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Abstract

Infections represent a major medical concern and have severe impact on the public health economy. Antimicrobial coatings represent one major solution and are the subject of many investigations in academic and industrial research. Polyelectrolyte multilayers (PEMs) consist in the step-by-step deposition of polyanions and polycations films on surfaces. The wide range of disposable polyelectrolytes makes this approach among the most versatile methods as it allows to design surfaces that prevent bacterial adhesion, and kill bacteria by contact or by releasing antibacterial agents. The present work focused on the release-killing effect of an active PEM coating of a polyethylene terephthalate (PET) textile support. This activity was obtained thanks to the PEM film build up using cationic and anionic polyelectrolytes both based on cyclodextrins (PCD- and PCD+) that provided a reservoir property and prolonged release of triclosan (TCS). To this effect, a PET non-woven preliminarily modified with carboxylate groups by applying a thermofixation process was then treated by dip-coating, alternating soaking cycles in cationic PCD+ and in anionic PCD- solutions. Samples coated with such PEM film were then loaded with TCS whose release was assessed in dynamic mode in a phosphate buffered saline solution (PBS) at 37°C. In parallel, TCS/PCD+ and TCS/PCD- interactions were investigated by Nuclear Magnetic Resonance (NMR) and phase solubility study, and the biocide activity was assessed against *S. aureus* and *E. coli*. Finally, the present study has demonstrated that our PCD+/PCD- PEM system presented release-killing properties that supplement the contact-killing effect of this system that was reported in a previous paper.

Keywords: β-cyclodextrin polymers, polyelectrolytes multilayer (PEM), textile, drug delivery system, antibacterial textile.

1. Introduction

Infections represent a major medical concern and has severe impact on the public health economy. Antimicrobial coatings of medical devices and implants is one major solution and is the subject of many investigations in academic and industrial research. Polyelectrolyte multilayers (PEMs) introduced by Decher *et al.* in 1992 (Decher et al., 1992) consist in the step-by-step deposition of polyanions and polycations building layer-by layer (LbL) films on surfaces. The wide range of disposable polyelectrolytes makes this approach among the most versatile methods for the design of antibacterial surfaces as PEMs present the great advantages (i) to be applicable to a large variety of substrates (Boudou et al., 2010; Hammond, 2012), (ii) to be tunable in terms of chemical, physicochemical and mechanical properties (Phelps et al., 2011) and iii) to embed and release a wide range of antimicrobial compounds. Three general strategies are commonly used in the design of LbL antimicrobial films (Lichter et al., 2009; Lichter and Rubner, 2009; Séon et al., 2015). The first approach consists in the prevention of the bacterial adhesion through the adjustment of the surface hydrophobicity (Chen et al., 2010; Genzer and Efimenko, 2006), the surface charge (Zhu et al., 2015) or the surface stiffness (Lichter et al., 2008). The second approach is based on contact-killing surfaces obtained by the immobilization into the LbL film of polymers carrying cationic groups. Antimicrobial peptides, synthetic polymers carrying amine groups such as polyallylamine (Lichter and Rubner, 2009), quaternary ammonium salts such as poly(diallyldimethylammonium chloride) (Zhu et al., 2015), chitosan biopolymer (Wang et al., 2012) and its quaternary ammonium derivatives (Graisuwan et al., 2012) are the most commonly used solutions (Lichter et al., 2009; Zhu and Loh, 2015). The third approach consists in the release of antimicrobial agents compound toward the tissues directly in contact

89 with the implant or the device such as copper or silver salts or nanoparticles (Dai and
90 Bruening, 2002), antibiotics especially cationic ones such as gentamicin (Chuang et al., 2008),
91 antimicrobial peptides (Shukla et al., 2010), nitric oxide (Cai et al., 2011), antiseptic agents
92 (Agarwal et al., 2012; Nguyen et al., 2007; Séon et al., 2015; Wang et al., 2014) (triclosan,
93 chlorhexidine) have been especially studied. Concerning the two later strategies, it is worth to
94 mention that the antibacterial effect of contact killing surfaces often lasts longer than release
95 based coatings since the LbL film degradation is in general slower than the release
96 phenomenon.

97 Recently, our group successfully developed contact-killing PEMs films based on the one
98 hand on cationic β-cyclodextrin polymers crosslinked with epichlorohydrin in the presence of
99 glycidyl trimethylammonium chloride (PCD+), and on the other hand on an anionic β-
100 cyclodextrin polymer crosslinked with citric acid (polyCTR-CD = PCD-). Such LbL system
101 was built onto a nonwoven PET textile whose surface was preliminarily modified with
102 carboxylic acid groups in order to provide the requested anionic character to the support
103 (Junthip et al., 2015). Thanks to their high glycidyl trimethylammonium chloride content,
104 such LbL systems presented a relevant intrinsic bacterial reduction up to 7.3 log against *S.*
105 *aureus* and 4.5 log against *E. coli* (Junthip et al., 2016).

106 In the present work, the contact-killing PCD+/PCD- LbL system described above was
107 loaded with triclosan (TCS), a broad-spectrum antimicrobial agent (Jones et al., 2000;
108 Yazdankhah et al., 2006) commonly used in personal care products (Cheng et al., 2011) that
109 conveniently exhibits a high host-guest complexation affinity with cyclodextrins (Duan et al.,
110 2005; Loftsson et al., 1999; Lu et al., 2001). Thereby, a dual antimicrobial action system was
111 expected, combining the contact-killing effect provided by the trimethylammonium groups of
112 PCD+ on the one hand, and the release-killing effect obtained TCS release on the other hand
113 (Figure 1). To our knowledge, only a few dual release and contact killing LbL systems with

multiple bactericidal components have been already described in the literature (Zhu and Loh, 2015). Despite PEM integrating CDs have been particularly studied in drug delivery (Fagui et al., 2014; Leguen et al., 2007; Smith et al., 2009) and in biomaterials applications (Benkirane-Jessel et al., 2004; Chen et al., 2011; Martin et al., 2013a; Teo et al., 2015), the only existing work dealing with the concept of dual functionality was reported by Wong et al. who prepared a dual functional PEM coating including a poly(carboxymethylcyclodextrin) and N,N-dodecyl,methyl-polyethyleneimine that displayed both prolonged release of anti-inflammatory diclofenac and contact microbiocidal activity (Wong et al., 2010).

To achieve our objective, a nonwoven PET coated with an LbL film made of five PCD+/PCD- bilayers was prepared and characterized by Scanning Electron Microscopy (SEM). The release-killing effect was obtained by loading TCS on samples, whose release kinetics was performed in phosphate buffered saline (PBS) at 37°C in a dynamic system. TCS inclusion in CD cavities present in both PCD+ and PCD- were investigated by proton Nuclear Magnetic Resonance (NMR) and by phase solubility study. The antibacterial activities of the samples loaded with TCS dipped in PBS medium over a period of 28 days were assessed in parallel by diffusion test.

2. Materials & Methods

2.1. Materials

βCD was a gift from Roquette (Kleptose®, Lestrem, France). Glycidyltrimethylammonium chloride (GTMAC), epichlorohydrin (EP), sodium dihydrogen hypophosphite ($NaH_2PO_2.H_2O$), sodium hydroxide (NaOH), citric acid (CTR), triclosan (TCS), phosphate buffered saline (PBS, for solution 0.15 M at pH=7.4) and potassium dihydrogen phosphate (for solution 50 mM at pH=6.5) were supplied from Sigma Aldrich (Saint-Quentin Fallavier, France).

139 The textile support was a polyethylene terephthalate non-woven (PET, thickness =
140 0.24 mm, surface weight = 65 g/m², reference NSN 365) donated by PGI-Nordlys (Bailleul,
141 France).

142 Anionic water-soluble polymer of β-cyclodextrin (polyCTR-CD or PCD-) was
143 prepared according to a method described by Martel et al. (Martel et al., 2005) by the
144 solubilization of β-cyclodextrin, sodium hypophosphite as catalyst and citric acid as
145 crosslinking agent in respective weight ratio 10g/ 3g/ 10g in 100 mL of water. After water
146 removal by rotary evaporator, the resulting solid mixture was then cured at 140°C during 30
147 min under vacuum. After water addition, the resulting suspension was filtered and the filtrate
148 dialyzed during 72 hours against water using 6–8 kDa membranes (SPECTRAPOR 1,
149 Spectrumlabs). Finally, the polyCTR-CD was recovered by freeze drying. Molecular masses
150 in number (Mn) and in weight (Mw), measured by aqueous size exclusion chromatography
151 (SEC) in water equipped with a light scattering detector, were 13.0 kg/mol and 22.6 kg/mol
152 respectively (PDI = 1.7). The charge density of PolyCTR-CD (or COOH groups content) was
153 4 mmol per gram (measured by acid-base titration). The weight composition determined by
154 1H NMR was 50 wt.% in CD moieties and 50 wt.% in citrate cross-links.

155 Cationic water-soluble polymer of β-cyclodextrin (polyEPG-CD-10 or PCD+) was
156 synthesized by reacting βCD with epichlorohydrin (EP) in the presence of
157 glycidyltrimethylammonium chloride (GTMAC), with a molar ratio GTMAC/ βCD = 10,
158 under basic conditions as previously described (Junthip et al., 2015). Briefly 5g (4.4 mmol) of
159 βCD was dissolved in 8 mL of NaOH (22% (w/v)) aqueous solution and left under
160 mechanical stirring overnight at room temperature. Then, 7.40 mL (44 mmol) of GTMAC
161 (90%(w/v) in water) and 3.45 mL (44 mmol) of EP were rapidly added to solution heated to
162 60°C during 3 hours before adding acetone. The aqueous phase was heated to 50°C overnight,
163 neutralized with HCl (6 N), dialyzed (cut-off of 6–8 kDa, SPECTRAPOR 1, Spectrumlabs)

and finally freeze-dried before collecting polyEPG-CD samples as white powders. Mn and Mw, measured by aqueous size exclusion chromatography (SEC), were 16.1 kg/mol and 25.8 kg/mol respectively (PDI = 1.6). The charge density (or trimethylammonium groups content) of polyEPG-CD-10 was 2 mmol/g of polymer (measured by colloidal titration). The weight composition determined by 1H NMR, was 58 wt.% in CD moieties, 16wt.% in EP cross-links and 26 wt.% in GTMAC.

2.2.Layer by Layer coating of the PET textile

The virgin textile PET sample was preliminary functionalized by thermofixation using the *pad-dry-cure* process in order to obtain the negative charges provided by the resulting carboxylic groups strongly anchored on the surface (Martin et al., 2013a, 2013b). Virgin textiles (3 x 3cm²) were impregnated in 100 mL of solution containing β-cyclodextrin (10 g), sodium hypophosphite as catalyst (3 g) and citric acid as crosslinking agent (10 g). The textile was then roll-padded (BHVP model, Roaches, UK) and cured at 150°C in a ventilated oven (Minithermo, Roaches, UK). Samples were finally washed by soxhlet with water. This thermofixation treatment yielded samples provoked a 20wt.% weight gain of the samples (abbreviated PET20) measured with a precision balance ($\pm 4.10^{-4}$ g, Precisa 240A), corresponded to a surface density of 6.5 µmol COO^-/cm² measured by using the calcium acetate titration method (Ducoroy et al., 2008).

The LbL build-up was carried out at room temperature by dipping process with both CD cationic and anionic polyelectrolytes solutions (4 g/L) in water at their natural pH, i.e 3.5 ±0.1 (polyCTR-CD) and 6.5 ±0.2 (polyEPG-CD-10) (Junthip et al., 2015). The thermofixed PET20 samples were firstly dipped during 15 minutes in 50 mL of cationic polymer solution PCD+, drying at 90°C for 15 minutes, rinsing again with 50 mL of distilled water for 15 minutes and drying at 90°C for 15 minutes. The similar sequence was then applied with the

189 anionic polymer solution PCD-. Both steps were repeated 4 times to obtain ten self-assembled
190 layers, or five PCD+/PCD- bilayers onto the thermofixed PET supports (abbreviated PET20-
191 PEM5) with a weight gain of 75% ± 3% (Junthip et al., 2016).
192 Finally, a thermal treatment of textiles was applied at 140°C in a ventilated oven
193 (MEMMERT, DIN 40050- IP20) for 8 hours in order to improve the PEM film stability in
194 contact with PBS medium.
195
196 **2.3.Triclosan adsorption**
197 The incorporation of TCS was performed by dipping the PET20 and PET20-PEM5
198 samples into 15 mL of a saturated TCS solution during 24 hours under stirring 150 rpm at
199 37°C. Then, samples were washed in 15 mL of distilled water during 5 minutes for three
200 times before drying at 45°C.
201 **2.4. Scanning Electron Microscopy (SEM)**
202 Textile samples were characterized by SEM apparatus SEM instrument (Hitachi S-
203 4700 SEM FEG (Field Emission Gun)), using an acceleration voltage of 5 kV. Textile
204 samples were covered with a carbon layer before their analysis.
205
206 **2.5. Triclosan release study**
207 Drug release was measured with a fully automated flow-through cell dissolution
208 apparatus (Sotax USP4, CE 7 Smart with CP7 piston pump, Switzerland) in a closed loop
209 configuration combined with a UV-visible spectrometer (Perkin Elmer LAMBDA 25). Textile
210 samples containing TCS (100 mg) were placed inside a cylinder flow cell (22.6 mm). The
211 dissolution medium (filtered solution of PBS 0.15 M at pH=7.4) under stirring (200 rpm) was
212 circulated by pumping it through each cell at a rate of 50 mL/min and the temperature was
213 maintained at 37 ± 0.5°C during testing. The concentration of TCS was measured at time

intervals and calculated at each time point based on calibration curves (specific extinction coefficient of TCS in PBS at λ=282 nm was 0.0149 L mg^{-1} cm^{-1} with r^2=0.9974).

2.6. Complexation study of triclosan with PCD+ and PCD-

2.6.1. Proton Nuclear Magnetic Resonance

The one and two dimensional ^1H NMR spectra were recorded in D_2O using a Bruker AV 300 spectrometer at 300 MHz with 8 increments for polymers and complexed TCS/polymers, except for TCS with 13056 increments. Two-dimensional NOESY (Nuclear Overhauser Effect SpectroscopY) experiments were operated at 300 K using the standard Bruker parameters and a spin-lock mixing time of 350 ms with TPPI method. 2D spectrum consisted of a matrix of 2048 (F2) by 2048 (F1) covering a sweep width of 1929 Hz and 16 increments were collected with 256 transients. A concentrated polymer solution was prepared (50mM) in D_2O before TCS addition in excess, and then it was maintained under agitation (150 rpm) 24 hours at 25°C. The supernatant was characterized by NMR.

2.6.2. Phase solubility of TCS with PCD+ and PCD-

Phase solubility of TCS in phosphate buffer (50 mM, pH 6.5) was carried out by adding an excess amount (5mg/mL) of TCS to 10 ml of polymer solution (0 to 8%(w/v)) and βCD solution (0 to 1.6%(w/v)). The mixtures were mechanically shaken at 25 °C for 24 hours (until equilibrium), centrifuged and the TCS concentration in the supernatant was determined by UV-visible spectroscopy (Shimadzu UV-1800) at 282 nm. Each experiment was performed in duplicate. In the case of the formation of a 1:1 inclusion complex, the stability constant $K_{1:1}$ was obtained from the equation described by Higuchi and Connors (Higuchi and Connors, 1965) :

$$K_{1:1} = Slope / (S_0 (1-Slope))$$

where S_0 represents the intrinsic solubility of TCS in PBS, and the molar concentration of CD polymers was calculated by considering that β-CD represented 58. wt % and 50. wt % in PCD+ and PCD- respectively.

2.7. Antibacterial tests

The diffusion test was applied to evaluate the antimicrobial activity through the measurement of the inhibition zone around the samples put on agar gel seeded with *Staphylococcus aureus,* CIP224 and *Escherichia coli,* K12. The textile samples of 11 mm diameter were sterilized with absolute ethanol during 1 minute before air-drying and then placed in a 24 well plate containing 1 mL of PBS sterilized solution (0.15 M, pH=7.4) under stirring (150 rpm) at 37°C. At the predetermined time points, the PBS solution of each well plate was removed and refilled with a new PBS solution, except at zero time. 100 µL of the bacterial suspension (1×10^4 colony forming unit (CFU)·mL^{-1}) were then plated on Müller-Hinton-Agar (MHA). Then, the textile samples were deposited on MHA and were incubated at 37°C during 24 hours. Inhibition zone radius (in cm) were measured and plotted as a function of the release time in PBS. The tests were repeated three times to obtain an average value.

3. Results & Discussion

3.1. LbL film build-up onto PET20 samples

LbL deposition of cationic polyEPG-CD-10 and anionic polyCTR-CD onto the nonwoven PET textile were realized as reported previously (Junthip et al., 2016, 2015). After the thermofixation step, textile samples underwent a weight gain of 20%wt and after the superimposition of five PCD+/PCD- bilayers, the weight gain increased up to 75%wt (Junthip et al., 2016). Micrographs displayed in Figure 2 show the evolution of the fibrous support

morphology at the fibers scale before treatment, after thermofixation (PET20 sample) and after the ten cycles of the dip coating process (PET20-PEM5). Both modification steps involved an increase of the diameter of the fibers synonym of their coating firstly by the thermofixed layer and then by the LbL film. In the latter case, ~~one can observe that~~ the textile structure was covered by the PEM coating, especially when focusing on the fibers crossings where fibers are bridged together. From this observation, ~~one can notice~~ a decrease of the textile porous volume ~~of the textile, and one can a~~ could also explain the stiffness increase noted upon samples handling, especially ~~observed~~ after the LbL process and the final thermal post-treatment at 140°C.

3.2. Study of the complexation of TCS with PCD+ and PCD- by NMR

TCS/polyEPG-CD-10 and TCS/polyCTR-CD interactions were investigated by Nuclear Magnetic Resonance (NMR). In Figure 3.a and 3.b the main signals in both cationic and anionic poly-cyclodextrins spectra could be attributed according to previous reports (Martin et al., 2013b) and (Junthip et al., 2016, 2015). Spectrum in figure 3.a relative to polyCTR-CD displayed the signal of the glucopyranosic units of cyclodextrins, H1 at 5 ppm, H3, H5, H6, H4 and H2 situated between 3.5 and 4 ppm, the methylene groups of the citrate crosslinks appearing between 2.7 and 3 ppm. Finally, two singlets at 6.25 and 7.8 ppm corresponding to cis and trans aconitic esters respectively issued from a side reaction consisting of the dehydration of citrate crosslinks. Spectrum in figure 3.b relative to polyEPG-CD-10 displayed the signal of anomeric proton (H-1) of cyclodextrin near 5 ppm, the protons of quaternary ammonium of GTMAC at 3.2 ppm and the rest of cyclodextrin protons and reactant protons (GTMAC and EP) in the range 3.2 to 4.5 ppm. In the presence of TCS, no signal shifts of H3 and H5 cyclodextrins protons could be observed in the 3.7 to 3.9 ppm interval. However, the spectrum magnification reported in Figure 3c displays spectral changes in the range from 6 to 8 ppm where aromatic protons

of TCS are situated (Jug et al., 2011; Qian et al., 2008). All aromatic protons of TCS shifted upfield or downfield (Figure 3c), in the presence of both polymers, revealing a possible complexation. This was confirmed by 2D-NOESY NMR spectra (Figure S1) where correlation tasks appeared consequently to the dipolar interaction between the TCS aromatic protons (6 to 8 ppm) with internal proton H3 and H5 of poly-cyclodextrins (around 3.8 to 3.9 ppm).

3.3.Phase solubility study of TCS in the presence of PCD+ and PCD-

Solubility enhancement studies of TCS in the presence of βCD, polyCTR-CD and polyEPG-CD-10 were carried out using the phase solubility method (Figure 4). TCS presented intrinsic solubility of 0.04 mM at 25°C that sharply increased by factors up to 10, 60 and 220 in the presence of βCD, polyCTR-CD and polyEPG-CD-10, respectively. More specifically, Figure 4a shows an increase in TCS solubility in function of the βCD concentration up to 4.4 mmol/L, and then levels around 0.4 mmol/L. This is typical of a B-type phase-solubility profile (Higuchi and Connors, 1965) indicating the formation of complexes with limited solubility in the aqueous medium. The apparent association constant calculated from the slope of the plot ($K_{1:1}$) was equal to 1870 M^{-1}, in accordance with the literature in which $K_{1:1}$ was equal to 2526 M^{-1} for the complex βCD: TCS (Jug et al., 2011). TCS solubility linearly increases with both βCD polymers types concentration, displaying in both cases A_L-type profiles (Figure 4b and 4c). The calculated association constants of TCS with polyEPG-CD-10 and polyCTR-CD were equal to 6650 and 1590 M^{-1} respectively. Elsewhere, the $K_{1:1}$ value of TCS with another type of cationic βCD polymer using choline chloride as cationizing agent was found to be equal to 3800 M^{-1} in water (Qian et al., 2008). So, this study confirmed that both PCD+ and PCD- can form inclusion complexes with TCS.

315 **3.3.1. TCS Release study**

316 PET 20 and PET20-PEM5 samples were loaded with TCS and their release profile were
317 assessed in PBS at 37°C and in dynamic mode at the flow rate of 50mL/min. Figure 5 shows
318 a meaningful influence of the mode of functionalization on the release patterns. As a matter of
319 fact, 4.6 mg of TCS /g of sample were loaded and then released by the textiles modified by
320 thermofixation and then with five self-assembled bilayers, while samples that only underwent
321 the thermofixation step adsorbed and then released only 1.1 mg/g. This result demonstrated
322 that the multilayer film increased of a 3.5 factor the reservoir capacity of the textile. This
323 result can be attributed to the inclusion complex formed between TCS and CD moieties
324 present in both PCD+ and PCD- in the PEM coating.

325 **3.4. Antibacterial activity of samples**

326 PET20 and PET20-PEM5 samples cut in 11mm disks and then loaded with TCS were put
327 in PBS batch at pH 7.4 at 37°C over a period of 28 days. The disks were withdrawn at
328 different time points from the batches and deposited on agar plates pre-inoculated with *S.*
329 *aureus* and *E. coli* (Figure 6a). After 24 hours of incubation, their residual antibacterial
330 activities were reported by plotting the inhibition diameters of halos appearing around
331 samples against the release time in PBS (Figure 6b and 6c). Despite TCS is a broad-spectrum
332 antimicrobial agent, all test samples displayed larger inhibition diameters in the presence of *S.*
333 *aureus* (minimum inhibitory concentration (MIC) of 0.025-1 mg/L) compared to *E. Coli* K12
334 (MIC of 1mg/L), due to the higher sensitivity of Gram+ toward TCS (Assadian et al., 2011;
335 Suller and Russell, 2000). On the other hand, inhibition diameters formed around PET20-
336 PEM5 samples were sharply superior to those observed around PET20 samples loaded with
337 TCS. This can be correlated with the kinetic study that showed that the release rates of TCS
338 from PET20-PEM5 samples were sharply superior compared to PET20 samples. In the course
339 of time of the batch experiments, PET20 samples displayed a fast loss of their antibacterial

activity against *E. coli* within 3 days as the inhibition diameters decreased from 2.3 cm to 1.1 cm (corresponding to the diameter of disks) within this period. On the contrary, the antibacterial activity of PET20-PEM5 samples against *E. coli* only slightly decreased during the 28 days period as inhibition diameters decreased from 3.1 cm down to 2.7 cm. Besides, PET20 and PET20-PEM5 samples displayed a sustained antibacterial effect even after 28 days against *S. aureus.* However, the antibacterial activity of PET20 samples against *S. aureus* quickly decreased within 3 days period as inhibition diameters decreased from 4.0 cm down to 2.2 cm. On the contrary, the antibacterial activity of PET20-PEM5 samples against *S. aureus* only slightly decreased during the 28 days period as inhibition diameters decreased from 5.0 cm down to 3.5 cm. So, diffusion tests realized after ageing samples in batch was maintained at high and almost constant level over at least 28 days against both bacterial strains. The test positive response in the diffusion test indicated that the amount of TCS released from aged samples in the agar gel was always maintained above the minimum inhibitory concentration (MIC). This slow and controlled release can be attributed to the high inclusion complex formation constants reported above between TCS and CD moieties in PCD+ and PCD-.

In our previous paper (Junthip et al., 2016), we reported that PET20-PEM5 sample without TCS, tested by the *kill-time* method, displayed bacterial reductions of 7.3 and 4.5 $\log_{10}$ against *S. aureus* and *E. coli*. This intrinsic antibacterial activity was found to be dependent of the trimethylammonium content of polyEPG-CD-10 in the self-assembled layer (Junthip et al., 2016). Interestingly, in our former studies dealing with PEM coatings based on PCD- as polyanion, and chitosan as polycations (Aubert-Viard et al., 2019; Martin et al., 2013a; Mogrovejo-Valdivia et al., 2019; Pérez-Anes et al., 2015) control samples not loaded with any antibacterial substances did not display such intrinsic antibacterial activity despite chitosan is often reported as an antibacterial polymer. The presence of quaternary ammonium

groups carried by PCD+ versus primary ammonium groups carried by chitosan can explain such result. These previous results combined to our new results showed that these PCD+/PCD- systems presented intrinsic contact killing property and also extrinsic release killing properties once loaded with TCS (up to 28 days).

Conclusion

Polyelectrolyte multilayer (PEM) assembly based on water-soluble cationic β-cyclodextrin polymer (polyEPG-CD) and anionic β-cyclodextrin polymer (polyCTR-CD) was built up by the layer-by-layer technique onto non-woven PET textile preliminarily modified by polyCTR-CD through a thermofixation process. TCS loading on the samples coated with 5 self-assembled bilayers (PET20-PEM5 samples) reached 4.6 mg/g of textile, against only 1.1 mg/g on the sample modified with one thermofixed layer consisting of polyCTR-CD (PET20 samples). The PEM system displayed the sustained release of TCS over 14 hours in dynamic conditions. Besides, diffusion tests realized after ageing samples in batch was maintained at high and constant level over at least 28 days. These properties could be explained by the reversible inclusion complex formation between TCS and CD moieties present in both cationic and anionic crosslinked polymers. These results combined to our previous published paper (Junthip et al., 2016) showed that these PCD+/PCD- systems presented intrinsic contact killing property and also extrinsic release killing properties once loaded with TCS. PCD+/PCD- systems offer consequently an excellent potential for the prevention and curing of peri-operative infections on biomedical devices which often display dramatical consequences.

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Figure captions

Figure 1. Schematic representation of cationic and anionic polyelectrolytes based on cyclodextrins (PCD- and PCD+) and of a nonwoven PET coated with an LbL film made of PCD+/PCD- bilayers, stabilized by a thermal crosslinking reaction at 140°C and loaded with Triclosan

Figure 2. SEM pictures of (a) and (d) virgin PET, (b) and (e) 20%wt thermofixed layer, (c) and (f) multilayers (10 layers). Magnification x 150 and x800 resolution; full scale 50 and 300 µm.

Figure 3. NMR study of TCS/polyEPG-CD-10 and TCS/polyCTR-CD complexation in D_2O. ^1H NMR of the TCS/polyCTR-CD complex (a), the TCS/polyEPG-CD-10 complex (b) and the magnification at 6-8 ppm of TCS (c1), TCS/polyCTR-CD complex (c2) and TCS/polyEPG-CD-10 complex (c3).

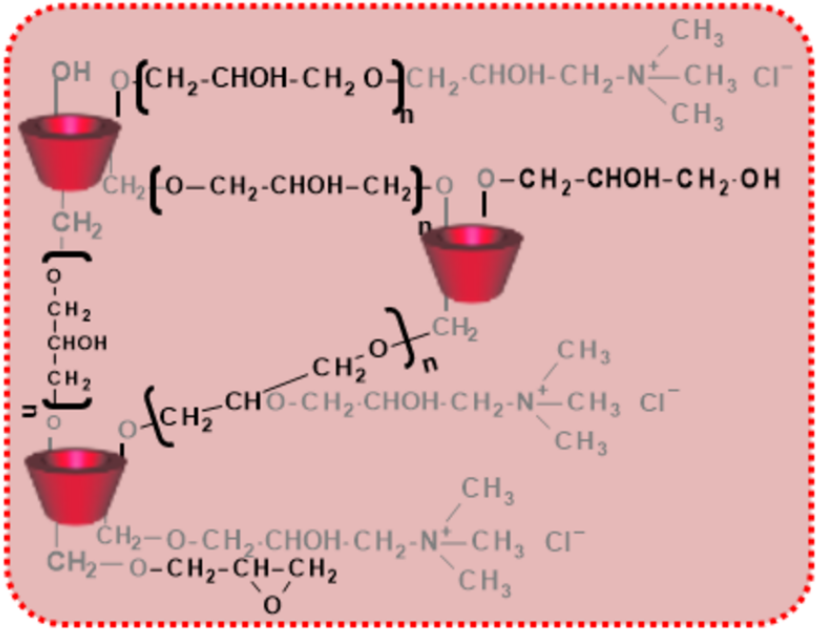
Figure 4. Phase solubility diagrams of TCS with (a) βCD and (b) polyEPG-CD-10 and (c) polyCTR-CD, in phosphate buffer (50 mM, pH 6.5) at 25°C.

Figure 5. Release kinetics study of TCS (dynamic mode at 50mL/min, in PBS at 37°C) from thermofixed (PET20) and 5 bilayers (PET20-PEM5) textile samples with (a) TCS released capacity in mg/g of sample, (b) TCS release capacity in %

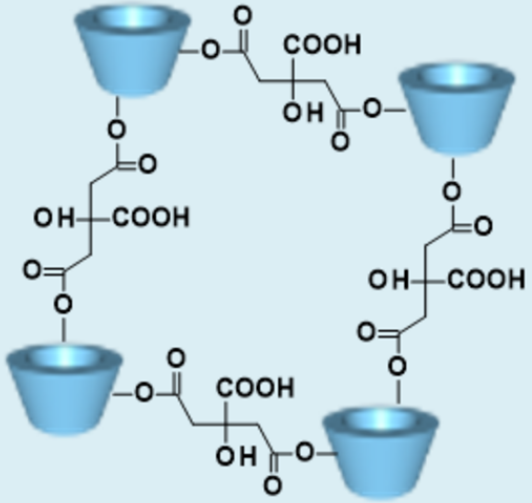
Figure 6. (a) Representative images of Kirby-Bauer test on TCS impregnated PET20 and PET20-PEM5 samples against *S. aureus* and *E. coli* with inhibition zone after 24 hours of TCS release in PBS at 37°C. Inhibition zone diameters around PET20 and PET20-PEM5 samples loaded with triclosan (TCS) against (b) *S. aureus* and (c) *E. coli* in function of contact time in PBS at 37°C after 24 hours incubation at 37°C over 28 days.

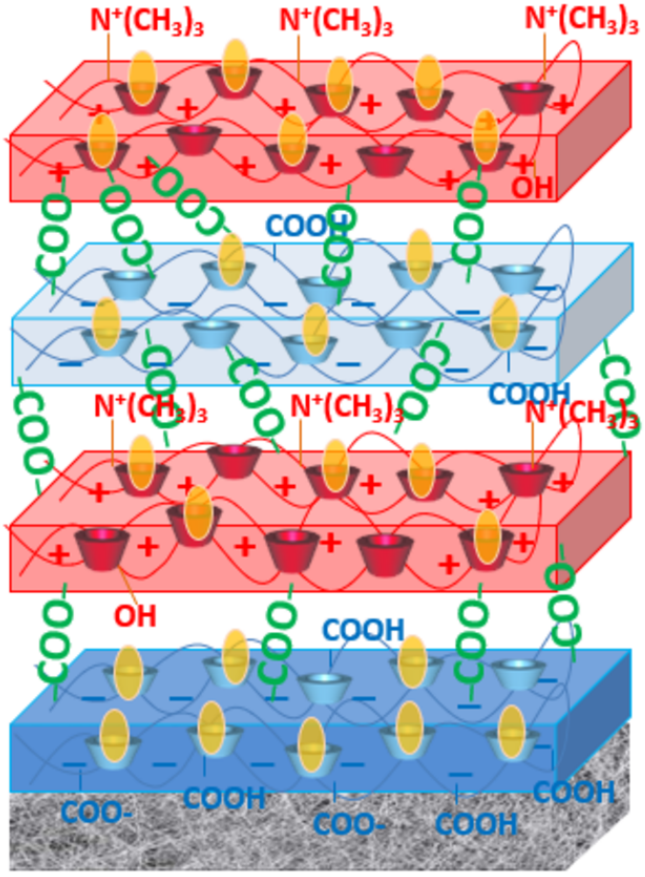
Figure S1. 2D-NOESY NMR spectra of TCS/polyCTR-CD (a) and TCS/polyEPG-CD-10 (b) complexes.

poly-EPG-CD-10 = PCD+



polyCTR-CD = PCD-





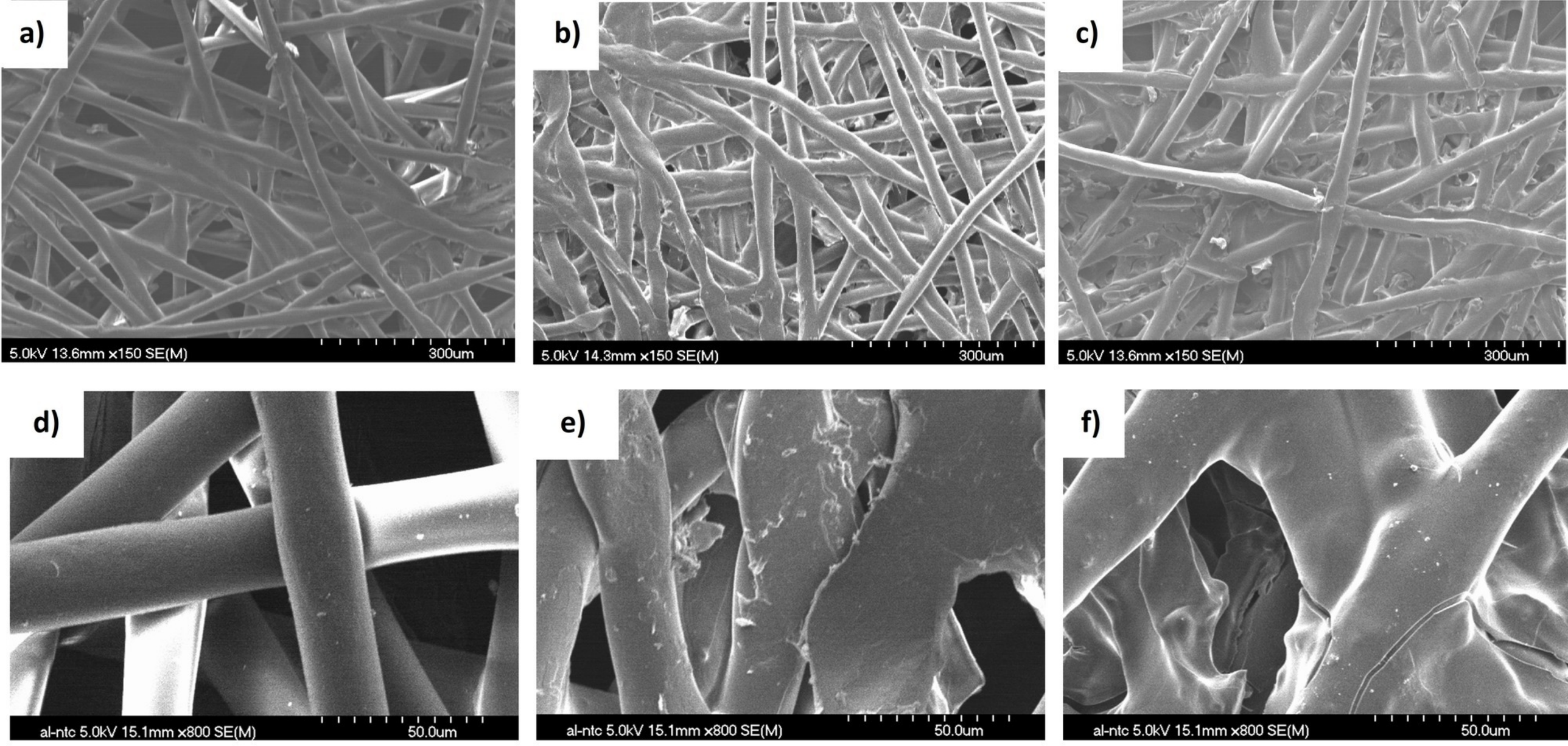
Triclosan

Self assembled polyCTR-CD

Self assembled poly-EPG-CD-10

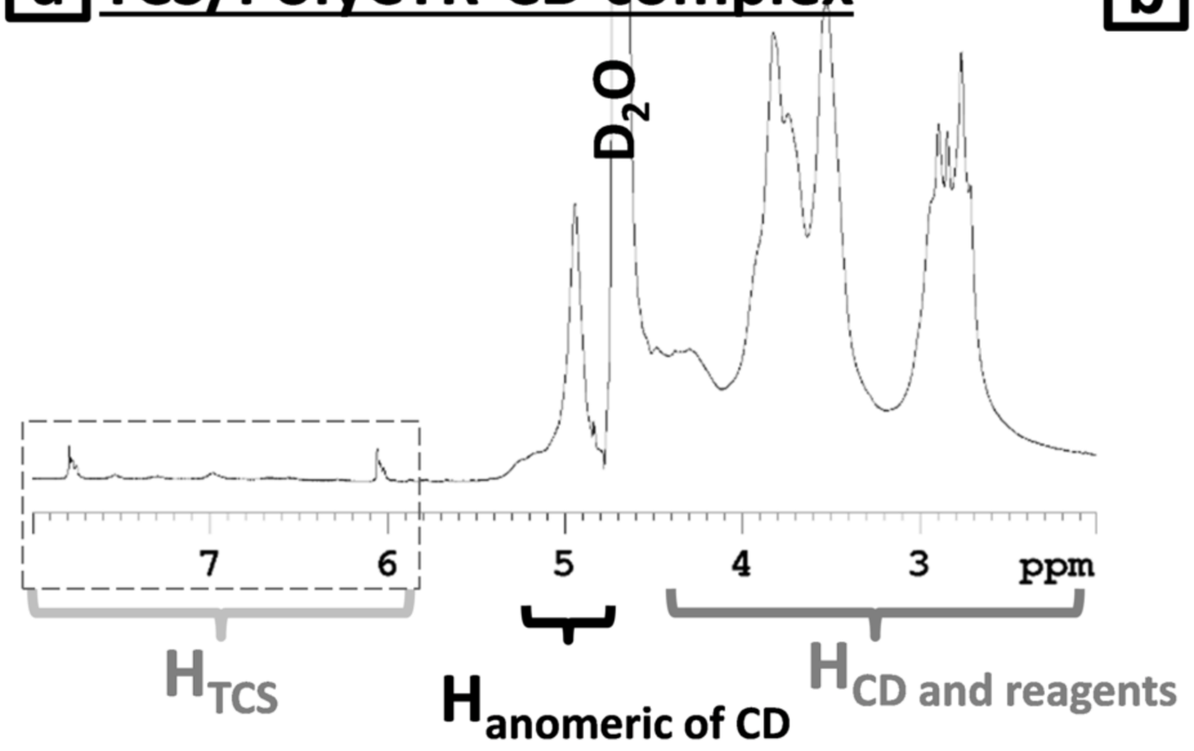
Ester link formed by post-treatment at 140°C

Nonwoven with thermofixed polyCTR-CD layer

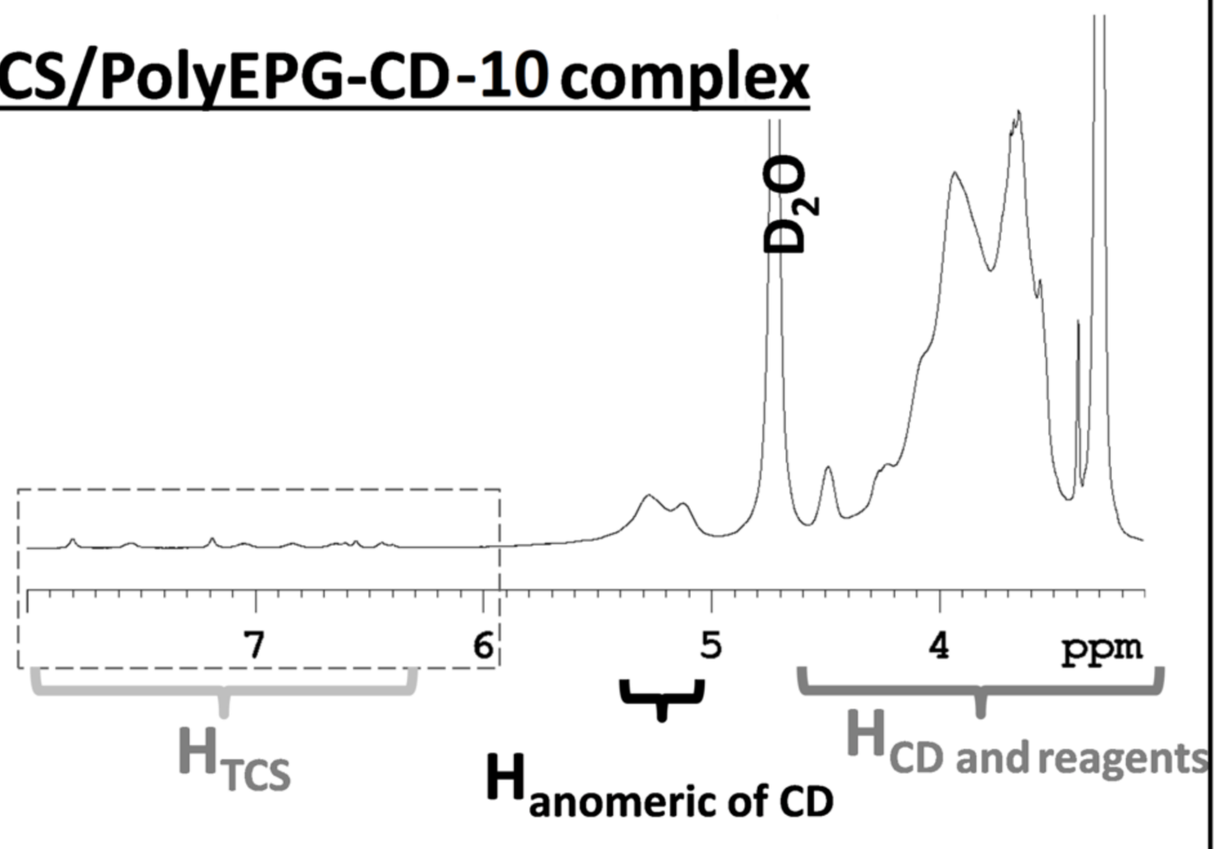

a)
5.0kV 13.6mm x150 SE(M)
300um
b)
5.0kV 14.3mm x150 SE(M)
300um
c)
5.0kV 13.6mm x150 SE(M)
300um
d)
al-ntc 5.0kV 15.1mm x800 SE(M)
50.0um
e)
al-ntc 5.0kV 15.1mm x800 SE(M)
50.0um
f)
al-ntc 5.0kV 15.1mm x800 SE(M)
50.0um

1D ^{1}H NMR (D_2O)

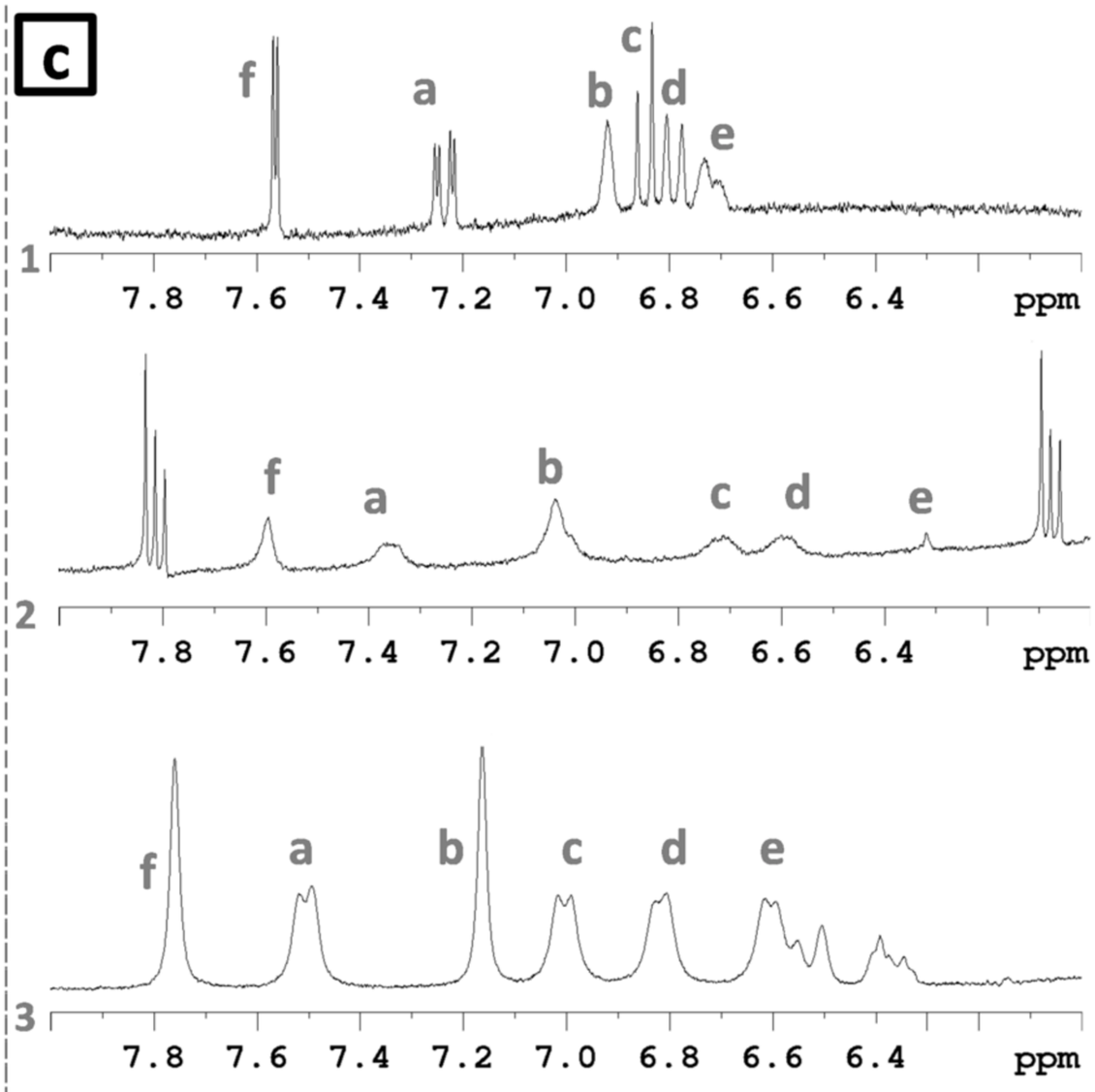
a **TCS/PolyCTR-CD complex**



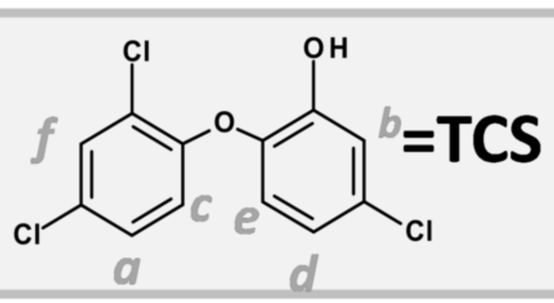
b **TCS/PolyEPG-CD-10 complex**

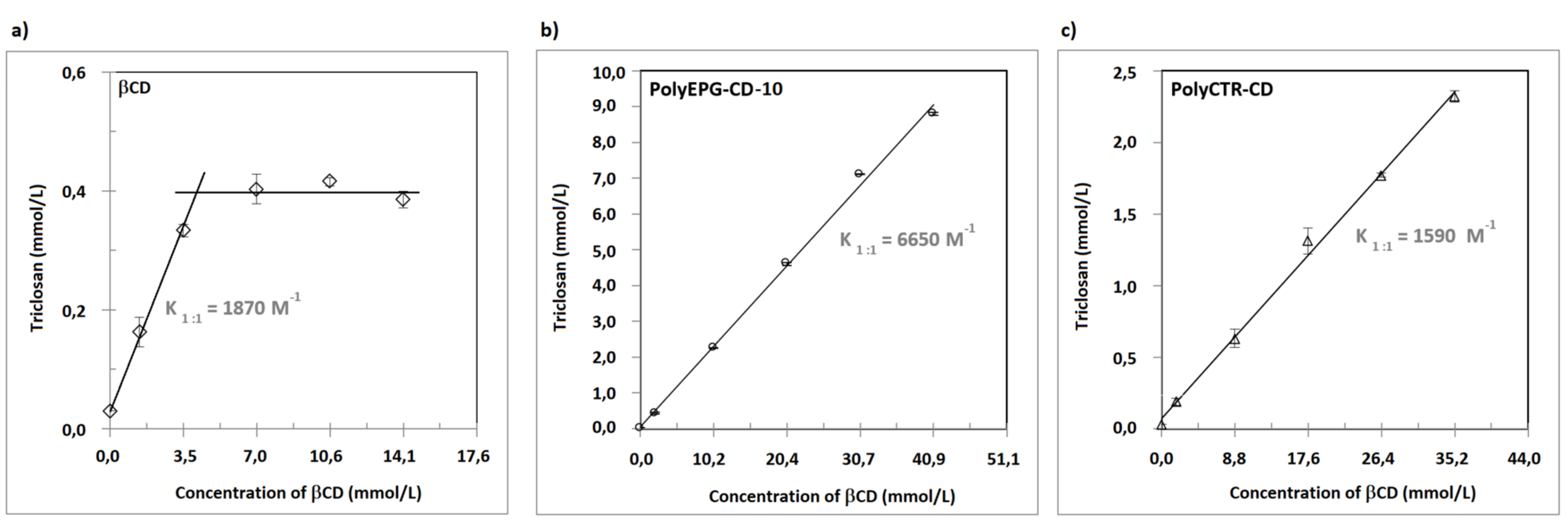


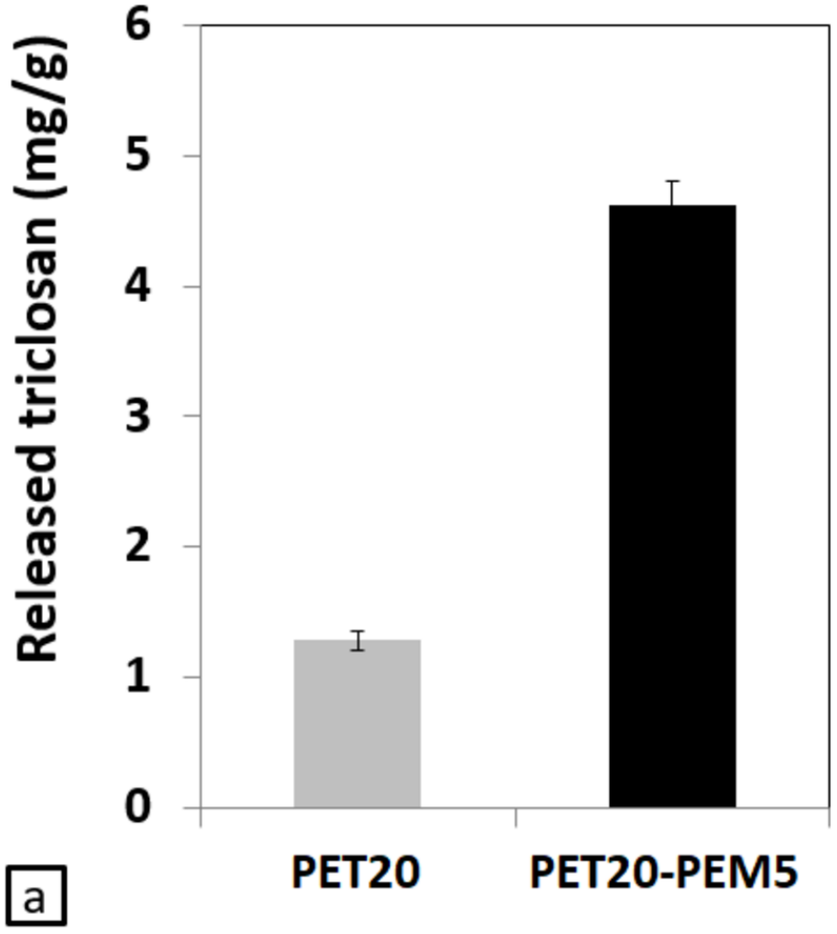
c

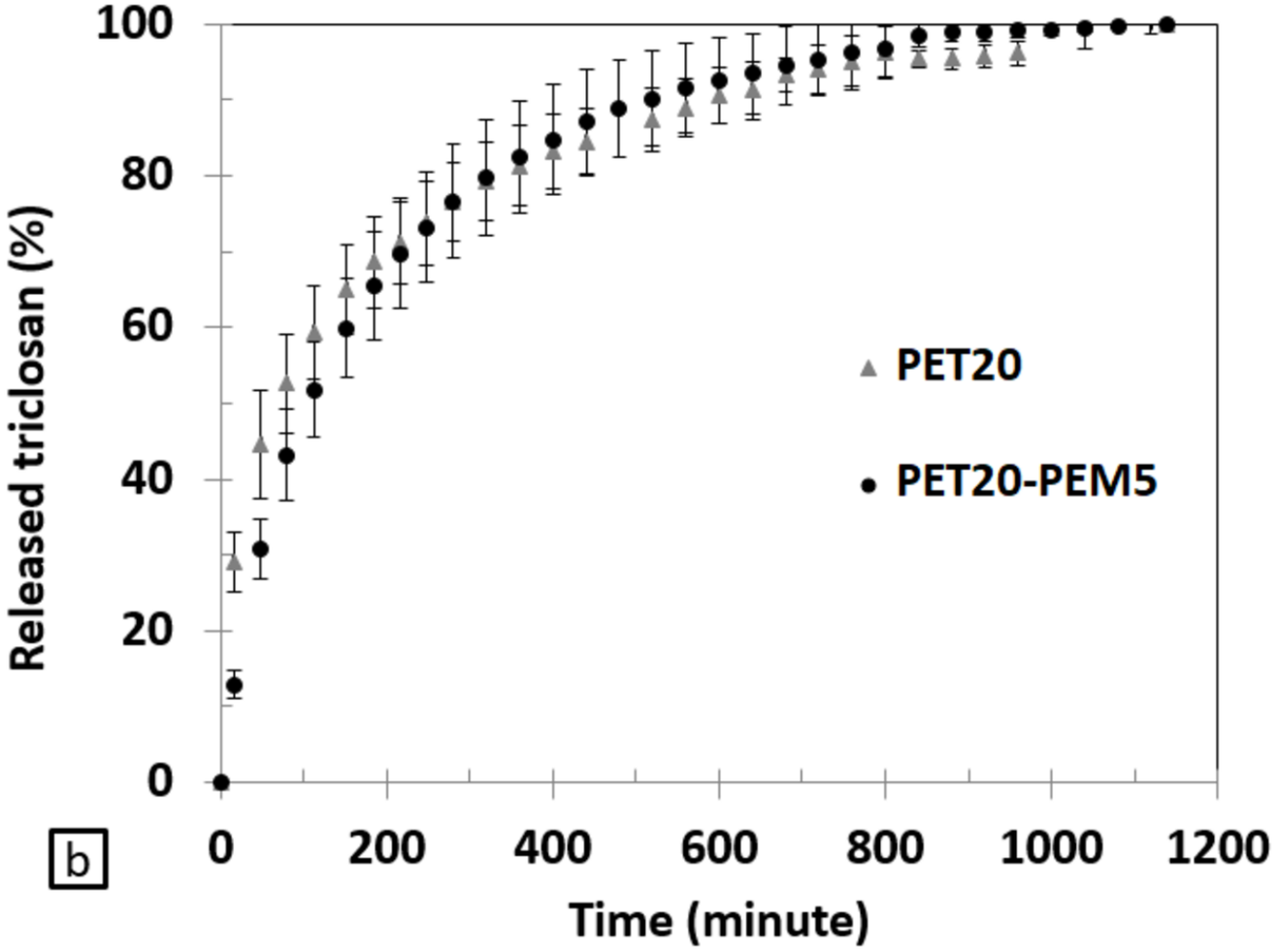


Zoom at 6-8 ppm

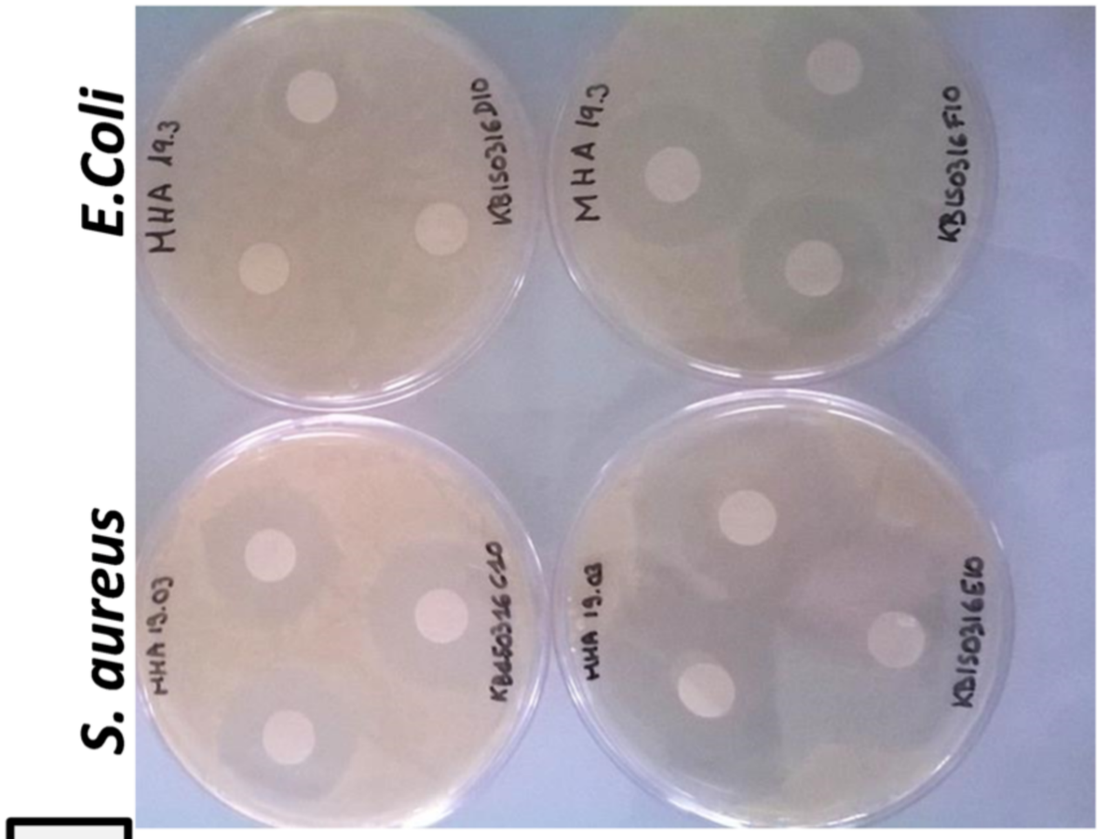



a)
βCD
Triclosan (mmol/L)
0,6
0,4
0,2
0,0
0,0
3,5
7,0
10,6
14,1
17,6
Concentration of βCD (mmol/L)
K 1:1 = 1870 M-1
b)
PolyEPG-CD-10
Triclosan (mmol/L)
10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
0,0
10,2
20,4
30,7
40,9
51,1
Concentration of βCD (mmol/L)
K 1:1 = 6650 M-1
c)
PolyCTR-CD
Triclosan (mmol/L)
2,5
2,0
1,5
1,0
0,5
0,0
0,0
8,8
17,6
26,4
35,2
44,0
Concentration of βCD (mmol/L)
K 1:1 = 1590 M-1


Released triclosan (mg/g)
6
5
4
3
2
1
0
PET20
PET20-PEM5
a

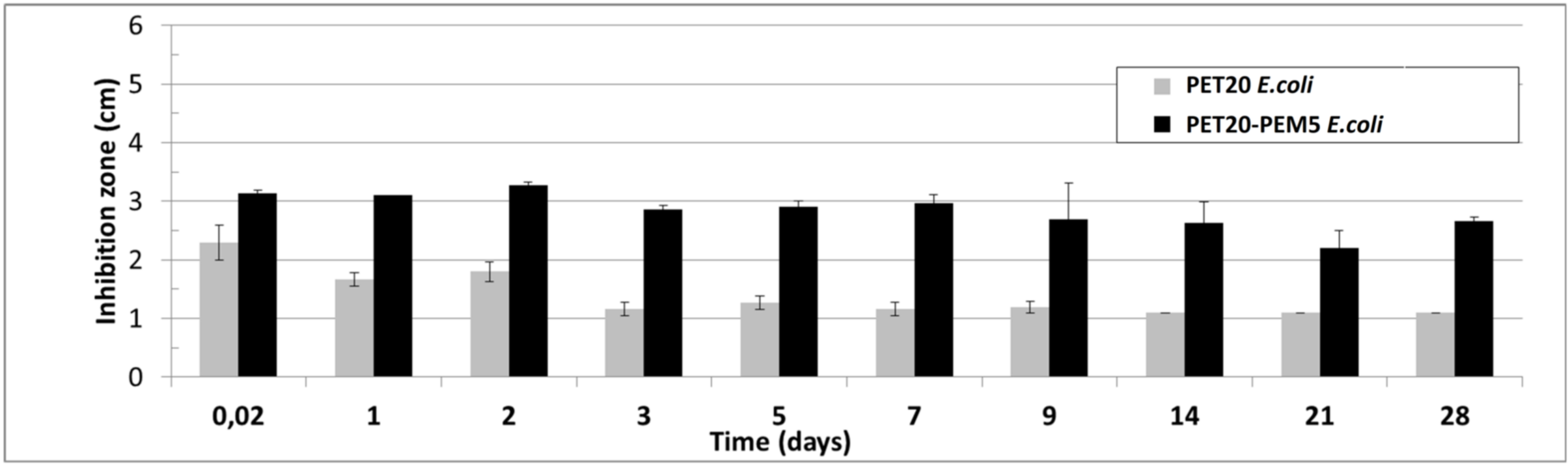

Released triclosan (%)
100
80
60
40
20
0
PET20
PET20-PEM5
0
200
400
600
800
1000
1200
Time (minute)
b

Samples after 24 hours of TCS release

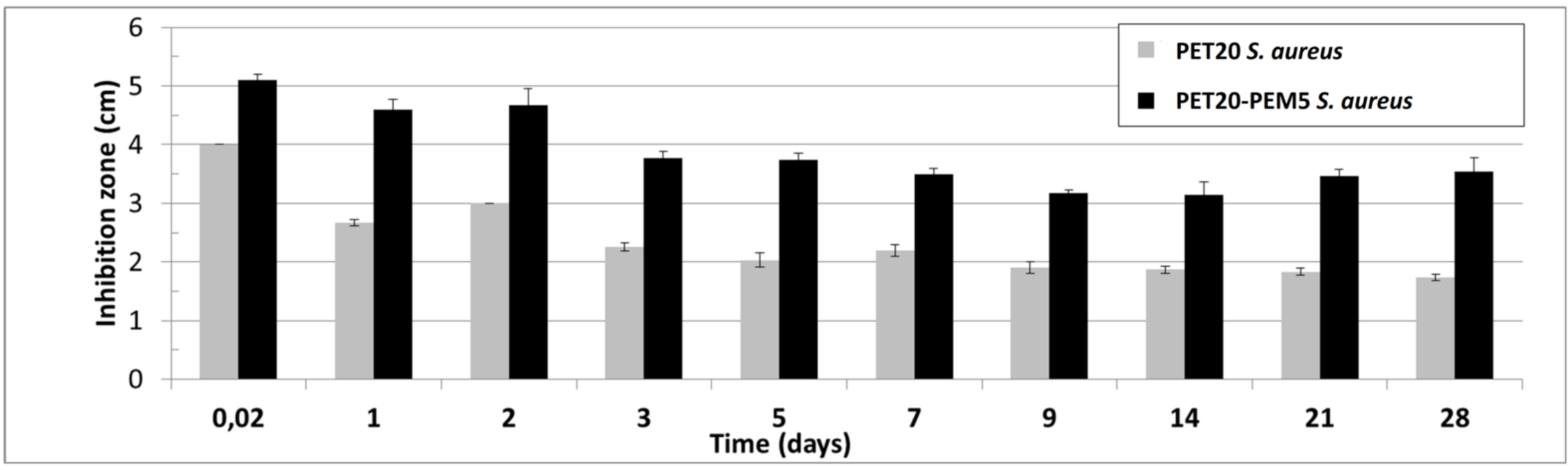


a

PET 20 PET20-PEM5

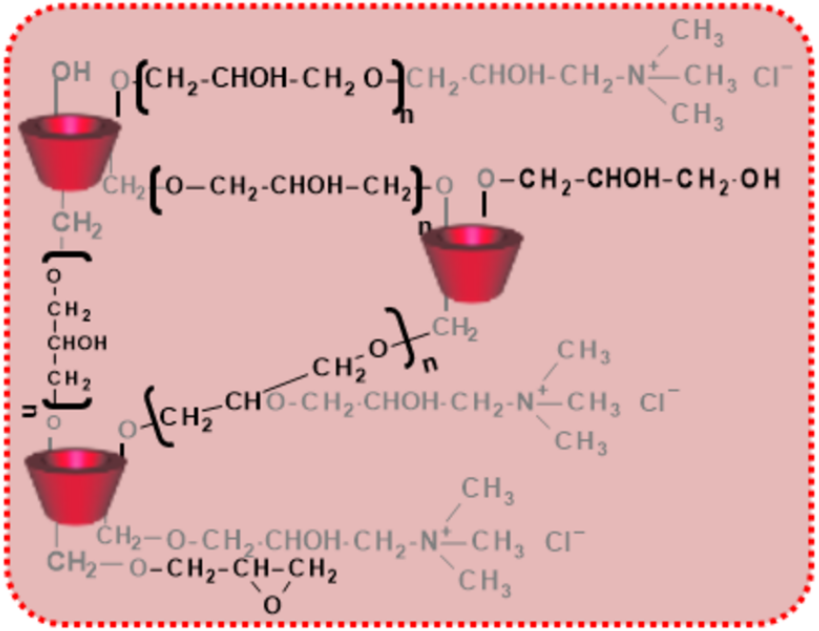


b

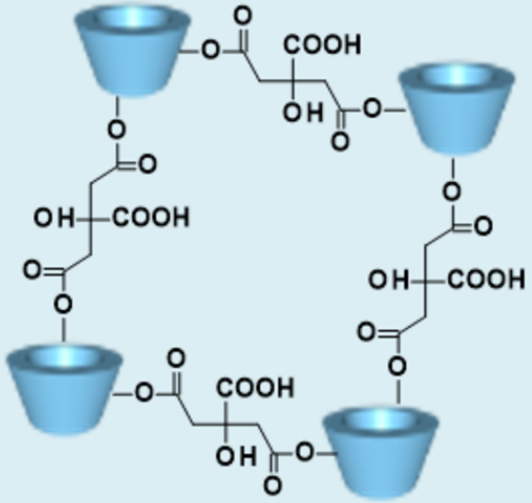


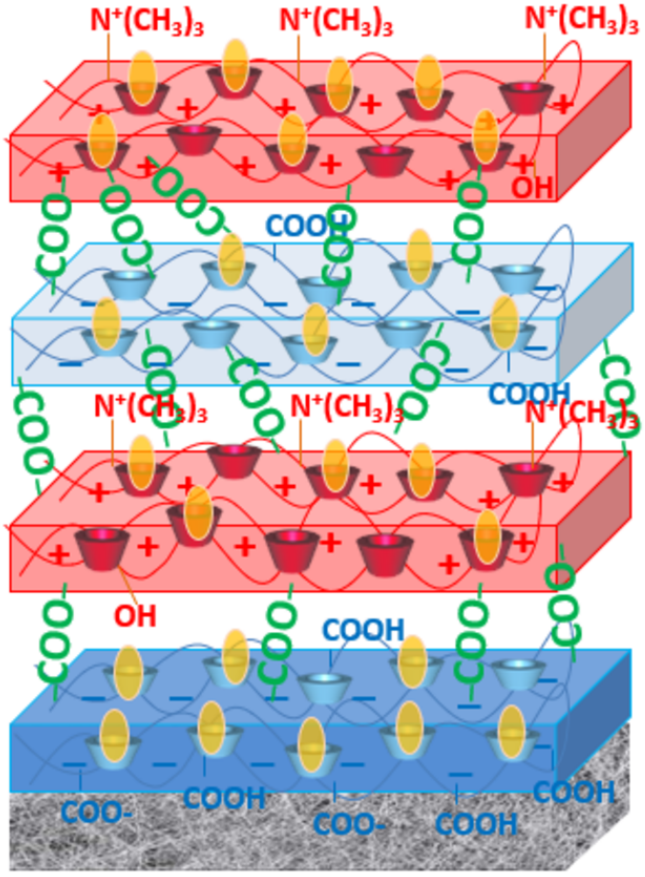
c

poly-EPG-CD-10 = PCD+



polyCTR-CD = PCD-





Triclosan

Self assembled polyCTR-CD

Self assembled poly-EPG-CD-10

Ester link formed by post-treatment at 140°C

Nonwoven with thermofixed polyCTR-CD layer