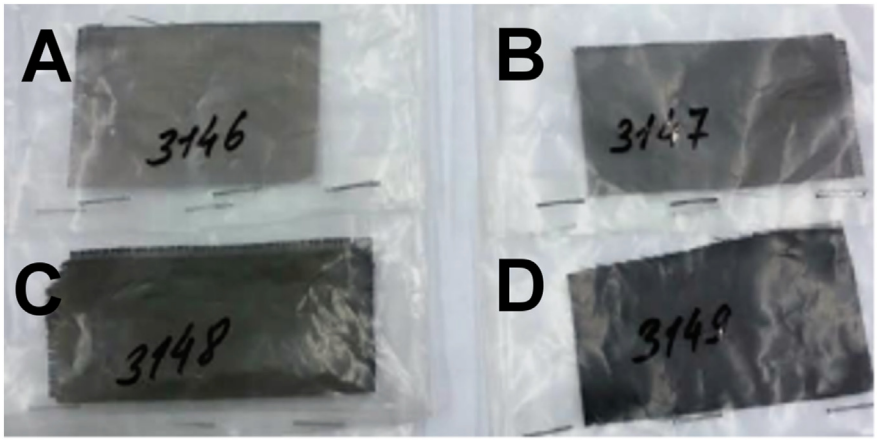


**Fig. 2.** Image of the PPy-coated fabrics A, B, C and D.

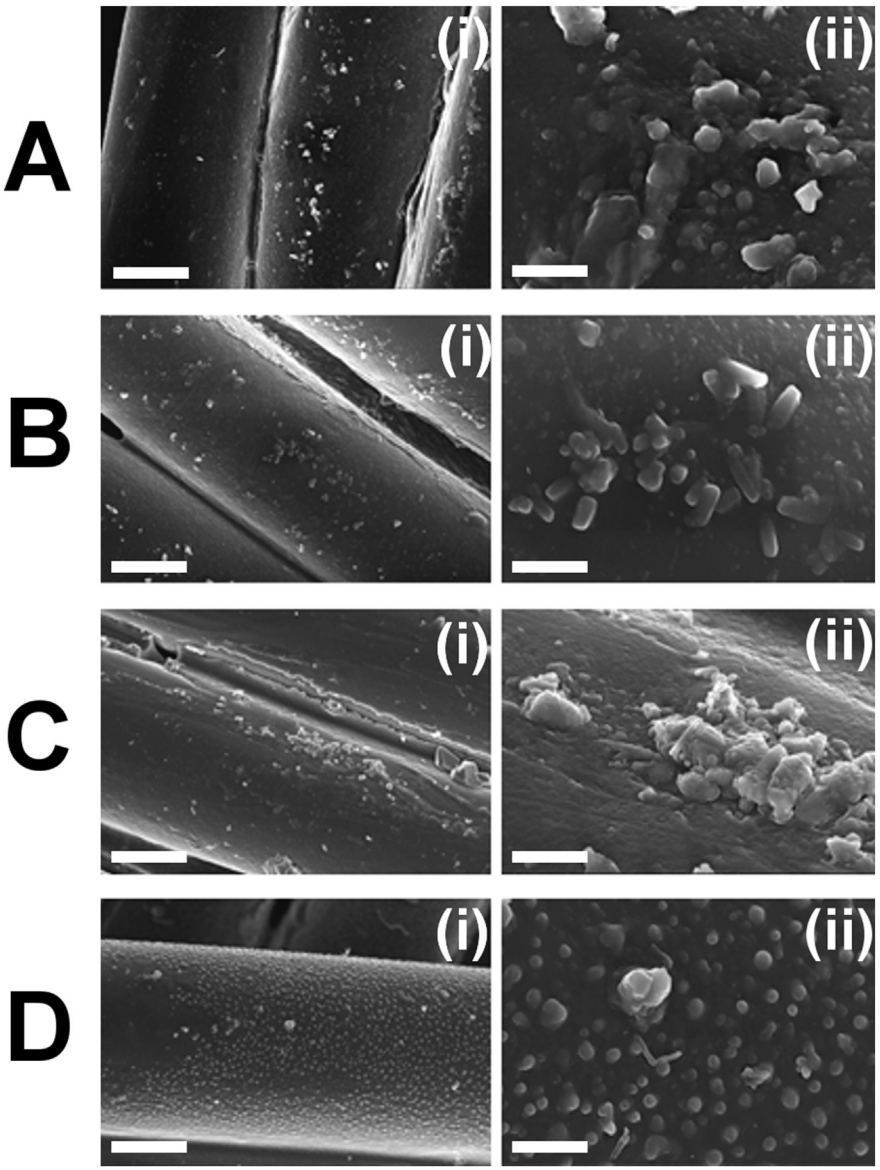


**Fig. 3.** HR-SEM images of the fabrics coated with PPy at different concentration (A-D). Scale bars are 5 μm (i) and 1 μm (ii).

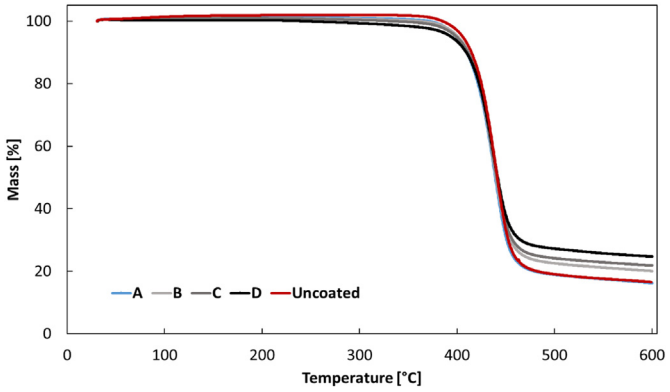


**Fig. 4.** TGA curves of the fabrics between 30 and 600 °C at heating rate of 10 °C min$^{-1}$ under nitrogen.

**Table 2**
PPy amount on fabrics evaluated by colorimetric analysis and residual mass at 600 °C by TGA.

| Samples | PPy (g/m$^2$)[a] | Residual mass @600 °C (%)[b] |
|---|---|---|
| Uncoated | – | 16.5 |
| A | 0.62 ± 0.15 | 16.2 |
| B | 1.42 ± 0.27 | 20.1 |
| C | 2.61 ± 0.18 | 21.8 |
| D | 4.33 ± 0.13 | 24.6 |

[a] Calculation from Datacolor results.
[b] TGA.

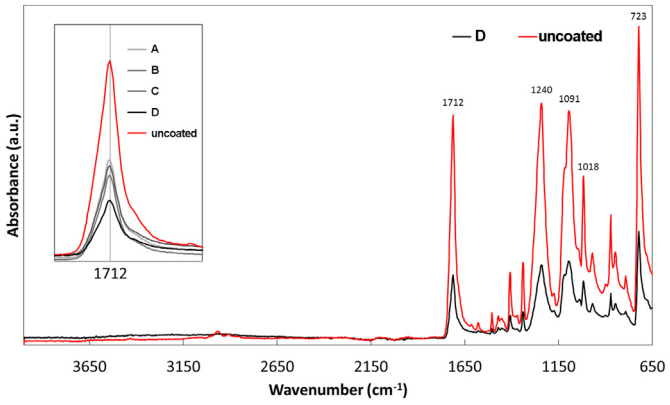


**Fig. 5.** Full scale FT-IR spectra of uncoated PET fabric (red) and sample D (black). In the box, the adsorption band at 1712 cm$^{-1}$ for all the samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

residual masses at 600 °C increased as the amount of PPy increased (Table 2). This is in agreement with previous works [37,71] that demonstrated the thermal resistance and flame-proofing properties of PPy-coated textiles.

Fig. 5 reports the full infrared spectra of uncoated PET fabric and sample D (the fabric with the highest amount of PPy) for comparison. Typical absorption bands of PET are the following [71]: the sharp and intense peak at 1712 cm$^{-1}$ is attributed to C=O stretching; the broad and strong bands at 1240 and 1091 cm$^{-1}$ are due to C–O, C–O–C stretching and CH in-plane deformation, respectively; the sharp peak at 1018 cm$^{-1}$ is associated to C–O–C asymmetric stretching, whereas the C–H out-of-plane deformation is responsible for the sharp peak at 723 cm$^{-1}$.

The bands of PET were attenuated by the PPy layer on the coated samples (as shown in the box in Fig. 5 for the band at 1712 cm$^{-1}$), but

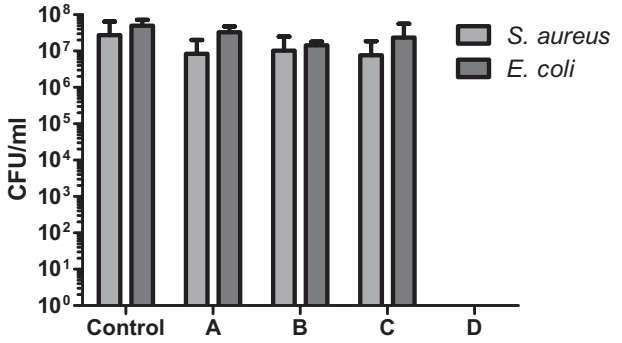


**Fig. 6.** Antibacterial activity of PPy-coated fabrics. *E. coli* and *S. aureus* bacteria were exposed to fabrics coated with PPy NPs at difference concentrations (A-D). Control designated uncoated fabrics. The results represent the average of three independent experiments.

no bands attributed to PPy were detected. The hypothesis is that the signals of PPy are too weak and broad to be observed in ATR mode taking into account the amount of PPy on the fabrics. Furthermore, as the concentration of PPy in the dispersion increases, the bands attributed to PET of the treated fabrics decreases in intensity. This is a sign of the increasing thickness of PPy layers on PET fibres [68], in agreement with DSC results.

### 3.4. Antibacterial tests

The minimal bactericidal concentration (MBC) of PPy nanoparticles' suspension was evaluated using *Escherichia coli* and *Staphylococcus aureus*, two common bacterial pathogens representing Gram-negative and Gram-positive bacteria, respectively. Both types of bacteria were exposed to a serially diluted PPy aqueous dispersions and the MBC was found to be 0.29% w/w for both bacteria.

Following which, PPy coated fabrics (A-D) were tested for their ability to attenuate bacterial growth. All the samples have slightly reduced the bacteria quantity while sample D was the most effective, eradicating *E. coli* and *S. aureus* completely (Fig. 6). These results are in agreement with the chemical characterizations, showing sample D has the highest concentration of PPy nanoparticles and the most homogenous coating.

## 4. Conclusions

Antimicrobial activity of polypyrrole produced by chemical oxidative polymerization is likely due to positive charges along the polymer chain. The positive charges are stabilized by the introduction of anions in the polymer matrix as counter-ions. The use of poly-anions, such as poly(styrene sulphate), allows also the formation of stable and homogeneous dispersions of antibacterial polypyrrole nanoparticles in water.

An ultrasound method was optimized to evenly coat polyester fabrics from the polypyrrole dispersions. The coating method is what has been previously called “throwing stones” method since the particles are prepared in advance and the ultrasound waves are only used to spread and fix them on the solid fibre surfaces.

The resulting coated fabrics were characterized and the antibacterial properties were evaluated. An optical colorimetric analysis was used to quantify the amount of polypyrrole nanoparticles on the treated fabrics. Morphological analysis by high-resolution scanning electron microscopy revealed nanostructures on the fibre surfaces of the treated fabrics, as well as infrared spectroscopy showed reduced intensity for the adsorption bands of the polyester substrate due to presence of the polypyrrole coating. Moreover, increases of the residual masses after thermal degradation at 600 °C were observed as the amount of polypyrrole on the fabric increased. Finally, antibacterial tests showed improved biocidal activities of the fabrics treated with

increased amount of polypyrrole. Excellent results with log reductions of 6.0 against *Staphylococcus aureus* and 7.5 against *Escherichia coli* were obtained on fabrics with 4.33 g/m$^2$ of polypyrrole nanoparticles.

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