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Investigation on Decontaminating Efficiency of *N,N*-Dichloropolystyrene Sulfonamide Nanofibers against Pathogenic Bacteria of Human Body Flora

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N,N-Dichloropolystyrene sulfonamide as a decontaminating agent against pathogenic bacteria that could be synthesized during five steps from styrene precursor. Structures of products of each step were characterized by FT-IR technique and finally the active chlorine (Cl^+) contents were checked by standard iodometry titration and determined to be 10 to 26 %. Nanofibers of *N,N*-dichloropolystyrene sulfonamide were electrospined from mixture of polystyrene: *N,N*-dichloropolystyrene sulfonamide (1:5) in DMF. Diameters of these fibers were estimated to be about 160-1900 nm by scanning electron microscopy (SEM). The decontaminant efficiency of these fibers was evaluated by treatment of three standard pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The results of this study, represented the bactericidal ability of *N,N*-dichloropolystyrene sulfonamide nanofibers.

Key Words: *N,N*-Dichloropolystyrene sulfonamide, Decontaminant, Nanofibers, Biological agent, Electrospinning.

INTRODUCTION

Nowadays, biological pathogens from hospitals are considered as great issues for human health. In order to protect the patients against these bacteria, new protective mechanisms are particularly needed[1]. Masks and protective clothing usually made of coal particles or plastics but have disadvantages considering their inability to eliminate the pathogens and inefficiency to exchange body evaporations along[1,2].

Applications of nanofibers in elimination of pollution are drastically emerging. Having extremely high surface to volume ratio, nanofibers are able to carry out highly efficient chemical reactions to eliminate microbial pathogens. So the nanofibers filters are capable of neutralizing biological pathogens with no reduction in air and water vapour permeability[2,3].

A layer of electrospined nanofibers have too many pores while the dimensions of the cavities are in the nano range. Containing such nano sized holes; nanofibers have very good resistance against passage of harmful environmental bacteria. Furthermore, nanofibers are capable of performing chemical reactions in order to destroy pathogens[3].

N,N-Dichloropolystyrene sulfonamide nanofibers can create and release active chlorine (HOCl) into environment. Hypochlorite acid (HOCl) hydrolyzes the peptide chain and causes loss of cell membrane of bacteria[4]. After penetration through cell walls of organisms, HOCl chlorinates (oxidation) proteins of the cell wall or enzymatic systems that can terminate the bacteria.

In this study in order to evaluate the performance of nanofibers against environmental pathogens, impacts of nanofibers have been investigated on three typical pathogenic standard bacteria including *Escherichia coli* (ATCC: M1787), *Pseudomonas aeruginosa* (ATCC: 25923) and *Staphylococcus aureus* (ATCC: 9027).

EXPERIMENTAL

Styrene used in this study was bought from Sigma and Aldrich Company and methanol, sodium chloride, chloroform, sulfuric acid, phosphorus oxytrichloride, ammonia, sodium hypochlorite solution and nutrient agar and nutrient broth were purchased from Merck. Three strains of the bacteria including *E. coli* ATCC: M1787, *P. aeruginosa* ATCC: 25923 and *S. aureus* ATCC: 9027 were utilized. Also, Shaker incubator was purchased from Jal Company. Synthesizing polystyrene, 15 mL of distilled styrene (without stabilizers) with 2 mL methanol, 5 % sodium chloride salt and 17 mL deionized water were poured into double neck boiling flask equipped with reflux condenser and nitrogen gas. The solution was stirred for 15 min in nitrogen gas environment. Then potassium

persulfate was added as the initiator at 72 ºC. The reaction was stirred for 12 h under the same conditions. Finally a white solid was obtained[5]. In order to synthesize sulfuric acid polystyrene 2 g of obtained polystyrene were dissolved in 20 mL of chloroform. Then 11 mL concentrated sulfuric acid were added at 50 ºC into the flask of reaction mixture and stirred for 2 h in this condition. Accomplishing the reaction, brown sulfonated polystyrene was obtained[5,6]. Chlorinating the produced sulfonyl polystyrene, 2 g of sulfonated polystyrene was mixed with 20 mL of $POCl_3$ and refluxed for 4-6 h. After completion of the reaction, $POCl_3$ were distilled in vacuum. Finally, obtained sulfonyl chloride polystyrene was washed with methanol. A brownish powder was remained as product of this phase[6].

At the next phase, 2 g of sulfonyl chloride polystyrene was refluxed with 30 mL concentrated ammonia for 4-6 h in order to produce yellow powder, which was polystyrene sulfonamide. Following mixing 2 g of polystyrene sulfonamides with sodium hypochlorite solution (which was acidified by acetic acid) for 2-4 h at 5 ºC resulted in formation of *N,N*-dichloro polystyrene sulfonamide[6].

Polymer of *N,N*- dichloro polystyrene sulfonamide was dissolved in DMF for electrospinning process, but obtaining an appropriate viscosity of this polymer in DMF was not possible. In order to achieve proper viscosity, combination of this polymer along with polystyrene was used in DMF. Electrospinning syringe contained a polystyrene mixture including 30 % (w/v) concentration of 1:3 ratios of *N,N*-dichloro polystyrene sulfonamide, which was dissolved in 2 mL of DMF. Obtaining good nanofibers, all spinning conditions such as electric potentials, spinning distance, polymer ratios and solvents were optimized experimentally. Two methods were used to examine the impact of nanofibers on the bacteria:

First method: Direct bactericidal impact of nanofibers on pathogenic *E. coli* bacterium and decontaminating time. In this study, nanofibers were directly added into the nutrients brass culture media to determine the bactericidal time of polymer. For this test two tubes of bacterial cultures with 0.6 OD were selected. One of the tubes was considered as blank witness and the other as test tube. 0.2 g of nanofibers was added to test tube. During first 2 h each 20 min, 10 µL of each culture media were sampled from tubes and cultured on agar in pour-plate manner. The plate was moved into an incubator at 37 ºC. After 48 h bacterial colonies were counted on the plates.

Second method: Certain concentrations of nanofibers were accommodated in specific disks and placed on nutrient agars. Their effects on growth of two bacteria including *S. aureus* and *P. aeruginosa* were studied. In this method the diameter of empty area around the nanofibers containing disks shows sensitivity of bacteria. Also, the least amount of nanofibers was used for exact determination of the nanofibers impact upon bacteria. Kirby-Bauer method was used in order to check the influence of these nanofibers on different families of pathogenic bacteria[7,8].

RESULTS AND DISCUSSION

In this study, the products of each polymer synthesis steps were confirmed by FT-IR technique. The spectrums of products at each stage are shown in Fig. 1. The FT-IR spectrum of polystyrene sulfonic acid has more peaks than spectrum of polystyrene. These peaks appear in the regions of 3345 cm^{-1} (OH stretching), 1413 and 1183 cm^{-1} (asymmetrical and symmetrical vibrations of SO_2 group). The OH stretching peak is so strong that covers several branched peaks of C-Hs of aromatic ring. The broad peak of OH stretching is eliminated in FT-IR spectrum of sulfonyl chloride polystyrene because of substitution of OH group with Cl. A broad peak of NH_2 group appears in the spectrum of polystyrene sulfonamide in 3448 cm^{-1}. Several peaks appear at spectrum of *N,N*-dichloro polystyrene sulfonamide in 3448 cm^{-1} (NH_2), 3200 cm^{-1} (Ar-H), 1382 and 1184 cm^{-1} (asymmetric and symmetric vibrations of SO_2) and 832 cm^{-1} (N-Cl). It is necessary to note that all mentioned peaks match the reported ones for these compounds[6]. Additionally, iodometry test determined that the polymer contains 10-26 % active chlorine[9].

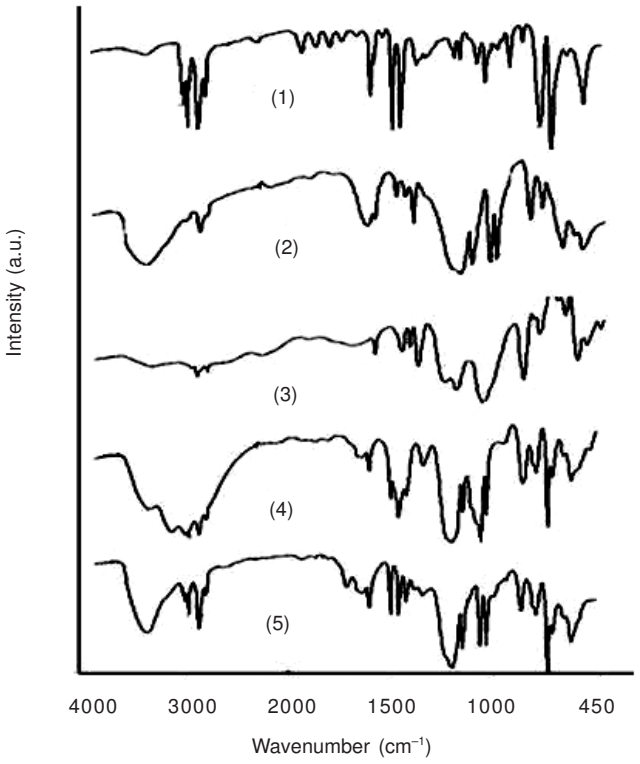

Fig. 1. FT-IR spectra of the products of *N,N*-dichloro polystyrene sulfonamide synthesis procedure: Poly styrene (**1**), polystyrene sulfonic acid (**2**), polystyrene sulphonyl chloride (**3**), polystyrene sulfonamides (**4**), *N,N*-dichloro polyester sulfonamide (**5**)

Best nanofibers achieved with applied electric potential of 22 kV and 13 cm distance between the syringe and the collector surface. It should be noted that different solvents (chloroform, NMP, DMSO) along with various ratios of the two polymers were also tested but the best conditions for electrospinning are the mentioned above. Using the SEM images, diameters of produced nanofibers were estimated at 160-1900 nm. Image of the nanofibers is shown in Fig. 2.

Direct effects of nanofibers on *E. coli* in culture represented complete eradication of bacteria in first 20 min. It was not possible to use photometry techniques in order to count colony forming unites of bacteria because not only presence

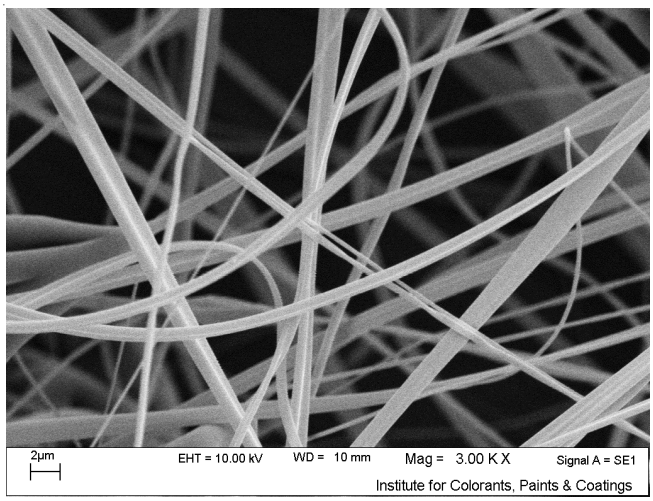

Fig. 2. SEM image of fibers produced by electrospinning from a mixture of polystyrene and *N,N*-dichloro polystyrene sulfonamide

of nanofibers interfere the process but also presence of dead bacteria can reduce the accuracy of counting. So translocation of bacteria into agar plates is more appropriate method.

The impact of nanofibers on the two types of bacteria such as *S. aureus* and *P. aeruginosa* are shown in Fig. 3. Using a ruler the halo around nanofibers disks was measured in mm (Table-1). The results showed that this polymer has high bactericidal activities. Comparing two species of bacteria revealed higher sensitivities of *S. aureus* to nanofibers. The result indicates that the effects of nanofibers are higher against gram-positive bacteria. It seems that if further testing would have been accomplished, nanofibers could have been used in the filters, clothes and tools that protect individuals against fatal germs.

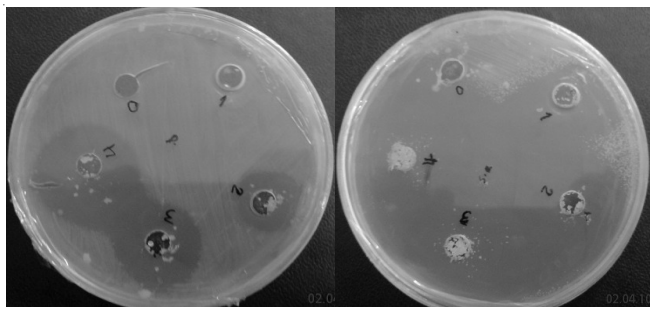

Fig. 3. Right picture is halo formation in *P. aeruginosa* culture; the left image is halo formation in *S. aureus* culture

As shown in Table-1, *N,N*-dichloro polystyrene sulfonamide nanofibers can kill both *Pseudomonas* and *Staphylococcus* bacteria. According to diameters of the formed halos around these bacteria, it could be realized that *P. aeruginosa* is less sensitive to the chlorine released from *N,N*-dichloro polystyrene sulfonamide nanofibers than *S. aureus*. This presents high ability of nanofibers in elimination of Gram-positive pathogenic bacteria. This nanofiber could be used in coating equipments and design of protective clothing that are resistant against biological agents used in unconventional warfare. Nanofibers could be used in order to construct filters needed to clean and disinfect drinking water in emergency[10].

TABLE-1
HALO DIAMETERS OF NANOFIBERS IN PRESENCE OF *P. aeruginosa* AND *S. aureus* IN THE CULTURE MEDIA (mm)

No sink	0	1	2	3	4
Nanofibers weight (mg)	0	0.0010	0.0025	0.005	0.0100
P. aeruginosa (mm)	0	9	15	20	25
S. aureus (mm)	0	25	30	Non-measurable	Non-measurable

Conclusion

As shown in Table-1, *N,N*- dichloro polystyrene sulfonamide nanofibers can kill both *Pseudomonas* and *Staphylococcus* bacteria. According to diameters of the formed halos around these bacteria, it could be realized that *P. aeruginosa* is less sensitive to the chlorine released from *N,N*-dichloro polystyrene sulfonamide nanofibers than *S. aureus*. This presents high ability of nanofibers in elimination of Gram-positive pathogenic bacteria. This nanofiber could be used in hospital equipment, to prevent the spread of common hospital infection. However, nanofibers could be used in order to construct filters needed to clean and disinfect drinking water in emergency.

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