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Antibacterial electrospun nanofibers from triclosan/cyclodextrin inclusion complexes

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ABSTRACT

The electrospinning of nanofibers (NF) from cyclodextrin inclusion complexes (CD-IC) with an antibacterial agent (triclosan) was achieved without using any carrier polymeric matrix. Polymer-free triclosan/CD-IC NF were electrospun from highly concentrated (160% CD, w/w) aqueous triclosan/CD-IC suspension by using two types of chemically modified CD; hydroxypropyl-beta-cyclodextrin (HPβCD) and hydroxypropyl-gamma-cyclodextrin (HPγCD). The morphological characterization of the electrospun triclosan/CD-IC NF by SEM elucidated that the triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF were bead-free having average fiber diameter of 520 ± 250 nm and 1100 ± 660 nm, respectively. The presence of triclosan and the formation of triclosan/CD-IC within the fiber structure were confirmed by ^{1}H-NMR, FTIR, XRD, DSC, and TGA studies. The initial 1:1 molar ratio of the triclosan:CD was kept for triclosan/HPβCD-IC NF after the electrospinning and whereas 0.7:1 molar ratio was observed for triclosan/HPγCD-IC NF and some uncomplexed triclosan was detected suggesting that the complexation efficiency of triclosan with HPγCD was lower than that of HPβCD. The antibacterial properties of triclosan/CD-IC NF were tested against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria. It was observed that triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF showed better antibacterial activity against both bacteria compared to uncomplexed pure triclosan.

1. Introduction

Electrospinning technique has received tremendous attention recently due to its versatility for producing multifunctional nanofibers (NF) from variety of polymeric and inorganic materials [1,2]. Electrospinning is a relatively simple and cost-effective setup for NF production when compared to advanced techniques such as self-assembly, lithography, etc, or conventional fiber spinning techniques such as melt-blown, wet spinning, etc. [1,2]. NF produced by electrospinning have unique properties including extremely high surface area, very lightweight, nanoporous features, and design flexibility for specific physical and chemical functionalization [1–3]. Due to their remarkable characteristics and the multifunctional nature of these electrospun NF, they have shown promising potentials to be used in a wide range of fields such as, health, environmental, textiles, electronics, energy, food, agriculture, etc. [1–13].

Electrospun NF having antibacterial functionality can be achieved by incorporating certain types of antibacterial agents in the polymeric NF matrix during the electrospinning process. As a polymeric NF matrix, natural polymers or biocompatible and biodegradable synthetic polymer types are often chosen for the electrospinning. The resulting nanofibrous webs having antibacterial properties can be quite applicable in biomedical practices such as wound dressing [14–18]. For instance, Nitanan et al. obtained electrospun poly (styrene sulfonic acid-co-maleic acid) (PSSA-MA)/polyvinyl alcohol (PVA) blend NF which was loaded with antibacterial agent neomycin [14]. In another study, Unnithan et al. produced antibacterial electrospun scaffolds by the electrospinning of dextran, polyurethane (PU) and ciprofloxacin HCl (CipHCl) including solution [15]. In the study of Karami et al., the electrospun poly (ε-capro-lactone) (PCL), poly (lactic acid) (PLA), and their 50/50 hybrid nanofibrous mats were obtained with the incorporation of herbal agent thymol [16]. In another related study, Lin et al. developed biocompatible nanofibrous membranes by the co-electrospinning of collagen/zein proteins which contain antibacterial agent berberine [17]. One of the associated study, Merrell et al. reported the electrospinning of curcumin loaded

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PCL NF [18]. While Qi et al. obtained antimicrobial NF by the combination of tetracycline hydrochloride (TCH) and halloysite nanotubes/poly (lactic-co-glycolic acid) (PLGA) composites [19], Nirmala et al. achieved the production of this functional NF by mixing triethylbenzylammonium chloride (TEBAC) into rosin NF [20]. In all the studies mentioned above, the antibacterial activity and/or the wound healing performance of these functional NF were investigated and it was revealed that, antibacterial agent incorporated electrospun NF mats have high potential for the wound dressing purposes.

Cyclodextrins (CD) are natural and non-toxic cyclic oligosaccharides which are produced from the enzymatic conversion of starch. CD which have toroid-shaped molecular structure can form non-covalent host-guest inclusion complexes (IC) with drugs and antibacterials [21–23]. Such CD-IC of bioactive agents are quite useful in medical application since CD-IC provide enhanced functionality by improving the stability, solubility, reactivity, and controlled release of the drugs and antibacterials [21,22,24]. In the literature, there are also reports about the incorporation of CD-IC of antibacterial and drug into polymeric matrix to utilize from the inclusion complexation property of CD molecules and to render them into more applicable form [25–29]. Huang et al. embedded neomycin sulfate and its β-CD-IC into PLLA and PCL films [25]. In another study, the mucoadhesive Buccal films (chitosan, KollicoatIR, glycerol) were developed by the IC of HPβCD and poorly soluble anti-inflammatory drug, flufenamic acid for the topical administration [26]. In the study of Sreenivasan, the salicyclic acid/β-CD-IC were loaded into PVA hydrogel film [27]. Plackett et al. produced IC of α-CD and β-CD with an antibacterial agent allyl isothiocyanate (AITC) and encapsulated into polylactide-co-polycaprolactone films for the use of cheese packaging [28]. In another related study, the antibacterial agent triclosan/β-CD-IC were incorporated into biodegradeable PCL films [29]. Instead of film, electrospun NF are better candidate as the carrier matrix for CD-IC of bioactive agents such as drugs and antibacterials. Since, very high surface area and highly porous structures of NF provide advantage during the release of these active agents and also by this functionalization the unique properties of CD molecules were combined with the above mentioned features of NF. There are few studies regarding to the incorporation of CD-IC of bioactive agents into the electrospun NF matrix. For instance, Vega-Lugo et al. obtained antibacterial NF by the electrospinning of AITC/β-CD-IC including protein isolate (SPI)/poly (ethylene oxide) (PEO) blend and PLA solutions [30]. In another study, the curcumin/β-CD-IC loaded electrospun PVA NF were produced by Sun et al. [31]. In a study of our research group, PLA NF incorporating IC of triclosan with native CD (α-CD, β-CD, and γ-CD) were obtained successfully [32]. Very recently, we have also reported preliminary findings on electrospun NF from triclosan/HPβCD-IC without using polymer carrier matrix [33].

Triclosan is a kind of hydrophobic antibacterial agent having very low water solubility [34,35]. There are studies in the literature about the enhancement of triclosan solubility by forming IC with various kinds of CD and CD polymers [36–39]. The CD types such as β-CD and HPβCD can efficiently form complexation with triclosan and enhance its solubility. Hence, in our previous study [33], triclosan was chosen as a model antibacterial agent to obtain polymer-free NF from CD-IC system by using HPβCD and triclosan. In the present study, we used two types of CD (HPβCD and HPγCD) for the complexation with triclosan and we have attained two types of polymer-free triclosan/CD-IC NF; triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF. Here, we have also compared these two triclosan/CD-IC NF from the point of inclusion complexation efficiency and antibacterial property in conjunction with the structural characterization. From the characterization by ^{1}H-NMR, FTIR, XRD, DSC and TGA, it was determined that IC formation between CD and triclosan was successfully obtained and the complexation efficiency was higher for triclosan/HPβCD-IC compared to triclosan/HPγCD-IC. Moreover, the antibacterial test results showed that triclosan/CD-IC NF have better bactericidal activity than the powder form of pure triclosan against both *E. coli* and *S. aureus* bacteria. In brief, in this study the very high surface area of NF was integrated with the unique functionality of CD-IC and polymer-free triclosan/CD-IC NF having better antibacterial effect was achieved compared to pure triclosan.

2. Experimental

2.1. Materials

Triclosan (>97%, Sigma, Germany) was obtained commercially. The hydroxylpropyl-beta-cyclodextrin (HPβCD) (degree of substitution: 0.6) and hydroxylpropyl-gamma-cyclodextrin (HPγCD) (degree of substitution: 0.6) were purchased from Wacker Chemie AG (Germany). The water used was from a Millipore Milli-Q ultrapure water system. The materials were used as-received without any further purification process.

2.2. Preparation of CD solutions and triclosan/CD-IC suspensions

The clear and homogenous solutions of HPβCD and HPγCD were prepared in water by dissolving CD (160%, w/v) at 40 °C and they were cooled down to the room temperature before electrospinning. The optimal CD concentration was 160% (w/v) for the electrospinning as it was determined from our previous study [40]. The inclusion complex (IC) formation of HPβCD and HPγCD with triclosan was carried out by using 1:1 molar ratio of triclosan/HPβCD and triclosan/HPγCD in their aqueous solution. First of all, triclosan was dispersed in water at 40 °C because it is not soluble in water. Then, HPβCD (160%, w/v) and HPγCD (160%, w/v) aqueous solutions were separately added to the triclosan aqueous dispersion. The amount of CD and triclosan was adjusted accordingly in order to have 1:1 molar ratio of triclosan/HPβCD-IC and triclosan/HPγCD-IC. After the addition of HPβCD and HPγCD to triclosan dispersion, the solutions became clear and homogeneous due to the dissolution of triclosan by IC formation. As the solutions were cooled down at room temperature and stirred overnight, highly turbid and white color suspensions of triclosan/HPβCD-IC and triclosan/HPγCD-IC were obtained (Fig. 1a,b).

2.3. Electrospinning

The clear solutions of HPβCD and HPγCD and, the suspensions of triclosan/HPβCD-IC and triclosan/HPγCD-IC were placed separately in a 3 mL syringe fitted with a metallic needle of 0.6 mm inner diameter. The syringe was fixed horizontally on the syringe pump (KD Scientific, KDS 101). The electrode of the high-voltage power supply (Spellman, SL Series) was clamped to the metal needle tip, and the cylindrical aluminum collector was grounded. The feed rate of solutions was 1 mL/h, the applied voltage was 15 kV, and the tip-to-collector distance was kept at 10 cm. Electrospun nanofibers (NF) were deposited on a grounded stationary cylindrical metal collector covered with a piece of aluminum foil (Fig. 1c). The electrospinning apparatus was enclosed in a Plexiglas box, and electrospinning was carried out at 25 °C at 30% relative humidity. The collected NF were dried at room temperature under the fume hood overnight.

2.4. Measurements and characterizations

A rheometer (Anton Paar, Physica CR 301) equipped with a cone/plate accessory (spindle type CP40-2) was used to

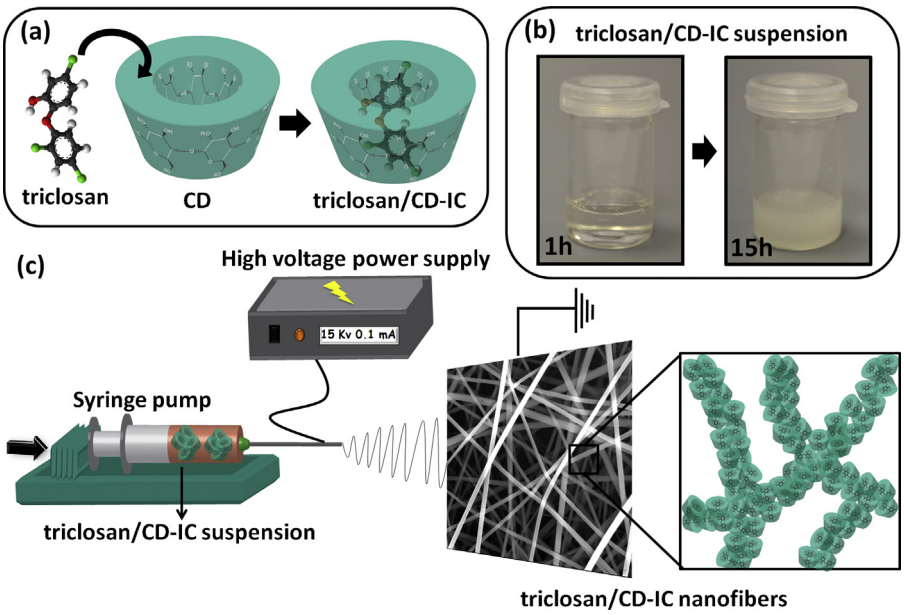

Fig. 1. Schematic representation of (a) IC formation between CD and triclosan. (b) the photograph of triclosan/HPβCD-IC suspension for 1 h and 15 h periods. (c) illustration of electrospinning of triclosan/CD-IC NF.

measure the rheological behavior of the HPβCD and HPγCD solutions and, triclosan/HPβCD-IC and triclosan/HPγCD-IC suspensions in the range of 0.1 to 100 1/s shear rate. The conductivity of the solutions and suspensions was measured with a Multiparameter InoLab ® Multi 720 (WTW) at room temperature. Both the viscosity and conductivity measurements were performed three times for each sample. The morphological analyses of the electrospun NF were performed by using scanning electron microscope (SEM) (FEI Quanta 200 FEG). Samples were sputtered with 5 nm Au/Pd prior to SEM imaging. The average fiber diameter (AFD) was determined from the SEM images, and around 100 fibers were analyzed. The infrared spectra of the NF were obtained by using a Fourier transform infrared spectrometer (FTIR) (Bruker-VERTEX 70). For measurement, the samples were mixed with potassium bromide (KBr) and pressed as pellets. The scans (64 scans) were recorded between 4000 cm^{-1} and 400 cm^{-1} at resolution of 4 cm^{-1}. The molar ratio between triclosan/CD was determined by using proton nuclear magnetic resonance (^{1}H NMR, Bruker D PX-400) system. The electrospun NF were dissolved in *d6*-DMSO at the 20 g/L concentration. The spectra were recorded at 400 MHz and at 16 total scan. The X-ray diffraction (XRD) (PANalytical X'Pert powder diffractometer) data of the electrospun nanofibrous mats were recorded by using Cu Kα radiation in a range of 2θ = 5°–30°. The thermal properties of electrospun NF were investigated by thermogravimetric analysis (TGA) (TA Q500) and differential scanning calorimetry (DSC) (TA Q2000). The TGA of the samples was carried out from 25 to 500 °C at 20 °C/min heating rate, and N_2 was used as a purge gas. DSC analyses were carried out under N_2; initially, samples were equilibrated at 0 °C and then heated to 200 °C at a heating rate of 10 °C/min.

2.5. Antibacterial tests

The antibacterial activities of triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF were tested against *Escherichia coli (E. coli)* RSHM 888 (RSHM, National Type Culture Collection Laboratory, Ankara, Turkey) and *Staphylococcus aureus* (*S. aureus)* RSHM 96090/07035 (ATCC 25923) representing Gram-negative and Gram-positive bacteria, respectively. The tests of activities were performed using disc agar diffusion method. *E. coli* and *S. aureus* were grown overnight and 150 μL of cultures were spread on Luria-Bertani (LB) agar. For antibacterial tests, the triclosan/HPβCD-IC and triclosan/HPγCD-IC nanofibrous webs were cut into 1.2 cm sized circular pieces and the weight of the NF samples were adjusted accordingly in order to have same amount of triclosan. Then, they were placed on *E. coli* and *S. aureus* spreaded agar plates and visualized after 24 h incubation. For comparison, the antibacterial activity of pure triclosan was also investigated. The same amount of triclosan powder was used as present in the CD-IC NF and the triclosan dispersion was prepared by using a very little amount of water. Then this dispersion was dropped on the agar plate in order to provide same diameter with the nanofiber samples. The tests were repeated three times for each sample. Diameters of zones in which there is no bacterial growth were measured after 24 h and the inhibition zones were compared.

3. Result and discussion

3.1. Electrospinning of CD NF and triclosan/CD-IC NF

In our previous study, we have optimized the electrospinning parameters for the formation of bead-free NF from HPβCD and HPγCD in which highly concentrated (160%, w/v) aqueous CD solutions were used [40]. Here, we used the same concentration (160% CD, w/v) for the electrospinning of triclosan/HPβCD-IC and triclosan/HPγCD-IC suspensions and we were quite successful for the electrospinning of bead-free NF from both IC systems. The representative SEM images of HPβCD NF, HPγCD NF, triclosan/HPβCD-IC NF, and triclosan/HPγCD-IC NF samples were displayed in Fig. 2a–d. Bead-free NF with smooth morphology were attained for all samples. While the HPβCD NF have average fiber diameter (AFD) of 695 ± 335 nm, the HPγCD NF were obtained with AFD of 1355 ± 475 nm. Although the viscosity of triclosan/CD-IC suspensions was higher than the pure HPβCD and HPγCD solutions, the triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF were thinner having AFD of 520 ± 250 nm and 1100 ± 660 nm, respectively (Table 1). This was possibly because of the higher conductivity of triclosan/CD-IC suspensions when compared to HPβCD and HPγCD solutions. This resulted in more stretching of the electrified jet, so thinner fibers are formed during the electrospinning process [1,41]. Additionally, the HPγCD based fibers

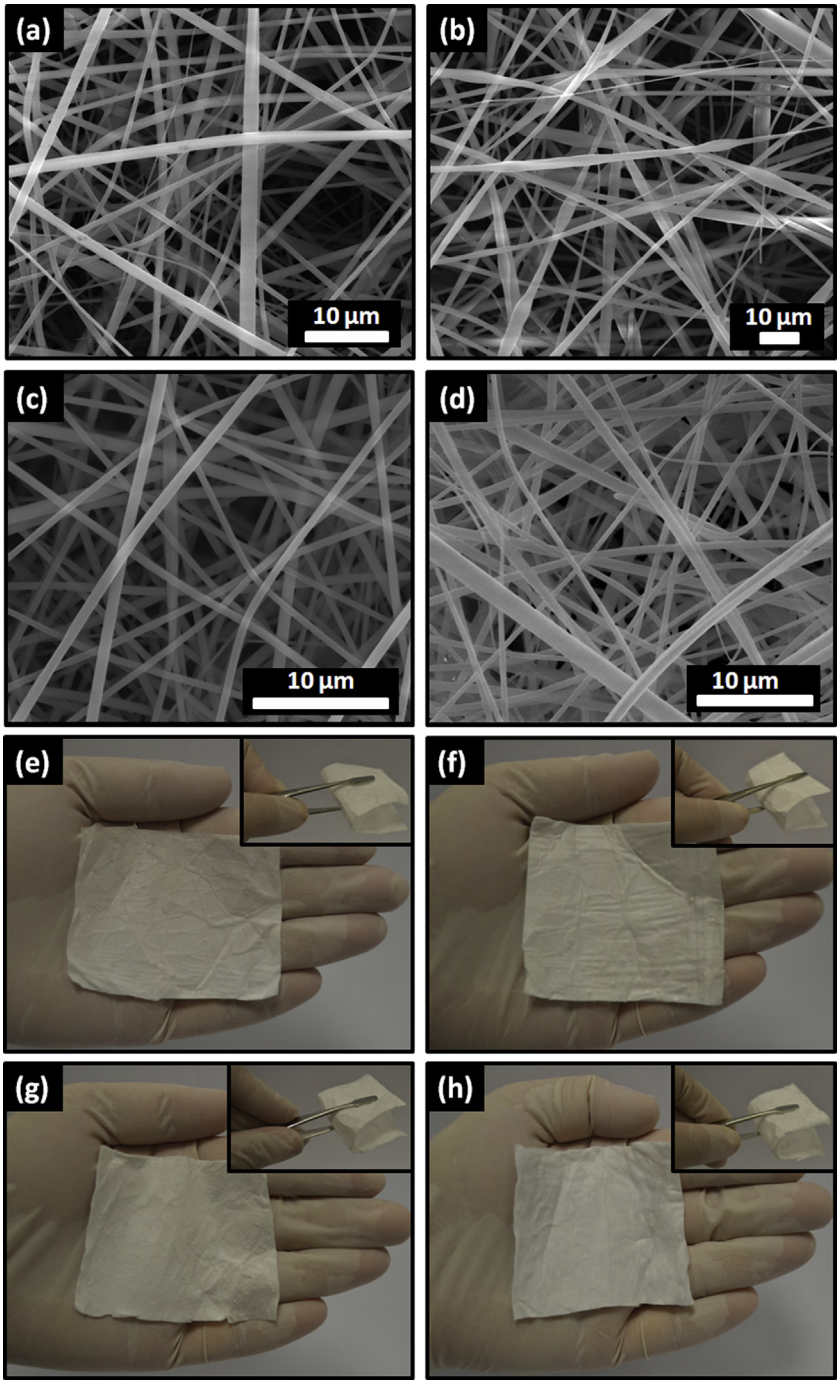

Fig. 2. Representative SEM image of electrospun NF obtained from (a) HPβCD, (b) HPγCD solutions, (c) triclosan/HPβCD-IC, and (d) triclosan/HPγCD-IC suspensions. The photographs of nanofibrous webs which can be easily handled and folded; (e) HPβCD NF, (f) HPγCD NF, (g) triclosan/HPβCD-IC NF, and (h) triclosan/HPγCD-IC NF.

Table 1
The characteristics of HPβCD and HPγCD solutions; triclosan/HPβCD-IC and triclosan/HPγCD-IC suspensions and the properties of resulting electrospun nanofibers.

Solution	Viscosity[a] (Pa s)	Conductivity[a] (μS/cm)	Fiber morphology	Average fiber diameter[b] (nm)	Fiber diameter range (nm)
HPβCD	0.26 ± 0.01	197.00 ± 1.52	Bead-free nanofibers	695 ± 335	240–1700
HPγCD	0.34 ± 0.06	4.89 ± 1.11	Bead-free nanofibers	1355 ± 475	505–2300
Triclosan/HPβCD-IC	0.86 ± 0.10	249.00 ± 8.88	Bead-free nanofibers	520 ± 250	250–1100
Triclosan/HPγCD-IC	1.29 ± 0.26	6.74 ± 0.07	Bead-free nanofibers	1100 ± 660	300–2100

[a] The measurements were repeated three times ($n = 3$) and the average results were given with standard deviations.
[b] The average fiber diameter and standard deviations were determined from the analysis of around 100 fibers.

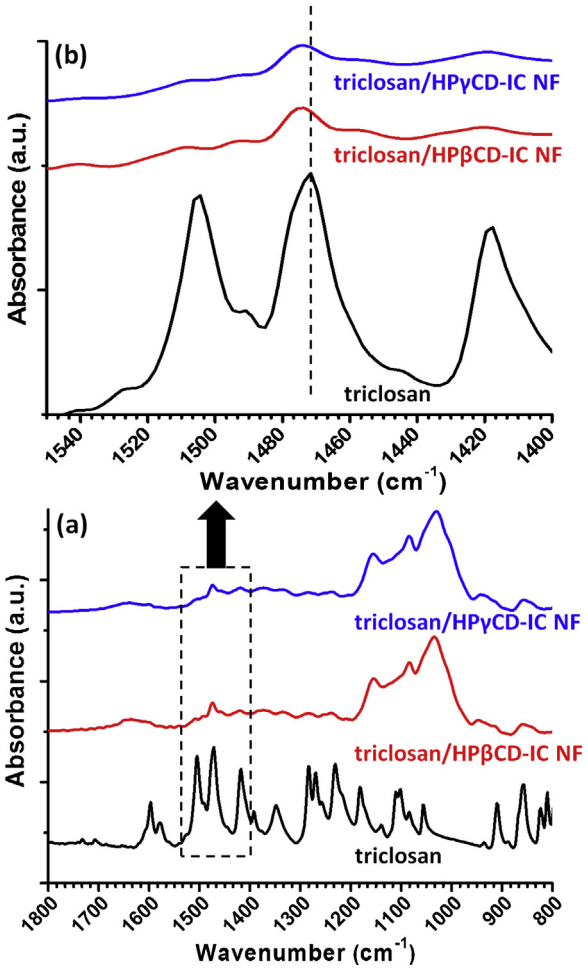

Fig. 3. FTIR spectra of pure triclosan, triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF.

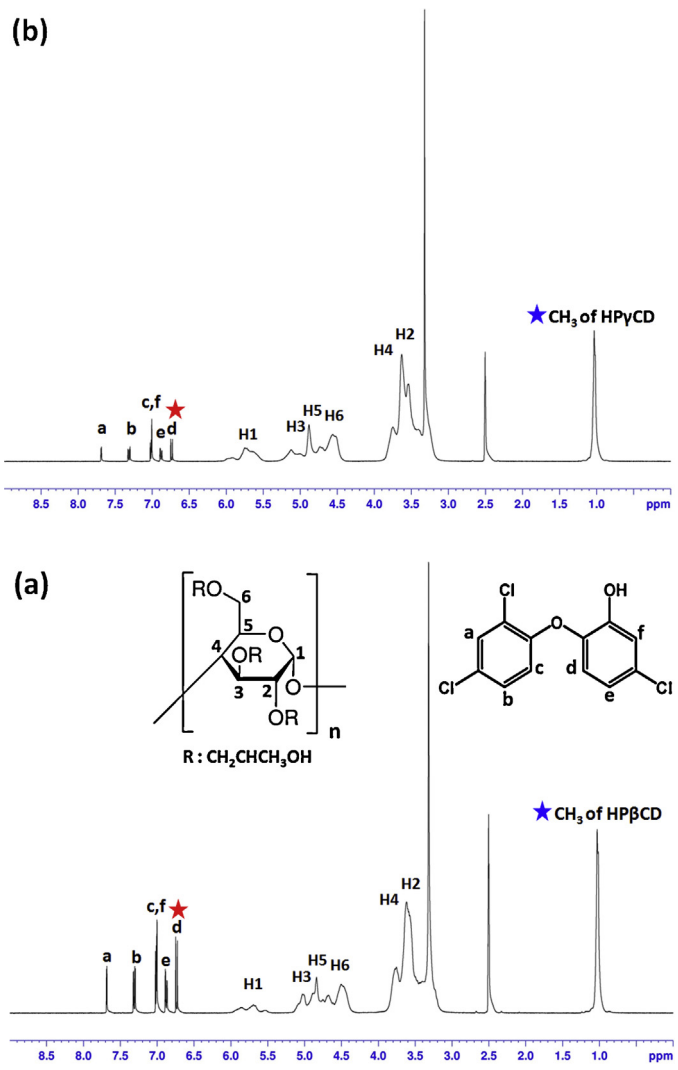

Fig. 4. ^{1}H-NMR spectra of (a) triclosan/HPβCD-IC NF and (b) triclosan/HPγCD-IC NF dissolved in *d6*-DMSO (red and blue stars are assigned for the calculated peak of triclosan and CD, respectively).

were thicker than the HPβCD based fibers for both pure CD and triclosan/CD-IC systems owing to the considerably lower conductivity and higher viscosity of the HPγCD based solutions. Fig. 2e-h indicates the photographs of HPβCD, HPγCD, triclosan/HPβCD-IC and triclosan/HPγCD-IC nanofibrous webs. As clearly seen from the photographs, HPβCD NF, HPγCD NF, triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF can be easily handled as a free-standing web and have some mechanical integrity and flexibility.

3.2. *Characterization of triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF*

The structural and thermal characterizations of the triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF were carried out by using FTIR, ^{1}H-NMR, XRD, TGA and DSC techniques. Fig. 3 shows the FTIR spectra of the electrospun triclosan/CD-IC NF samples and pure triclosan. The triclosan/CD-IC NF samples have prominent absorption peak at around 1020, 1070 and 1150 cm^{-1} that correspond to the coupled —C—C/C—O stretching vibrations and the antisymmetric stretching vibration of the C—O—C glycosidic bridge of CD molecules, respectively (Fig. 3a) [32,42]. The triclosan spectrum shows the characteristic absorption bands at 1598, 1579, 1507, 1471, 1417, and 1392 cm^{-1} that correspond to C—C stretching of the benzene ring [32,35]. As reported in the literature, some characteristic peaks of triclosan can shift to the higher wave number owing to IC formation between CD molecule and triclosan [32,37]. We have also observed small shifts for triclosan peaks in FTIR spectra of triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF, for instance, absorption peaks at 1471 and 1417 cm^{-1} shifted to 1474 and 1420 cm^{-1}, respectively (Fig. 3b). So, the FTIR study suggested the presence of host-guest interactions between CD cavity and triclosan in triclosan/CD-IC NF samples.

The presence of triclosan and the molar ratio between triclosan and CD (HPβCD and HPγCD) in NF samples were determined by ^{1}H-NMR study (Fig. 4). The molar ratio was calculated by integrating the peak ratio of the characteristic chemical shifts (δ) corresponding to triclosan, HPβCD and HPγCD by using the NMR software. The molar ratios were calculated by taking account the integration of triclosan aromatic peak at about 6.7 ppm [43] and the CD's characteristic peak at about 1 ppm [44]. For CD molecules, the chemical shift (δ) (1 ppm) that was used for calculation corresponds to the CH_3 of hydroxypropyl groups and the number of H belonging to this part were determined by using molar substitution of hydroxypropyl as 0.6 for both CD types. It was found from the calculation that the triclosan:CD molar ratio in triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF was 1:1 and 0.7:1, respectively. The triclosan:HPβCD 1:1 molar ratio for triclosan/HPβCD-IC NF was quite well agreeable with our initial 1:1 mixing ratio in the solution. This indicates that triclosan was fully complexed with HPβCD and

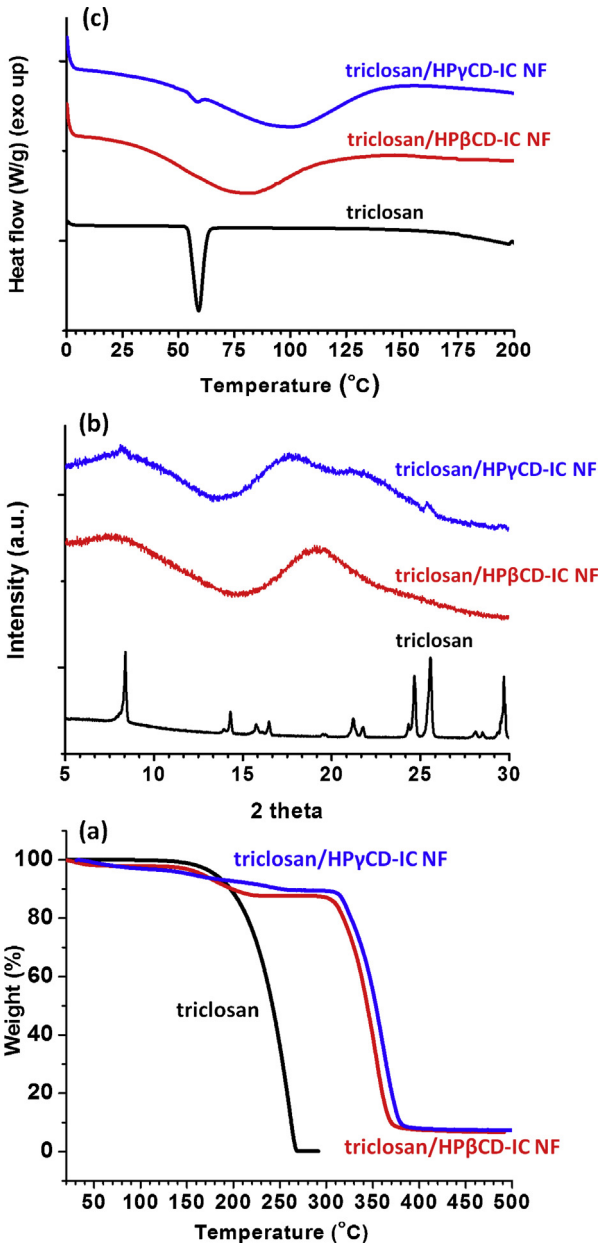

Fig. 5. (a) TGA thermograms, (b) XRD patterns, and (c) DSC thermograms of pure triclosan, triclosan/HPβCD-IC NF, and triclosan/HPγCD-IC NF.

its loss was protected by inclusion complexation during the electrospinning process and/or during storage. However, in the case of triclosan/HPγCD-IC NF, the triclosan:HPγCD molar ratio in NF sample (0.7:1) was lower than the initial ratio (1:1) and this is probably because of some uncomplexed triclosan in triclosan/HPγCD-IC suspension that could not be effectively preserved and some amount of triclosan was lost during the electrospinning process and/or during storage.

TGA thermograms of pure triclosan powder and triclosan/CD-IC NF samples are shown in Fig. 5a. The thermal evaporation/degradation of triclosan started at about 140 °C (Td onset) and continued till 250 °C. For triclosan/HPβCD-IC NF, three stages of weight losses were observed between 25 and 400 °C. The weight losses at 100 and 300 °C correspond to the water loss and main thermal degradation of HPβCD, respectively (Fig. 5a). On the other hand, triclosan/HPβCD-IC have additional weight loss having onset point (Td onset) at 150 °C and belong to the evaporation/degradation of triclosan. The Td onset for pure triclosan was 140 °C, however, in the case of triclosan/HPβCD-IC, Td onset of triclosan slightly shifted to the higher temperature (150 °C) suggesting the complexation between triclosan and HPβCD. The triclosan amount in triclosan/HPβCD-IC NF was calculated as ~10% (w/w, with respect to HPβCD) that is correlated with the 1:1 molar ratio complexation and proved the preservation of triclosan during the electrospinning. This result was also supported by the ^{1}H-NMR analysis. For triclosan/HPγCD-IC NF, four stages of weight losses are observed; the initial step below 100 °C belongs to water loss, the second step started at about 140 °C corresponds to uncomplexed triclosan degradation, the third step having onset point at 210 °C due to degradation of complexed type of triclosan, in last step, the main degradation of the HPγCD is observed at about 300 °C. From TGA data of triclosan/HPγCD-IC NF, we have detected the existence of triclosan which could not make IC and wane at the triclosan amount during the electrospinning because the total of the calculated triclosan from TGA thermogram (~7.5%-w/w, with respect to HPγCD) is less than the used quantity (~9%-w/w, with respect to HPγCD for the 1:1 molar ratio). This finding indicates that, certain parts of the guest molecule formed IC and the other part could not be preserved because of the uncomplexation. The obtained TGA outcomes also clarified the results taken from ^{1}H-NMR analysis for triclosan/HPγCD-IC NF in terms of molar ratio (1:0.7). Furthermore, for triclosan/HPγCD-IC NF, the thermal degradation of complexed triclosan delayed to the higher temperature (210 °C) compared to complexed triclosan which existed in triclosan/HPβCD-IC NF, indicating that the interaction between triclosan and HPγCD was much stronger in comparison with HPβCD.

Fig. 5b shows the XRD pattern of pure triclosan powder and triclosan/CD-IC NF samples. Triclosan is a crystalline material having major diffraction peaks at $2\theta = 8.2^{\circ}$, 24.4° and 25.4° (Fig. 5b). The uncomplexed pristine HPβCD NF and HPγCD NF have amorphous structure just like their powder forms [40]. For triclosan/HPβCD-IC NF, the similar amorphous XRD pattern was obtained owing to complexation between HPβCD and triclosan molecules, because guest molecules are separated from each other inside the CD cavity so they cannot form crystal structure [32,42]. However, triclosan/HPγCD-IC NF have diffraction peak with low intensity for the uncomplexed triclosan part, additively to the amorphous HPγCD pattern and this situation also supported the TGA results.

DSC is a useful technique to supply evidence for evaluating the IC formation between CD and guest molecules. In the case of complexation generally, the thermal transition of guest molecules such as melting or sublimation can shift or disappear [32,42]. The DSC graphs of triclosan powder, triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF are given in Fig. 5c. The pure triclosan scan indicates a melting at around 60 °C, on the contrary, there is no endothermic peak for triclosan/HPβCD-IC NF, confirming the fully IC formation. For triclosan/HPγCD-IC NF, the melting point of triclosan (Tm ~ 60 °C) was observed that is originated from the uncomplexed triclosan part, so DSC also proved the existence of uncomplexed triclosan in the triclosan/HPγCD-IC NF.

In brief, TGA, XRD, and DSC measurements demonstrated that, triclosan/HPγCD-IC NF have some uncomplexed triclosan molecules unlike triclosan/HPβCD-IC NF. So, the initial 1:1 molar ratio of triclosan:HPβCD was optimal and it was preserved during the electrospinning for triclosan/HPβCD-IC NF. However, 1:1 molar ratio of triclosan:HPγCD was excessive and some uncomplexed triclosan was detected for triclosan/HPγCD-IC NF. In addition, from the TGA data, we observed that the HPγCD make more influential interaction with triclosan compared to HPβCD, most probably due to the better size match between HPγCD and triclosan molecules [24].

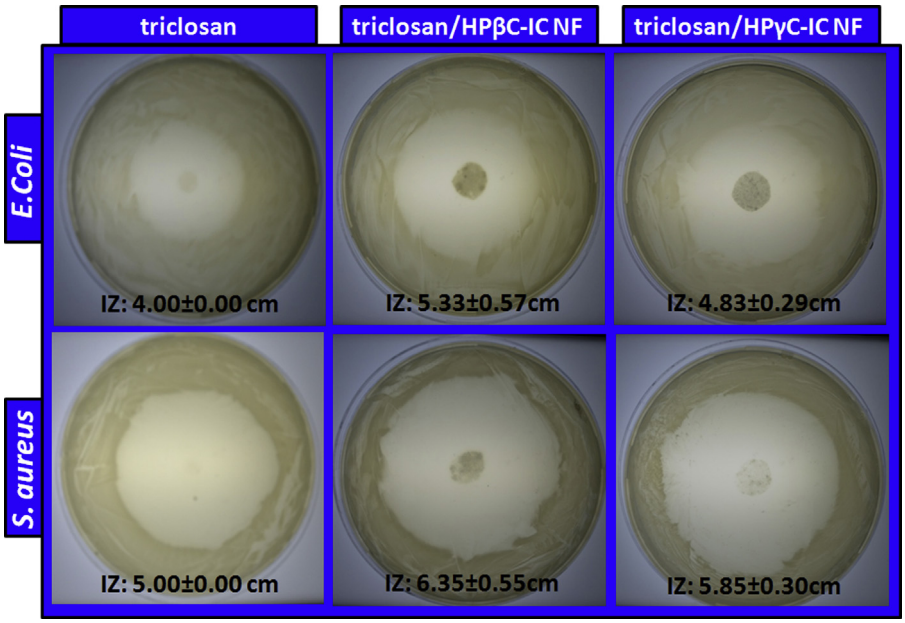

Fig. 6. The representative photographs of antibacterial test plates with the average inhibition zone (IZ) and standard deviation calculations for *E. coli* and *S. aureus* treated with pure triclosan, triclosan/HPβCD-IC NF, and triclosan/HPγCD-IC NF.

3.3. Antibacterial activity of triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF

The antibacterial activity of triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF were investigated against Gram-negative (*E.coli*) and Gram-positive (*S. aureus*) bacteria. Pure HPβCD and HPγCD do not have antibacterial activity so their NF were not compared [45]. Fig. 6 indicates the representative photographs of antibacterial test plates with the average inhibition zone calculations for zero and 24 h time intervals. It was observed that triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF show efficient antibacterial activity against both of the bacteria type. In the pure triclosan plates, the inhibition zones were measured as 4.00 ± 0.00 cm and 5.00 ± 0.00 cm for *E.coli* and *S. aureus* bacteria, respectively. For triclosan/HPβCD-IC NF, the inclusion zones were wider, 5.33 ± 0.57 cm and 6.35 ± 0.55 cm for *E.coli* and *S. aureus* bacteria, respectively. The inhibition zones are smaller in triclosan/HPγCD-IC NF plates, and they were determined to be 4.83 ± 0.29 cm for *E.coli* and 5.85 ± 0.30 cm for *S. aureus*. The increased antibacterial activity of triclosan is caused by the IC mechanism that increases the solubility and therefore provided efficient release of the hydrophobic agent in the agar medium. Triclosan/HPγCD-IC NF contains uncomplexed triclosan part, so the solubility improvement could not be very efficient and this lead to a slightly smaller inhibition zone compared to triclosan/HPβCD-IC NF. The stronger interaction between HPγCD and triclosan molecules could be another reason for the lower antibacterial activity, which results in a delay of the release of triclosan. Furthermore, the inhibition zone areas are larger for *S. aureus* compared to *E. coli* due to the cellular wall content differences between Gram negative and Gram positive bacteria [46].

4. Conclusion

Here, the electrospinning of NF from triclosan/HPβCD-IC and triclosan/HPγCD-IC systems were achieved, although it is quite a challenge to obtain NF from these non-polymeric systems. The FTIR, ^{1}H-NMR, TGA, XRD and DSC analyses proved the host-guest IC formation between triclosan and CD (HPβCD and HPγCD) in the electrospun CD-IC NF. Moreover, it was observed that triclosan was fully complexed having 1:1 molar ratio of triclosan:HPβCD in triclosan/HPβCD-IC NF. Yet, in the case of triclosan/HPγCD-IC NF, some uncomplexed triclosan was detected for this sample indicating that the 1:1 initial molar ratio of triclosan:HPγCD could not be preserved during the electrospinning. We have performed the antibacterial tests for triclosan/CD-IC NF against Gram-negative (*E.coli*) and Gram-positive (*S. aureus*) bacteria. We have observed more efficient antibacterial effect for triclosan/HPβCD-IC NF and triclosan/HPγCD-IC NF against *E.coli* and *S. aureus* compared to pure triclosan powder. Electrospun CD-IC NF are very intriguing materials owing to the unique properties by combination of very high surface area and the specific functionalities of CD-IC supramolecular structures. Moreover, CD-IC NF can have higher amount of active component when compared to polymer-templated NF incorporating CD-IC. Thus, better bioactivity may be provided by polymer-free CD-IC NF due to the higher content of active component. In brief, due to the non-toxic nature of CD, and the enhanced functionality of CD-IC along with fibrous morphology, the electrospun triclosan/CD-IC NF or CD-IC NF containing other types of bioactive agents would be applicable in biotechnology area such as wound dressing and drug delivery, etc.

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